PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

SELENIUM

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Public Health Goal for
Selenium
in Drinking Water

NOT A REGULATORY STANDARD

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PREFACE

Drinking Water Public Health Goals
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This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.

8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
9. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Public Health (DPH) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs).

PHGs are not regulatory requirements, but instead represent non-mandatory goals. Using the criteria described above, PHGs are developed solely for use by the California Department of Public Health (DPH) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Thus, PHGs are not developed as target levels for cleanup of ground or ambient surface water contamination, and may not be applicable for such purposes, given the regulatory mandates of other environmental programs.

Whereas PHGs are to be based solely on scientific and public health considerations, drinking water standards adopted by DPH are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DPH shall be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. Each primary drinking standard adopted by DPH is required to be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. MCLs established by DPH must be at least as stringent as the federal MCL, if one exists.

Additional information on PHGs can be obtained at the OEHHA web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR SELENIUM IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) hereby establishes a public health goal (PHG) of 30 micrograms per liter [µg/L or parts per billion (ppb)] for water-soluble and bioavailable selenium compounds in drinking water. The no observed adverse effect level (NOAEL) is 0.015 milligrams per kilogram of body weight per day (mg/kg-day) for hair loss and nail damage observed in a human population. The evaluation is focused on water-soluble and bioavailable selenium because this is the form of selenium most commonly found in drinking water.

The primary water-soluble selenium forms in aqueous media that are bioavailable to humans are inorganic selenate (SeO_4^{-2}) and selenite (SeO_3^{-2}) ions. Compared to drinking water, food is the major overall source of selenium for humans. Moderately water-soluble organic selenium compounds such as selenomethionine and selenocysteine are the major selenium species in plants and in food for human consumption. Selenium is an essential trace element for human nutrition. Drinking water should not be regarded as a nutritional source of selenium, and intake from drinking water should be less than the total tolerable exposure through all routes. The PHG is based on a comprehensive assessment of animal and human studies on both toxicity and essentiality of water-soluble and bioavailable selenium compounds.

The extensive field studies on humans by Yang *et al*. (1981; 1982a,b; 1983; 1987; 1988a,b; 1989a,b), Yang (1987), Yang and Zhou (1994), and Yang and Xia (1995) provide a database for the best estimates of the toxic doses to adult humans for chronic oral exposures to selenium, including dietary and drinking water intakes. These studies reported adverse effects in approximately 400 adult villagers exposed to excess selenium in a remote mountainous region of China. This population was exposed to environmental concentrations of selenium more than 10 times the normal dietary intake. The exposure was mainly in the form of selenomethionine in locally grown grains and vegetables from seleniferous soils, and to a much lesser extent in the drinking water. The toxic effects of selenium, called selenosis, included increased frequency of thickened and brittle fingernails, garlic odor in the breath, hair loss, skin lesions, and disturbances of the digestive tract. Nervous system abnormalities such as hemiplegia, peripheral anesthesia, acroparesthesia and pain in extremities were observed in some cases. Yang (1987) also reported lowered hemoglobin levels in residents of a chronic selenosis area, compared with residents of a nearby area without selenosis. Measurements of selenium intakes and whole blood concentrations indicate that the NOAEL is 0.015 mg/kg-day or 15 µg/kg-day. This dose led to disappearance of selenosis symptoms in recovering adults (Yang and Zhou, 1994).

We include an uncertainty factor (UF) of three, to account for potentially sensitive subpopulations such as infants, pregnant women and their fetuses, the elderly, the

undernourished with respect to proteins and methionine, and patients with liver diseases, to convert the NOAEL to an acceptable human intake of 5 µg/kg-day. The uncertainty factor is also recommended because the human exposures that the NOAEL is based on were mainly to selenomethionine (in food). The U.S. Environmental Protection Agency derived their reference dose (RfD) of 5 µg/kg-day for selenium compounds (U.S. EPA, 1991) in the same way. Assuming an adult body weight of 70 kg, a total acceptable daily oral intake of 350 µg/day was calculated (5 µg/kg-day × 70 kg = 350 µg/day).

The National Research Council (NRC, 2000) provided selenium dietary and supplement intake data based on the Third National Health and Nutrition Examination Survey (NHANES III) market-basket survey data of 1982 to 1994. For adults, the overall mean selenium intake from diet alone was estimated as 114 µg/day. Most individuals in the U.S. readily meet their nutritional needs for selenium and do not consume selenium supplements. However, supplements are available in doses up to 300 µg/day. The 90th percentile selenium intake for diet plus supplements is estimated as 175 µg/day. In order to account for this variability of intakes, OEHHA chose to consider two dietary intake values for selenium. For the typical consumer who does not take selenium supplements in excess of the Recommended Dietary Allowance, the mean selenium intake from diet alone at 114 µg/day is used as the background dietary selenium consumption rate. For consumers who do take selenium supplements in excess of the Recommended Dietary Allowance, the 90th percentile selenium intake for diet plus supplements at 175 µg/day is used as the background dietary selenium consumption rate.

Another consideration is adequate protection for zero- to six-month-old infants, due to their potential exposure to selenium from drinking water-diluted powdered formula. Intake for infants is derived from values for selenium in human breast milk and in powdered infant formulas, supported by data from NHANES III (NRC, 2000) and other sources for total daily infant exposures to selenium. The NRC derived a selenium Tolerable Upper Intake Level for infants aged zero to six months of 45 µg/day. Tolerable Upper Intake Level is defined as the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. For selenium, this is based on data showing that a human milk selenium concentration of 60 µg/L was not associated with known adverse effects. The NRC identified an average concentration of selenium in human milk in the U.S. as 18 µg/L. Commercially available powdered formula in the U.S. contains about the same equivalent selenium content. However, the average total daily selenium consumption in infants zero to six months of age was estimated by NRC (2000) as 35 µg/day, which would correspond to selenium derived from breast milk or formula, water, and any supplemental foods.

The subtraction method has sometimes been used to calculate an acceptable drinking water concentration of chemicals (U.S. EPA, 1989), especially for nutrients. With this technique, the portion of the allowable daily intake of a chemical which is derived from all sources except water is estimated. The remainder can be allocated to exposure from drinking water. For adults, subtraction of the background dietary intake of 114 µg/day or 175 µg/day (NRC, 2000) from the total daily oral intake of 350 µg/day (U.S. EPA, 2009), divided by the assumed water consumption rate of 2 L/day would result in an allowable intake from drinking water of approximately 88 to 118 µg/L [(350 µg/day – 114 µg/day) / (2 L/day) = 118 µg/L or (350 µg/day – 175 µg/day) / (2 L/day) = 88 µg/L]. A

comparable calculation for infants who consume formula yields a much lower value of 27 µg/L [45 µg/day – 18 µg/day) / (1 L/day) = 27 µg/L]. No adverse effect has been reported for infants aged zero to six months at up to 45 µg/day, and at 60 µg/L in human milk. No specific toxicity has been observed in infants, and overall data for infants is inadequate to more accurately determine a maximum level.

Calculation of a health-protective level based on the NOAEL (0.015 mg/kg-day) and the UF of three rather than this subtractive method provides similar values. For adults, the default parameters included a relative source contribution of 0.2 from drinking water, i.e., that twenty percent of total exposure is assumed to be provided by drinking water. A 70 kg body weight and a drinking water consumption rate of 2 L/day yields a value of 35 µg/L [0.015 mg/kg-day × 70 kg × 0.2 / (3 × 2 L/day) = 35 µg/L]. For infants, the default parameters including a relative source contribution of 0.6 from drinking water and a drinking water consumption rate of 1 L/day yield a value of 30 µg/L [0.015 mg/kg-day × 10 kg × 0.6 / (3 × 1 L/day) = 30 µg/L]. In order to be adequately protective of infants, we therefore set the PHG at 30 µg/L.

The U.S. EPA has promulgated a Maximum Contaminant Level Goal (MCLG) of 0.05 mg/L (50 µg/L or 50 ppb), and a Maximum Contaminant Level (MCL) of 0.05 mg/L for selenium in drinking water (U.S. EPA, 1991). The California MCL for selenium, established in 1994 by the California Department of Health Services (DHS, now Department of Public Health, or DPH), is also 50 ppb. The action level for selenium in drinking water recommended by WHO is 10 ppb (WHO, 1996a, 2001).

INTRODUCTION

The purpose of this document is to establish a PHG for selenium in drinking water. Selenium is an essential element for humans, animals, and some species of microorganisms only in minute amounts; toxicity can follow if the intake exceeds the tolerable levels by relatively small amounts. The range of selenium intake between the level that causes deficiency and the level that causes toxicity is relatively small (Fordyce, 2005; Reilly, 1996, 2006; Renwick, 2006). Selenium is currently regulated under the Safe Drinking Water Act (SDWA) with a federal MCL of 50 ppb established by the U.S. EPA (1991). The California MCL for selenium is 50 ppb or µg/L, adopted by DHS in 1994 from the 1991 U.S. EPA rule.

In this document, background information on chemistry and biology of selenium have been summarized and data on the toxicity of water-soluble and bioavailable selenium compounds, primarily administered by the oral route, have been evaluated. Our review of the available data includes current information via Medline, Toxline, and Web of Science, as well as TOMES PLUS® (Toxicology and Occupational Medicine System) (Hall and Rumack, 2004). New data on selenium, especially on the benefits of selenium as a food supplement, are plentiful. However, toxicity studies on excess selenium useful for risk assessment are limited and no new studies have been identified that would be more appropriate for the quantitative risk assessment than the ones by Yang and his colleagues from their studies in China.

Development of the PHG considered both essentiality and toxicity. Of all essential elements, selenium has one of the most narrow ranges between dietary deficiency and toxic levels (Renwick, 2006). The deficiency intake level is generally considered as about 40 µg/day or less, and the toxic intake level is generally considered as about 400 µg/day or more (Fordyce, 2005).

In PHG development, special considerations are given to potentially sensitive subgroups that comprise a meaningful portion of the general population, including but not limited to infants, children, pregnant women, lactating women, and the elderly. Selenium nutritional status, age, gender, physical activity, ethnicity, and genetic variations in selenoproteins can play a role in sensitivity to selenium toxicity. It is possible that those undernourished with respect to protein or methionine, or patients with hepatitis or other liver diseases, could have compromised abilities to methylate and excrete selenium before it reaches toxic levels. These people are likely to be more sensitive to selenium toxicity. Exercise alters selenium status and selenoenzyme activity differently in young and elderly (Rousseau *et al.*, 2006). Mutations or polymorphisms in selenoprotein-related genes may cause differential sensitivity to selenium. There are some data or speculations that some small children may be more or less sensitive to selenium than adults (ATSDR, 2003; Chen *et al.*, 1980; Yang *et al.*, 1988a).

Selenium status can be a major factor affecting potential sensitive subpopulations' response to selenium toxicity, due to its influence on selenium distribution and expression of selenoproteins, as well as selenoenzyme activity and turnover (Squires *et al.*, 2007). Premature babies, infants and toddlers of smoker parents, the young, the elderly, women, obese females, lactating mothers, athletes, sick people, cancer patients, cardiovascular patients, smokers, alcoholics, coal mine workers, the economically poor, and the uneducated tend to have lower selenium levels than other groups. However, data are inadequate to document whether any of these subpopulations are more or less sensitive to selenium toxicity than other groups.

Selenium is found in various chemical forms with highly variable distributions in the environment (Lakin and Davidson, 1973; Reilly, 1996, 2006). The primary forms in aqueous media are soluble inorganic selenate and selenite ions. However, diet is the predominant source of human selenium exposure. Intake is mainly organic selenium in the form of selenomethionine from plants, and to a lesser degree selenocysteine in meats and dairy products (Finley, 2005a). Bioaccumulation of selenium in the food chain through aquatic plants and invertebrates has resulted in deformities and reduced survival of various fish species and water fowl. This has made selenium the primary element of concern in many environmental contaminant situations (Capman *et al*., 2009; Hamilton, 2004; Lemly, 2004; U.S. EPA, 2004a).

CHEMICAL PROFILE

Chemical Identities

Selenium, chemical symbol Se, CAS Number 7782-49-2, is a semi-metallic element with an atomic number of 34 and an atomic weight of 78.96. In the periodic table, selenium is

located in the oxygen group between nonmetallic sulfur and metallic tellurium (McNeal and Balistrieri, 1989). Selenium, discovered in 1817 in Sweden (Barceloux, 1999), occurs naturally in the earth's crust, usually in the presence of the sulfide ores of the heavy metals (U.S. EPA, 1980). Selenium, one of the three chalcogen "ore-generating" elements, possesses a wide range of essential biological functions similar to the other chalcogens, oxygen and sulfur. Because of the position of selenium between the metals and nonmetals in the periodic table, selenoproteins are ideal catalysts for biological redox transformations (Jacob *et al.*, 2003).

Selenium reactivity and bioavailability depend not only on its total content but also its speciation (oxidation state and molecular structure) (Liang *et al.*, 2006; Templeton, 1999). Selenium can assume five oxidation states (-2, 0, +2, +4, +6) and occurs in many forms such as selenide Se^{-2}, elemental selenium Se^{0}, selenite Se^{+4}, or selenate Se^{+6} (ATSDR, 2003; Cobo *et al.*, 1994; Johansson *et al.*, 2005; McNeal and Balistrieri, 1989; Robberecht and Van Grieken, 1982; WHO, 1996a). Se^{+2} compounds have not been found in nature (Sarquis and Mickey, 1980) and only a few unstable compounds are in +2 states (Rosenfeld and Beath, 1964). Table 1 lists commonly occurring chemicals and their formulae at the four common oxidation states. Determination of selenium species in water, food, tissues, and functional proteins is essential for assessment of selenium status and potential toxic effects (Pyrzynska, 2002).

Table 1. The Four Common Oxidation States of Selenium Compounds

State	Representative compounds	Chemical formula
-2	hydrogen selenide	Na_2Se
	sodium selenide	H_2Se
	dimethyl selenide	$(CH_3)_2Se$
	dimethyl diselenide	$CH_3SeSeCH_3$
	selenomethionine	$CH_3Se(CH_2)_2CH(NH_2)COOH$
	selenocysteine	$HSeCH_2CH(NH_2)COOH$
	selenohomocysteine	$HSe(CH_2)_2CHCOOH(NH_2)$
	selenocystine	$COOH(NH_2)CHCH_2SeSeCH_2CH(NH_2)COOH$
0	elemental selenium	Se^0
	selenenic acid	H_2SeO
	sodium selenenate	Na_2SeO
+4	selenium dioxide	SeO_2
	selenious acid	H_2SeO_3
	sodium selenite	Na_2SeO_3
+6	selenium trioxide	SeO_3
	selenic acid	H_2SeO_4
	sodium selenate	Na_2SeO_4

There are six stable isotopes of selenium, [^{74}Se], [^{76}Se], [^{77}Se], [^{78}Se], [^{80}Se], and [^{82}Se] (Reilly, 1996). The most abundant natural forms are [^{80}Se] at about 49.6 or 49.82 percent and [^{78}Se] at about 23.8 or 23.52 percent (Edmonds and Morita, 2000; Johansson *et al.*, 2005; Reilly, 2006; Sarquis and Mickey, 1980). [^{82}Se] accounts for about 9.19 percent,

[^{76}Se] accounts for about 9.02 percent, [^{77}Se] accounts for about 7.58 percent, and [^{74}Se] accounts for about 0.87 percent (Johansson *et al.*, 2005; McNeal and Balistrieri, 1989; Sarquis and Mickey, 1980). None of the naturally occurring selenium isotopes are radioactive. Four artificial radioactive isotopes of selenium are prepared by neutron activation, [^{72}Se], [^{73}Se], [^{75}Se], and [^{79}Se], with half-lives of 8.4 days, 7.1 hours, 120.4 days, and 60,000 years, respectively (Johansson *et al.*, 2005). The gamma (γ) emitting [^{75}Se] is used as a diagnostic marker in biochemical analysis, for example, as an *in vivo* label for proteins and a medical diagnostic agent. Neutron activation of selenium-containing samples creates radioactive [^{75}Se] and nonradioactive [^{77}Se], which can be utilized in a specific and sensitive method for trace element analysis. Selenium compounds enriched with stable nonradioactive [^{74}Se] and [^{77}Se] have also been used for studies on selenium metabolism (Finley *et al.*, 1999; Janghorbani *et al.*, 1982, 1999a,b).

Selenium can exist in the environment as inorganic species such as elemental selenium Se^{0}, selenide Se^{-2}, selenite Se^{+4}, and selenate Se^{+6}, and as organic species such as methylated selenium compounds, selenoamino acids, selenoproteins, and their derivatives (Pyrzynska, 2002). The selenium cycle in the environment involving plants, animals, and microorganisms has been presented by Frost (1972). Selenite Se^{+4} and selenate Se^{+6} in the environment can be transformed into volatile organic compounds in air or organic selenides in soil and water, i.e., selenide Se^{-2}, through microbial action of fungi and plants. Selenides Se^{-2} can be used for the formation of selenoamino acids Se^{-2} or recycled. Ruminant animals can convert dietary selenium, including selenoamino acids Se^{-2} and selenite Se^{+4} to elemental selenium in their digestive tract, which is unavailable to plants without conversion, and excrete it through feces. Selenates and selenites can be reduced to elemental selenium, and vice versa, elemental selenium may be oxidized to selenite Se^{+4} and selenate Se^{+6} in some soils at certain pH, possibly through bacterial actions, to become available to plants (Frost, 1972).

Organic selenium species are usually found in biological systems and organic selenides are found in soil and water to a lesser extent. Selenium forms covalent carbon-selenium (C-Se) bonds *in vivo*. It can be present in selenoproteins in the form of selenocysteinyl residue and in selenium-containing proteins in the form of selenomethionyl residues. Selenium also may be present in inorganic forms such as selenite and selenate, in methylated selenium compounds such as monomethyl selenol, dimethyl selenide, and trimethylselenonium ions, and selenoamino acids such as selenocysteine, selenomethionine, selenocystine, selenium-methylselenocysteine, and selenoglutathione (Lobinski *et al.*, 2000). In natural water samples, inorganic species such as selenite and selenate ions appear to predominate, and are the most environmentally mobile and biogeochemically important oxidation states of selenium (Cobo *et al.*, 1994). Low concentrations of organic dimethyl selenide were detected in seawater, and low concentrations of trimethylselenonium ion were detected in ambient water (Oyamada and Ishizaku, 1986; Tanzer and Heumann, 1990). Organic compounds such as the volatile methyl selenides, and selenosugars, trimethylselenonium ion, and selenoamino acids can be found in air, soil, and plants (Pyrzynska, 2002).

The -2 oxidation state of selenium exists in reducing environment as hydrogen selenide (H_2Se) and metal selenide with copper, lead, and iron. It tends to exist in acid and

organic-rich environments. The low water solubility and oxidation potential of these species make them largely unbioavailable to plants and animals.

When heated to temperatures at or above 400 °C, selenium can become hydrogen selenide, the principal gaseous inorganic selenium compound. The properties of hydrogen selenide are similar to hydrogen sulfide (H_2S) and hydrogen disulfide (HS_2), but hydrogen selenide is a stronger acid than hydrogen sulfide with a pKa of 3.73 versus 6.96, even though hydrogen selenide is a weak acid in water (Johansson *et al.*, 2005; McNeal and Balistrieri, 1989). Hydrogen selenide is more reactive in air than hydrogen sulfide and hydrogen disulfide and therefore hydrogen selenide is not usually detectable in ambient air samples. Hydrogen selenide is soluble in water at about 7.3 mL/L at 20 °C, however, it has not been detected in drinking water and ground or surface water because it oxidizes to non-water-soluble elemental selenium in water. Hydrogen selenide, a volatile colorless foul-smelling gas, is more poisonous than hydrogen sulfide but oxidizes to nontoxic elemental red selenium on the mucous membranes of the nose and in the alveoli of the lungs (McNeal and Balistrieri, 1989; Sarquis and Mickey, 1980).

The inorganic forms of selenium in the earth's crust occur mainly as selenide minerals like CaSe, Ag_2Se, or CdSe. The most common is clausthalite, or lead selenide (PbSe). Metal selenides are found in sulfide ores, particularly sulfides of copper, nickel, cobalt, and molybdenum. Selenium occurs with free sulfur and in sulfide ores; thus, it is obtained as a byproduct in the refining of copper sulfide ores. Metal selenides, as well as selenium sulfides, are virtually insoluble in water and have not been found in drinking water (McNeal and Balistrieri, 1989). Sodium selenide (Na_2Se) decomposes in water. The formation of insoluble mercuric selenide may be a major mechanism of detoxification of methylmercury by dietary selenite (Sarquis and Mickey, 1980).

Microbes, plants, and animals can convert inorganic selenium compounds like selenates and selenites to volatile methylated derivatives of selenide such as dimethyl selenide and dimethyl diselenide or to organic selenide compounds (McNeal and Balistrieri, 1989; Terry *et al.*, 2000). Some accumulator plants such as certain species of *Astragalus* contain significant amounts of volatile selenium compounds like dimethyl selenide and give out garlicky odor. Yeast and plants such as alfalfa, white grain, mustard, cabbage, broccoli, and cauliflower can bioaccumulate selenium, primarily in the form of organic Se^{-2} like selenomethionine and selenocysteine, along with dimethyl selenide. Animals metabolize selenium and release dimethyl selenide into expired air (ATSDR, 2003). A variety of organoselenides possesses antioxidation activity and induces neurotoxicity (Nogueira *et al.*, 2003).

Selenomethionine is two to four fold more bioavailable to plants than selenite, and selenocysteine is less bioavailable than selenomethionine (Fordyce, 2005; Jacobs, 1989). Selenate in soil is more mobile and soluble than selenite, and selenate is less adsorbed than selenite, although both compounds are well adsorbed. Considering the interconversion of these forms, selenium in soil is more bioavailable to plants under oxidizing alkaline conditions and less bioavailable under reducing acid conditions.

Selenocysteine, selenomethionine, and methylselenocysteine are selenides, while selenocystine is a diselenide (Mishra *et al.*, 2006). Methylated organic excretory products of selenium metabolism in humans include volatile and water-soluble dimethyl

selenide [$(CH_3)_2Se$] through respiration, and almost nonvolatile and water-soluble trimethylselenonium ion [$(CH_3)_3Se^+$] and methylselenol (CH_3SeH), and nonvolatile and water-soluble selenosugars through urine. The carbon-selenium (C-Se) and selenium-selenium (Se-Se) covalent bonds are less strong than those of their sulfur counterparts (336 versus 243 kJ/mole and 226 versus 172 kJ/mole) (Sunde, 1997). The pKa for selenocysteine, about 5.2 to 5.7, is lower than that for cysteine, about 8 to 9, as a result, selenocysteine is more reactive at physiological pH than cysteine (Johansson *et al.*, 2005; Kim *et al.*, 2000). The redox activity of the amino acids selenocysteine and selenomethionine under physiological conditions allows a variety of posttranslational protein modifications, metal free redox pathways, and unusual chalcogen redox states that increasingly attract the attention of biological chemists.

Selenocysteine, the predominant form in animals given inorganic selenium, has been identified in viruses, bacteria, archea, plants, and animals (Fu *et al.*, 2002; Stadtman, 2000, 2002). Selenomethionine is the major selenoamino acid in nonaccumulator plants like cereal grains and legumes. Grains and beans incorporate selenium largely as L-selenomethionine (Levander, 1986). Garlic, onions, broccoli florets and sprouts, and wild leeks have the ability to accumulate selenium from soil. Two selenoamino acids identified in garlic and onion are selenium-methylselenocysteine and γ-glutamyl-selenium-methylselenocysteine (Arnault and Auger, 2006). Selenium-methylselenocysteine is the major selenoamino acid in selenium accumulator plants. Other related selenoamino acids are selenocystine, selenohomocysteine, selenocystathione, and γ-glutamyl-selenium-methylselenocysteine, which are found in edible plants mostly in nutritionally insignificant amounts (Schrauzer, 2001). These five seleno-analogues of sulfur-containing amino acids and their derivatives as well as several unknown selenium species have been detected in selenium accumulator plants (Arnault and Auger, 2006; Spallholz, 1994; Terry *et al.*, 2000; Whanger, 2002). Plants that may accumulate selenium include various species of mushrooms and algae, multiple *Brassica* species, and Brazil nuts (Finley, 2005a).

Selenium in the Se^{-2} state exists mainly in the form of selenol (-SeH) in organic biological compounds. Occasionally -SeH oxidizes to -Se-Se-, however, -Se-Se- is not stable. Volatile dimethyl diselenide ($CH_3SeSeCH_3$) was detected in environmental water samples in low concentrations, however, no data are available regarding its water solubility (ATSDR, 1996, 2003; U.S. EPA, 1980, 1991). There are also no data available regarding the water solubility of trimethyl selenide or selenocysteine, and no data on dimethyl selenide, trimethyl selenide, selenomethionine, or selenocysteine in drinking water (ATSDR, 1996, 2003; U.S. EPA, 1980, 1991). However, the Aldrich (2005) catalog lists the water solubility of L-selenomethionine, a 98 percent pure white to off-white powder, as 50 mg/mL or 5 g/100 mL. Selenium-methylselenocysteine, selenocystathionine, selenotaurine, selenocystamine, and selenocystine have not been detected in drinking water and no data are available regarding their water solubility (ATSDR, 2003; U.S. EPA, 1980). Because they have not been detected, no toxicity assessment of these compounds is included in this document.

Elemental selenium (Se^0) or selenide (Se^{-2}), if present in aqueous media, may be in a colloidal state and pass through a 0.45 µm filter. Elemental selenium and metal selenides, however, have negligible water solubility and readily oxidize in the presence of

oxygen. These Se^0 and Se^{-2} species have not been detected in drinking water. Elemental selenium (Se^0) in the redox state of zero, rarely found naturally, is virtually insoluble in water and is generally considered to be chemically stable as well as biologically inert, especially in reducing environments (McNeal and Balistrieri, 1989). It forms from the burning of ores with a mixture of sulfur in sulfur dioxide (SO_2) and selenium in selenium dioxide (SeO_2). It exists in several allotropic forms in the natural environment: a brick red powder; a brownish black, glassy, amorphous mass called vitreous selenium; red monoclinic crystals of specific gravity 4.5 called red selenium; and gray to black, lustrous hexagonal crystals called gray selenium. Microbes in the environment oxidize elemental selenium to selenite Se^{+4} and selenate Se^{+6}, for example, the bacterium *Bacillus megaterium* is known to oxidize elemental selenium to selenite. Gray selenium melts at 217 °C, boils at 685 °C, and has a specific gravity of 4.81. Like sulfur, selenium can react to form ionic and covalent bonds. Elemental selenium, in the presence of oxygen, burns with a blue flame to form selenium oxides and smells like rotten horseradish upon combustion (ATSDR, 1996, 2003; U.S. EPA, 1980). Monoclinic elemental selenium is the major selenium form present in the evaporation ponds in Kesterson Reservoir, California, with aqueous selenite as another abundant form (Ryser *et al.*, 2006).

Particles of black or gray elemental selenium, and red elemental selenium formed in some bacteria in sizes greater than 300 nm, are less bioavailable than other selenium compounds (Combs *et al.*, 1996; Garbisu *et al.*, 1996; Oremland *et al.*, 2004; Schlekat *et al.*, 2000), e.g., gray selenium Se^0 had a bioavailability of two percent of selenite. However, a novel selenium Se^0 form, termed elemental nano red or nano selenium, at particle sizes of 20 to 60 nm, is equally bioavailable as selenite in rats (Gao *et al.*, 2000; Zhang *et al.*, 2001a, 2004, 2005); and less toxic than sodium selenite in an acute test in mice (Zhang *et al.*, 2001a), in short-term tests in mice (Zhang *et al.*, 2004), and in a 13-week subchronic test in rats (Jia *et al.*, 2005). Nano selenium is produced from the reduction of selenite or oxidation of selenosulfate, aggregating into nano red elemental selenium only in the presence of a biological macromolecule, such as proteins, acting as a dispersion agent. Huang *et al.* (2003) found that nano selenium with different particle sizes had marked differences in scavenging an array of free radicals *in vitro*; the smaller the particle, the better scavenging activity. No information is available on detection of nano selenium in drinking water, and therefore no toxicity assessment of this compound is included in this document. No data are available on the water solubility of dipeptide glutathione selenotrisulfide nor on its detection or measurement in drinking water (ATSDR, 1996, 2003; U.S. EPA, 1980), and thus no toxicity assessment is included here.

Selenites Se^{+4} occur in a mildly oxidizing neutral pH environment. Most selenite salts are less soluble in water than the corresponding selenates (McNeal and Balistrieri, 1989). At pH values below seven, which is common in bottom sediments, selenites are reduced to elemental selenium under mildly reducing conditions. Biselenite ion is the predominant ion in water between pH 3.5 and nine. Sodium selenite (Na_2SeO_3), a white crystal, is soluble in water but no data are available regarding its exact value of water solubility (ATSDR, 2003; U.S. EPA, 1980, 2004). However, ferric selenites such as $Fe_2(SeO_3)_3$ have extremely low water solubility; in fact, basic ferric selenite [$Fe_2(OH)_4SeO_3$] is considered insoluble in water (Rosenfeld and Beath, 1964). Selenites form stable adsorption complexes with ferric oxides, and the complexes are less soluble

in water than ferric selenites. Thus, precipitation techniques can be used to remove selenites in water. The adsorption of selenites by the oxides of metals like iron and manganese attributes to the low level of selenium in ocean waters (NRC, 1976; U.S. EPA, 2004a). Bacterial resistance to high levels of selenite is achieved through metabolic conversion to organic selenomethionine, selenocysteine, or dimethyl selenide; or reduction to insoluble elemental selenium (Combs *et al.*, 1996).

Selenate Se^{+6} is the major inorganic selenocompound in most foods of plant and animal origin. Selenates occur in alkaline aerated surface water. Selenates can leach from soil, transport to groundwater, and is the form of selenium most readily taken up by plants (McNeal and Balistrieri, 1989). Selenate is stable in a well oxidized environment. Water solubility of sodium selenate (Na_2SeO_4), a white crystal, is about 84 g/100 mL or 840 mg/L at 35 ^oC, which is greater than 40 percent by weight. Potassium selenate, a colorless crystal, is soluble in about one part of water. Water solubility of the silver selenates ranges from 16 to 33 mg/L (ATSDR, 2003; U.S. EPA, 1980, 2004). Selenate and selenite can be reduced to red amorphous selenium by numerous bacteria and yeasts (NRC, 1976). Bacterial reduction of selenate to insoluble elemental selenium is an important remedial technology to remove selenium from contaminated water and sediment (Zhang *et al.*, 2004). Inorganic selenites and selenates were used in floriculture as pesticides from about the mid-1930s, but are now banned (Frost, 1972).

Selenium dioxide (SeO_2), a white solid, dissolves in water and absorbs water from moist air to form selenious acid (H_2SeO_3) and the corresponding selenite (Se^{+4}) salts. Selenium trioxide (SeO_3), also a white solid, reacts vigorously with water producing selenic acid (H_2SeO_4) and the corresponding selenate (Se^{+6}) salts (Rosenfeld and Beath, 1964). Selenious acid ($pK_1 = 2.75$, $pK_2 = 8.5$, $\log K_1 = -2.57$, $\log K_2 = -7.03$), a colorless crystalline hygroscopic solid, is a weaker acid than selenic acid ($pK_1 = -3.0$, $pK_2 = 1.66$, $\log K_1 = 1.97$, $\log K_2 = -1.91$), a white crystalline solid (ATSDR, 2003; McNeal and Balistrieri, 1989; U.S. EPA, 1980, 1991). Selenium dioxide, selenium trioxide, selenious acid, and selenic acid are soluble in water, however, no data are available regarding their exact water solubility values (ATSDR, 2003; U.S. EPA, 1980, 1991). They have not been detected in drinking water because each becomes the corresponding selenite or selenate once it dissolves in water (ATSDR, 1996, 2003; U.S. EPA, 1980). Therefore no toxicity assessment of these chemicals or a gun blueing mixture containing selenious acid (Lombeck *et al.*, 1987) is included in this document.

Drinking water has a normal pH range of 6.5 to 8.5. For selenium in the Se^{+4} state, biselenite is more abundant than selenite at the lower pH range. At pH eight, biselenite and selenite are at about equal concentrations. In alkaline solution, selenite oxidizes slowly to selenate if oxygen is present, but not in an acid medium. Selenite is reduced to elemental selenium by sulfur dioxide or other reducing agents. Selenite binds to iron and aluminum oxides, and thus becomes insoluble in soils and is generally not present in water in any appreciable amount (WHO, 1987). For Se^{+6}, selenate would be the only species present (McKeown and Marinas, 1986). Sodium selenate (Na_2SeO_4) in neutral or alkaline conditions is soluble in water, stable, and available for plant or microbial absorption and utilization. Selenate is stable under both alkaline and oxidizing conditions. Selenate is the usual form of selenium in water, and is especially the most common form found in alkaline water (ATSDR, 1996, 2003; U.S. EPA, 1980). Only

Se^{+4} and Se^{+6} were detected in rainwater from sites in the Valparaiso region in Chile impacted by mining activities of copper ores providing the most favorable selenium species for vegetation uptake (De Gregori *et al.*, 2002). The selenate and sulfate anions are structurally similar, both being surrounded by four oxygen atoms in a tetrahedral configuration. Microbial and botanic sulfate transporter systems do not distinguish between the two anions; hence, uptake and transformation of selenate occurs in these biological systems (Terry *et al.*, 2000).

Selenium in soil is absorbed by plants mainly as selenates and to a lesser degree as selenites. Selenium accumulator and nonaccumulator plants as well as soil microflora metabolize inorganic and organic selenium to volatile dimethyl selenide with offensive odor (Spallholz, 1994). Selenate is the major inorganic selenium compound found in animal and plant tissues (Whanger, 2002). Plants metabolize selenate and selenite to selenoamino acids such as selenomethionine, selenocysteine, selenocystathionine, methylselenocysteine, and selenium-methylselenocysteine. Selenoamino acids and dimethyl selenide are less toxic than inorganic selenates and selenites in general, thus, plants can tolerate high concentrations of selenium by forming almost exclusively organic selenoethers with little accumulation of inorganic selenates and selenites (Spallholz, 1994). Selenium-enriched yeast, the most widely analyzed selenium material, contains more than 20 selenocompounds including selenocysteine, selenomethionine, selenium-methylselenocysteine, selenium-adenosylselenohomocysteine, and some inorganic forms (Lobinski *et al.*, 2000).

Mammalian selenium-containing proteins can be divided into three groups: specific selenocysteine-containing proteins designated as selenoproteins, specific selenium binding proteins, and proteins containing nonspecifically incorporated selenium (Behne and Kyriakopoulos, 2001; Gromer *et al.*, 2004, 2005; Hatfield, 2001; Kyriakopoulos and Behne, 2002; Papp *et al.*, 2007). Selenoproteins refer only to selenocysteine-containing proteins, and do not include selenium binding proteins or selenomethionine-containing proteins. Incorporation of selenocysteine into these proteins requires an elaborate translation machinery, whereas selenomethionine is randomly incorporated into proteins in place of methionine (Jacob *et al.*, 2003). Approximately 25 selenoproteins are known so far to be encoded in the human genome, about 24 in mice and rats, and about 30 in fish. Between 30 and 50 mammalian selenoproteins are expected to exist. Functional characteristics have been defined for at least five types of selenoproteins, but functions are unknown for most of this protein class.

Physical and Chemical Properties

The physical and chemical properties of the principal water-soluble selenium compounds including selenite and selenate, as well as elemental selenium and selenomethionine discussed in this review, are listed in Table 2, as adapted from ATSDR (1996, 2003).

Table 2. Chemical Identity of Elemental Selenium, Selenite, Selenate, and Selenomethionine

	Selenium	Sodium selenite	Sodium selenate	Selenomethionine
Synonyms	elemental selenium; selenium base; selenium dust; colloidal selenium; selenium homopolymer	disodium selenite; disodium selenium trioxide; selenious acid disodium salt; sodium selenium oxide	disodium selenate	methionine, seleno; 2-amino-4-(methylselenyl) butyric acid; 2-amino-4-(methylseleno)butanoic acid
Formula	Se	Na_2SeO_3	Na_2SeO_4	$CH_3Se(CH_2)_2CH(NH_2)COOH$
Molecular weight	78.96	172.95	188.94	196.11
Color	metallic gray to black; hexagonal crystals	white tetragonal crystals	colorless rhombic crystals	transparent, hexagonal sheets or plates; metallic luster of crystals
Physical state	solid	solid	solid	solid
Melting point	144 ^oC; 221 ^oC	no data	no data	D, L form: 265 ^oC (decomposes) L form: 266-268 ^oC (decomposes)
Boiling point	685 ^oC	no data	no data	not applicable
Density (g/cm^3)	4.81 (20 ^oC)	no data	3.213 (17.4 ^oC)	no data
Water solubility	insoluble	soluble	84 g/100 mL at 35 ^oC	L form: 5 g/100mL
CAS Number (No.)	7782-49-2	10102-18-8	13410-01-0	1464-42-2
RTECS No.	VS7700000	VS7350000	no data	ES7100000
OHM/TADS No.	7216880	7217299	no data	no data
HSDB No.	4493	768	no data	no data

Environmental chemistry (Frankenberger and Engberg, 1998) as well as occurrence and distribution (Ihnat, 1989) of selenium were well reviewed in ATSDR (2003). Selenium will form explosive products when it reacts with metal amides and may trigger the violent decomposition of nitrogen trichloride. Mixtures of sodium peroxide and selenium are explosive due to organic impurities formed during conversion of selenium to the dioxide form with oxygen and heat (Hall and Rumack, 2004).

Production

Most of the commercial selenium produced in the U.S. is recovered from copper ores. Primary selenium is recovered from anode slimes generated in the electrolytic refining of copper. Two copper refineries in Texas accounted for the domestic production of primary selenium (USGS, 2001). In 1981, 256 metric tons were produced domestically; an additional 338 metric tons were imported (U.S. EPA, 1985a). The domestic refinery production was 379 metric tons for 1996 and 373 metric tons for 1995. No production data were reported or available since 1997. The apparent consumption of primary selenium for 1996 was 564 metric tons. There was no domestic production of secondary selenium. Scrap photographic materials were exported for recovery of the contained selenium. The average price of refined elemental selenium to dealers in 1999 was $2.55 per pound (USGS, 2001). For 2000, the import volume was 452 metric tons and the export was 89 metric tons; in 2001 the import volume was 500 metric tons and the export was 75 metric tons (ATSDR, 2003).

Uses

Elemental selenium is used in rectifiers, photoelectric cells, blasting caps, xerography, electrostatic printing, stainless steel, optical lenses, exposure meters, and as a dehydrogenation catalyst (ATSDR, 2003; Lakin and Davidson, 1973). Gray selenium conducts electricity better in light than in darkness, which has made it useful in photoelectric devices. The use of high purity selenium as a photoreceptor on the drums of plain paper copiers has reached the “replacement only” stage because alternative materials are used in currently manufactured copiers (USGS, 2001).

In 1973, nearly 30 percent of the annual consumption of selenium went into the manufacture of glass to decolorize the undesired greenish tinge produced by ferrous compounds, to give a desirable pink tinge, or to produce black glass (Lakin and Davidson, 1973). In the form of red selenium or as sodium selenide, selenium compounds are used to impart a scarlet red color to clear glass, glazes, and enamels. It is also used to reduce solar heat transmission in architectural plate glass. Ammonium selenite is also used in the manufacture of red glass (ATSDR, 1996, 2003). In 1973, about 14 percent of the annual consumption of selenium went into the manufacture of inorganic pigments to be used in plastics, paints, enamels, inks, and rubber (Lakin and Davidson, 1973). Selenium dioxide is a catalyst for oxidation, hydrogenation, or dehydrogenation of organic compounds (ATSDR, 1996, 2003).

Combinations of bismuth and selenium are added to brasses to replace lead in plumbing applications. Small amounts of selenium are added to vulcanized rubber to increase its

resistance to abrasion. The estimated consumption of selenium by industrial end use in the U.S. was as follows: glass manufacturing, 35 percent; chemicals and pigments, 20 percent; electronics, 14 percent; and other, including agriculture and metallurgy, 31 percent (ATSDR, 1996, 2003). The amount of selenium used in the U.S. in 1968 was estimated to be about 1,065,600 pounds (Lakin and Davidson, 1973).

Selenium supplements, in the form of selenite, selenate, or selenomethionine, for human and livestock diets, are the largest pharmaceutical and agricultural uses of selenium. Such use is expected to continue to grow (Lakin and Davidson, 1973). Sodium selenate was used at one time as an insecticide, but this use is now obsolete and no registration for sodium selenate as a pesticide was found for California or the U.S. Sodium selenate is also used in medicinals to control animal diseases. Sodium selenite is used as a soil additive in fertilizers for selenium deficiency areas (ATSDR, 1996, 2003).

Release

The major source of selenium in the environment is the weathering of rocks and soils, but human activities contribute about 3,500 metric tons per year in the U.S. (U.S. EPA, 1980). Anthropogenic sources for selenium such as fossil fuel burning and industrial waste from metal smelters and steel plants account for about 41×10^6 kg/year (Nriagu and Pacyna, 1988). Acid and reducing conditions reduce inorganic water-soluble selenites to insoluble elemental selenium, whereas alkaline and oxidizing conditions favor the formation of water-soluble selenates. Selenates occur in alkaline soils and are available to plants. In acid soils, selenium is usually found as selenite bound to iron and aluminum oxides in compounds of very low water solubility (WHO, 1996a). The ultimate sink for selenium is the marine environment.

The U.S. EPA (2001) reported, in its Toxics Release Inventory (TRI) for selenium and selenium compounds in 1998 for 29 facilities, two of which were in California, the release of 8,074 pounds into air, 132 pounds into water, 17,937 pounds underground injection release, and 6,648,279 pounds onto land. Offsite waste transfer was 1,491,357 pounds, giving a total environmental release and offsite waste transfer of 7,995,769 pounds. As reported in the TRI in 1999 for 25 facilities, two of which were in California, 69,832 pounds of selenium were released into air, 285 pounds into water, none for underground injection release, and 268,425 pounds onto land. Offsite waste transfer was 19,410 pounds, giving a total environmental release and offsite waste transfer of 357,952 pounds. Three facilities in California were reported in the TRI in 2000 (U.S. EPA, 2001).

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

The atmospheric occurrence of selenium has been documented and reviewed by Mosher and Duce (1989), Chasteen (1998), Cahill and Eldred (1998), and ATSDR (1996, 2003). Selenium exists in the atmosphere in the aerosol phase, as a gas, and in precipitation. In the aerosol phase, selenium may be derived from sea spray, windblown mineral dust,

biogenic materials, volcanic effluvia, or direct anthropogenic emissions. Analysis of samples collected from a cascade impactor indicated that the median mass diameter of airborne selenium particles was 0.8 micrometer (µm), indicating a size that is able to reach the lower respiratory tract. The vapor phase selenium in the atmosphere is operationally defined as that portion of atmospheric selenium that passes through particulate collection filters. Examples of vapor phase compounds that might be present in air are selenium dioxide, hydrogen selenide, and dimethyl selenide.

The oceanic background atmospheric selenium level above the open sea and in the poles is estimated to be about 0.04 nanograms per cubic meter (ng/m^3) (WHO, 1987). The level of selenium in most urban air, mostly bound to particles, ranges from about 0.1 to 10 ng/m^3. Higher levels, in a low mg/m^3 range, may be found in areas such as the vicinity of copper smelters (WHO, 1996a). The highest urban concentration recorded in a location outside of the U.S. in the WHO (1987, 1996a) reviews was 116 ng/m^3 at four sites in Ankara, Turkey. The atmospheric selenium levels in Mumbai, India, varied between 0.02 and 1.92 ng/m^3 with a mean concentration of 0.21 ng/m^3 (Mahapatra *et al.*, 2001). A British study estimated the approximate mean concentration of selenium in air to be 3 ng/m^3 (Bennett, 1986). In China, the atmospheric selenium level varies from lower than 1 ng/m^3 in the Nanjiabawa area in remote Tibet to 0.7 to 44 ng/m^3 in metropolitan Beijing, where burning coals and fossil fuels are major sources of selenium in the air (Wang and Gao, 2001).

In the U.S. urban environment, the aerosol concentrations of selenium range from 1 to 10 ng/m^3 at standard temperature and pressure. For example, for nine San Francisco Bay Area sites, 24-hour samples collected on July 23, 1970, showed a mean selenium concentration of 1.1 ng/m^3. In the urban Maryland atmosphere, 10 to 40 percent of selenium was found in the vapor phase, with total concentrations of 0.69 to 1.1 ng/m^3. In semi-urban and rural locations such as in Vermont and Montana, selenium concentrations of 0.3 to 1 ng/m^3 are more typical. Samples from Mauna Loa, Hawaii ranged from 0.016 to 0.021 ng/m^3. Sea spray may contribute selenium to the air, but concentrations at most marine sampling sites are less than 0.5 ng/m^3. One study showed the levels near a coal-fired power plant, which is a major source of selenium to air, as less than 3 ng/m^3. Three studies have estimated a daily respiratory selenium intake of 0.02, 0.07 and less than 1 mg/day, respectively, indicating that inhalation is not a major route of exposure in comparison to ingestion from food (U.S. EPA, 1985a).

The release of selenium in the U.S. air has been summarized by ATSDR (2003) based on U.S. EPA's TRI report (U.S. EPA, 2001). Lakin and Davidson (1973) estimated the annual release of selenium from coal combustion in the U.S. as about eight million pounds based on the total selenium of 177,600 pounds in the 54.5 millions tons of coal to be burnt in one year in 10 power plants in 1968. An estimated total of at least 65,481 pounds of elemental selenium and 576,929 pounds of selenium compounds were discharged to air from manufacturing and processing facilities in the U.S. in 2000 (ATSDR, 2003). The overall release of selenium into the world's atmosphere is estimated to be 1 to 10×10^9 g (10,000 metric tons) per year.

The use of selenium in photocopiers may create elevated airborne levels in the workplace. Harkin *et al.* (1976) reported selenium concentrations of 20 to 60 ng/m^3 in an unventilated room of 48 m^3 housing a photocopier that made 450 to 475 copies daily.

Others have found levels of 3.5 to 45 ng/m^3 in an air-conditioned photocopier room, with reference outdoor concentrations at the same location of less than 1 ng/m^3.

Biomethylation of selenate and selenite ions in the soil by microbes volatilizes selenium to the air, mainly in the form of dimethyl selenide. Microbial volatilization of selenium has been considered as a method for detoxification of soils at the Kesterson Reservoir. Dimethyl selenide has been reported to be released in the air by plants such as cabbage, alfalfa, rice, and onion (Wu, 2004). Volatile selenium compounds may be generated by plants that accumulate selenium, such as garlic, cabbage, and broccoli, but the amounts released from these sources are too small to influence ambient air concentrations (Chasteen, 1998). These plants metabolize inorganic selenium compounds to volatile organic dimethyl selenide and dimethyl diselenide. Animals are also capable of metabolizing selenium and releasing dimethyl selenide in expired air (ATSDR, 2003). Dimethyl selenide has been identified in vapor phase samples collected near sewage works at concentrations of 0.2 to 5.4 ng/m^3, however, hydrogen selenide and selenium dioxide have not been directly measured in the ambient atmosphere. Dimethyl selenide has been detected at 0.45 µg/m^3 in the breath of six human subjects fed garlic (Cai *et al.*, 1995; Feldmann *et al.*, 1996).

The two principal sources of selenium in the atmosphere are anthropogenic activities such as combustion of coal or other fossil fuels, and emissions from natural sources such as windblown mineral dust, sea spray, and gaseous emissions from marine, continental and volcanic sources. Incineration of rubber tires, paper, and municipal waste is an additional source of atmospheric selenium. Selenium concentrations in atmospheric dust were estimated as 0.05 to 10 mg/kg in a study published in 1941 (McNeal and Balistrieri, 1989). Hashimoto *et al.* (1970) reported that, from Tokyo, Japan, and the vicinity, rubber tires contained selenium at a average of about 1.3 mg/kg, raw petroleum about 0.92 mg/kg, heavy petroleum fractions about 0.99 mg/kg, and coal about 1.18 mg/kg (Lakin, 1972). The average selenium content of U.S. coal was about 1 to 3 mg/kg and of petroleum about 0.2 mg/kg (Lakin, 1973). Selenium concentrations in Pennsylvania coal were estimated as 0.46 to 10.65 mg/kg in a study published in 1969 (Lakin, 1972; McNeal and Balistrieri, 1989). The selenium content of coal was about 2 to 4 mg/kg and 0.5 to 0.8 mg/kg for fuel oil (ATSDR, 2003).

In China, the selenium content of coal is about 0.04 to 10 mg/kg, petroleum is about 0.01 to 1.4 mg/kg, bituminous coal is 5.7 mg/kg, and brown coal is 4.4 mg/kg (Wang and Gao, 2001). The selenium content of coal in Ziyang County, Shaanxi Province, a seleniferous area with selenium poisoning, was about 32 mg/kg in high incidence Shuanan, about 37 mg/kg in high incidence Sanhomoutsego (Chen and Mei, 1980; Yang *et al.*, 1982a, 1983), about 19 or 30 mg/kg in high incidence Shuang'an, about 4.7, 6, 37, or 77 mg/kg in low incidence Huigouhe, and 1.3 to 12 mg/kg in low incidence Mixiliang (Fang and Wu, 2004). The stone coal and smokeless coal in selenosis Ziyang contained selenium at 5.66 to 32.06 mg/kg, compared to 0.17 to 0.27 mg/kg in nearby nonselenosis Xuyi, Shaanxi Province (Mei, 1985). The selenium content of coal in Enshi area, Hubei Province, another seleniferous area with selenium poisoning, was 185 or 155 mg/kg (Chen and Mei, 1980). The average selenium content of Enshi coal was reported as about 291 mg/kg in 1966 and 367 mg/kg in 1978. An additional 19 samples ranged from 13.2 to 1,332 mg/kg with an average of 329 mg/kg, with three others at 3,632, 66,100,

and 84,123 mg/kg (Yang *et al.*, 1981, 1982a, 1983). Another report found selenium content as high as 1,524 mg/kg in coal and 23 mg/kg in bone coal in the E'xi seleniferous region in Hubei Province (Wang and Gao, 2001). The selenium concentration of 129 E'xi stone coal samples averaged 144 mg/kg with a range from 6 to 1,150 mg/kg (Mao *et al.*, 1990). Samples collected in 10 households in E'xi revealed selenium at 158 mg/kg in stone coal, 200 mg/kg in burnt ash, and as high as 5,036.25 mg/kg in the smog dust during burning (Mao *et al.*, 1990).

Water

Dissolved selenium is operationally defined as material that passes through filters with 0.45 µm openings. Particulate selenium is material equal to and greater than 0.45 µm particle size (Cutter, 1989). Dissolved selenium exists primarily as Se^{+4} or Se^{+6} in the form of biselenite, selenite, and selenate ions. In water, the biselenite and the selenite ions are both dissolved at pH six. At pH nine, the fractional composition changes to 0.31 selenite and 0.69 selenate. Sodium selenite is rapidly reduced in sediment and binds to metal oxides with high affinity, so its concentration in natural waters is low. Ferric selenite is sparingly soluble, with a low solubility-product constant Ksp of $10^{-30.7}$, and the presence of iron reduces the concentration of selenite ions in water. Dissolved selenite is frequently oxidized over time to form the selenate ion. NRC (1976) noted that the hazards of sodium selenite in water are minimized by the probability that selenite will either form insoluble compounds such as adsorbates with ferric oxides or be reduced to insoluble elemental selenium. Dissolved selenate in fresh waters is relatively stable at pH six to nine and has properties similar to the sulfate ion.

Drinking water in the U.S. generally has low selenium concentrations. Cutter (1989) reported a selenium range of 0.12 to 0.44 µg/L, with selenate as the primary selenium species. The total dissolved selenium level was less than 0.32 µg/L in one U.S.study, less than 1 to 2 µg/L in another study, and less than 0.2 µg/L in New York (Robberecht and Van Grieken, 1982). More than 90 percent of 4,200 tap water samples collected throughout the U.S. were below the method detection limit of 1 µg/L (Greathouse and Craun, 1979); the mean selenium concentration of the rest (less than 10 percent) was 3.5 µg/L, with a range from 1 to 6.5 µg/L. The NRC (1980a) reported that less than 0.5 percent of the samples from public water supply systems in the U.S. exceeded 100 µg/L.

The U.S. EPA (2003), in the six-year review of existing National Primary Drinking Water Regulations, reported that about 77 percent of the U.S. population in the studies received selenium levels in drinking water less than 5 µg/L, about 23 percent received levels between 5 and 50 µg/L, and about 0.002 percent received levels greater than 50 µg/L (WHO, 2005). The California municipal water supply monitoring results showed that selenium was detected in 731 of 11,740 water sources sampled from 1984 to 2001 with a detection limit for purposes of reporting (DLR) of 5 µg/L, as updated on June 11, 2002 at the California DHS (now DPH) website (data now offline).

In certain situations, however, drinking water levels of selenium may be elevated. For example, during a 1975 drought in rural southeastern Colorado, selenium levels in well water used for drinking ranged from 50 to 125 µg/L (Tsongas and Ferguson, 1977). Smith *et al.* (1936) surveyed 50 rural families residing in seleniferous areas of South

Dakota and Nebraska and found selenium in only 23 percent of the water samples, at 50 to 330 µg/L. Valentine *et al.* (1978) reported that 19 home wells in the small New Mexico community of Milan contained selenium at 537 ± 650 µg/L (mean ± standard deviation, or SD), ranging from 26 to 1,800 µg/L. A later paper (Valentine *et al.*, 1980) provided values of 327 ± 549 µg/L with the same range of 26 to 1,800 µg/L. A nearby uranium mill-tailing pond was a potential source of selenium contamination. The drinking water in Jade Hills and Red Butte, Wyoming, contained selenium levels of 190 ± 10 µg/L (mean ± SD, n = 33), with a range of 174 to 202 µg/L, and 493 ± 55 µg/L (n = 36), with a range from 363 to 560 µg/L, respectively. The control community in Casper, Wyoming had selenium in drinking water at 1.71 ± 0.38 µg/L (n = 67), and a range of 0.60 to 2.90 µg/L (Valentine *et al.*, 1987).

Forty percent of all surface water samples collected from 26 National Irrigation Water Quality Program project areas during 1986-1994 in 14 U.S. western states had selenium concentrations that were at or below the laboratory reporting level of 1 µg/L. Less than one percent of all surface water samples had selenium levels exceeding 1,000 µg/L. The mean selenium levels in water samples from three National Irrigation Water Quality Program study areas were 35, 20, and 7 µg/L (Engberg *et al.*, 1998). In 112 wells producing water from the Gulf Coast Aquifer System and comprising coastward sloping beds of clay, silt, sand, and gravel in an irrigated agricultural region of south Texas, 21 percent had selenium concentrations that exceeded the 20 µg/L advisory level for irrigation water, and five percent surpassed the 50 µg/L standard for drinking water. Both irrigation practices and prevailing groundwater chemistry significantly influence selenium concentrations in the study area (Hudak, 2004).

Selenium levels can be high in well water in seleniferous areas and in rivers, ponds and reservoirs where irrigation drainage from seleniferous soils gathered (Reilly, 2006). In the shallow drain and groundwater flowing into the Kesterson Reservoir in the San Joaquin Valley of California, the average selenium value was 300 µg/L, with the maximum level detected at 3,800 µg/L (Laüchli, 1993). In the drainage water entering the Reservoir at an average of 300 µg selenium/L, about two percent of the selenium was selenite. In pond waters, 20 to 30 percent of the selenium was selenate (Wu, 2004).

Ten private drinking water wells were tested for selenium in a rural area adjacent to abandoned mines near Twisp, Washington, in samples collected between October 1999 and June 2001. The average selenium concentrations from two of the wells were 146 and 390 µg/L, with selenium nondetected in the other eight wells (Peplow and Edmonds, 2004). Selenium in the wastewater from a Wyoming uranium mine ranged from 340 to 450 µg/L; the mean concentration was 15 times higher than at a reference site (Ramirez and Rogers, 2002). Selenate concentrations up to 4,200 µg/L were reported in water draining irrigated seleniferous rocks and soils in the San Joaquin Valley (McNeal and Balistrieri, 1989).

Valentine (1997) summarized selenium levels in various water bodies and sources, such as drinking water from less than 0.01 to 0.017 µg/L, rain water at approximately 0.003 µg/L, surface and ground water supplies from 0.0007 to 44 µg/L, river water from 0.0007 to 2.4 µg/L, lake water from less than 0.05 to 0.23 µg/L, and ocean water from 0.09 to 0.11 µg/L. In general, well water had selenium from 1.9 to 44 µg/L; however, high values from 194 to 494 µg/L were reported in Wyoming, and a Colorado well had 9,000

µg/L. Spring water of an area rich in uranium in Grand County, Utah, had 1,000 to 3,000 µg/L. Colorado surface water had selenium levels from 140 to 400 µg/L and drinking water had 92 µg/L. Surface water from South Dakota had selenium as high as 3,370 µg/L. Irrigation water in California had selenium at 140 to 4,200 µg/L (Fan *et al.*, 1988). Irrigation drainage from seleniferous soils may result in water selenium concentrations up to 400 µg/L (NRC, 1976).

The average selenium concentration in effluent discharges from oil refineries into San Francisco Bay was 67 µg/L, with a range of 6.6 to 156 µg/L; approximately 50 to 76 percent of the total selenium was selenite (ATSDR, 2003; Barceloux, 1999). Based on the TRI report (U.S. EPA, 2001), an estimated total of 1,019 pounds of elemental selenium and 56,048 pounds of selenium compounds were discharged to surface water from manufacturing and processing facilities in the U.S. in 2000 (ATSDR, 2003).

Selenium levels in potable natural water are normally low, usually less than 1 µg/L in fresh water and less than 0.1 µg/L in sea water (Robberecht and Van Grieken, 1982; Bratakos *et al.*, 1988; Cutter, 1989; McNeal and Balistrieri, 1989; Shibata *et al.*, 1992; ATSDR,1996, 2003; Tamari, 1998; Wang and Gao, 2001; He *et al.*, 2005; WHO, 2005; Reilly, 2006). Total dissolved selenium ranged from less than 0.001 µg/L in the West Pacific, 0.025 µg/L in the Northeast Atlantic, 0.1 to 0.25 µg/L in Greece coastal areas, except polluted locations, to 4.4 µg/L in the North Sea. The selenium concentration was about 0.14 µg/L in the Mississippi River and about 0.21 µg/L in the Amazon River.

In China, the total selenium concentration in river water ranges from 0.04 to 5 µg/L and the soluble selenium concentration ranges from 0.01 to 0.8 µg/L (Wang and Gao, 2001). Levels were reported as less than 3 µg/L in Belgium in ground, lake, swimming pool, rain water, and snow, and between 0.182 µg/L and 9.3 µg/L in sewage water. Total dissolved selenium was about 0.8 µg/L to 100 µg/L in Lake Michigan, about 30 µg/L in the Colorado River, 20 µg/L in Nebraska in river water, and up to 480 µg/L in Nebraska groundwater. Higher levels in other areas included 48 and 67 µg/L in Argentina groundwater, 104 µg/L in Navigliaccio River in Italy, up to 200 µg/L in well water in France, 200 µg/L in the Guanajuato River in Mexico, and 280 µg/L in sewage water in the U.S. and Japan (Robberecht and Van Grieken, 1982).

Robberecht and Van Grieken (1982) reported that total dissolved selenium in drinking water ranged from less than 0.05 to 0.66 µg/L in four studies in Belgium and one each in Germany, Israel, Sweden, the Netherlands, and Russia; less than 1 µg/L in Australia and England; between 1.1 and 3.3 µg/L in England; less than 2 to 10 µg/L in France; and less than 0.12 to 3 µg/L in three other studies in Germany. One German study reported total dissolved selenium of 5.3 µg/L in mineral water and 1.6 µg/L in tap water in Stuttgart (Robberecht and Van Grieken, 1982). A British study estimated the mean concentration of selenium in drinking water to be 0.2 µg/L (Bennett, 1986). Average concentration of selenium in drinking water in Toronto, Canada, from 1978 to 1984 was 0.16 µg/L (Davies, 1990; Hall and Rumack, 2004). The concentration of selenium in tap or bottled drinking water in Greece was 0.1 to 0.2 µg/L and showed little seasonal fluctuation.

In rain water having a pH from 4.3 to 4.7 in the Valparaiso region in Chile impacted by copper ore mining and smelting, selenium distribution was a function of the distance from the industrial area, indicating that the selenium arises from the smelter of copper ore

and the thermoelectric plant (De Gregori *et al.*, 2002). Mean concentrations of inorganic selenium in rain water collected in 1998 to 1999 were up to about 20 µg/m^3.

For the studies of Yang *et al.* (1983) of selenosis in the Enshi area in China where selenium deficiency Keshan disease and selenosis occurred within 20 to 30 km of each other, 11 drinking water samples were analyzed. Four were surface water from a village with a high prevalence of selenosis and averaged 139 µg/L, with a range from 117 to 159 µg/L. The other seven samples from a variety of sources had an average of 5 µg/L. In the study of Fordyce *et al.* (2000) in the Enshi area, the maximum selenium concentration in 72 samples was 275 µg/L, from spring water in Huabei village, one of the five villages with high selenosis. The selenium concentrations in two well water samples from the E'xi seleniferous region were 8,400 and 72,000 µg/L (Wang and Gao, 2001). Yang *et al.* (1981) reported average selenium content of nine drinking water samples in the Enshi area of about 56 µg/L, with a range from trace to 158 µg/L.

Yang *et al.* (1989a,b) studied Enshi residents in three sites with low, medium, and high blood selenium levels that correlated with the soil selenium levels chosen from the seleniferous areas. The mean drinking water selenium levels were about 0.37 µg/L (n = 8), 1.72 µg/L (n = 26), and 12.27 µg/L (n = 22), respectively. The highest selenium concentration in the drinking water samples from the high selenium area was 40.9 µg/L (Yang *et al.*, 1989a). In comparison, the average selenium concentration in drinking water is 0.65 ± 1.06 µg/L for most Chinese cities and urban cities, 0.11 ± 0.08 µg/L in rural low selenium Kaschin-Beck disease region, and 0.16 ± 0.20 µg/L in rural Keshan disease region in the selenium deficient belt (Wang and Gao, 2001). Mei (1985) reported that selenium in water from the Enshi selenosis area was about 56 µg/L, compared to 0.54 µg/L from the nonselenosis area.

Soil

Selenium is estimated to range from 0.05 to 0.09 mg/kg in the earth's crust, however, the geographical distribution of selenium in soils is extremely varied (Wang and Gao, 2001). In addition, the proportion of water-soluble and bioavailable selenium does not necessarily correlate with the total selenium level in soil (Zhao *et al.*, 1993). Plant selenium uptake depends on the soluble fraction, but in general, selenium concentrations in plants correlate well with total selenium concentrations in soil (Fordyce, 2005). Chemical speciation determines selenium solubility and therefore its bioavailability and potential for transport in the environment. Bioavailability of selenium in soil tends to correlate negatively with clay content, iron content, and organic matter in soils (Fordyce, 2005). Analysis of crops from various parts of the world showed that seleniferous soils are widespread over the surface of the earth. Significant differences can be found between selenium concentrations in plants grown in selenium-deficient and in selenium-adequate regions, and the differences are more significant than the differences of selenium contents in soils of these regions (Wang and Gao, 2001).

Selenium occurs in soils in different forms including elemental selenium, selenides, selenites, selenates, and organic selenium (Ryser *et al.*, 2006). Elemental selenium is stable in soils and coprecipitates with sediments. Elemental selenium is formed by bacteria, fungi, and algae, which are capable of reducing selenites and selenates.

Elemental selenium and selenides occur in reducing, acid and organic-rich soils, and are largely insoluble and not available for uptake by plants. Selenites usually occur in soil in association with metal hydroxides, e.g., selenite is associated with insoluble ferric oxide and oxyhydroxide complexes in acid and neutral soils as a ferric oxide selenite complex, possibly with the formula $Fe_2(OH)_4SeO_3$ (Fordyce, 2005). The presence of sulfur, iron, and aluminium negatively affects the uptake of selenium by plants (Papp *et al.*, 2007). In acid soils at pH 4.5 to 6.3, selenites are usually bound as the metal selenite complex, which is of low solubility, and is not available to plants. Under more alkaline conditions at pH 7.5 to 8.5, the reduced selenium species like selenite are oxidized to the more water-soluble selenate, which is readily available to plants and is one of the more mobile species of selenium in the environment (Ryser *et al.*, 2006). Selenate is more soluble than selenite and thus available for plant uptake. Selenate occurs mainly under oxidizing, alkaline conditions (Fordyce, 2005). Organic selenium compounds can also be formed in soil by the actions of microorganisms; the primary product formed and released is the volatile dimethyl selenide (Berrow and Ure, 1989). Selenium must be present in the bioavailable forms in the soil before it is taken up by plants, and water-soluble inorganic and organic selenium compounds can be absorbed by plants (Terry *et al.*, 2000).

The amount of selenium in soils is mainly determined by geochemical processes of natural weathering and leaching, as well as by anthropogenic activities (Fordyce *et al.*, 2000). The earth crust is made up of about 95 percent igneous rocks and about five percent sedimentary rocks. Of the sedimentary rocks, about 80 percent are shales, 15 percent sandstones, and five percent limestones. Soils derived from sandstone, granite, and limestone are generally low in selenium. Soils with increased organic content, e.g. shale or peat, have a higher selenium content. Sedimentary rocks have higher selenium concentrations than igneous rocks. Shales contain more selenium than limestones or sandstones. The NRC (1976) estimated the selenium concentration as 1 to 300 mg/kg in phosphate rocks. McNeal and Balistrieri (1989) summarized selenium concentration estimates of about 0.05 mg/kg in the earth's crust, from 0.01 to 0.05 mg/kg in granite, about 0.08 mg/kg in limestone, less than 0.05 mg/kg in sandstones, and about 0.06 mg/kg in shales. Selenium concentrations have also been estimated as about 0.05 to 0.11 mg/kg in basaltic igneous rock, about 0.05 to 0.06 mg/kg in granitic igneous rock, about 0.08 mg/kg in limestone, and about 0.05 mg/kg in sandstone (He *et al.*, 2005).

The selenium levels in soils depend on the selenium concentration of the fundamental soil-forming rock, on the redox conditions of the soil, the pH, soil drainage, and on the general climatic features (Sarquis and Mickey, 1980). The amount of selenium in soils is also affected by the properties of the parent materials of the soils. Atmospheric deposition contributes to selenium in soil. Rain, irrigation, and drainage may transport selenium from one place to another. Mining, smelting, and burning of coal and oil may contribute to local selenium deposition on soil. Agricultural activities including application of fertilizers contribute to selenium concentration in soil and sediment. Marine aerosol may also contribute to the soil selenium content (ATSDR, 1996, 2003; Berrow and Ure, 1989).

The selenium concentration in soils rarely exceeds 0.1 mg/kg (Moreno Rodriguez *et al.*, 2005). Reilly (2006) reviewed the selenium levels in soil as about 0.05 mg/kg to 0.2 mg/kg, with some exceptionally high concentrations in soils in drier regions, such as the

North American great plains from Mexico north to Canada, especially in Wyoming and South Dakota. The majority of U.S. soils contain less than 2 mg/kg of selenium, ranging from 0.03 to 0.8 mg/kg, with little difference between the eastern and western states. The more alkaline soils in the west tend to make selenium more soluble in water and as a result, increase plant uptake and accumulation in the food chain (U.S. EPA, 1985a). The mean selenium concentration is estimated to be about 0.29 mg/kg in Chinese soils, about 0.2 mg/kg in world soils, and about 7 mg/kg in metal rich soils (He *et al.*, 2005).

The average selenium concentration of 500 samples from seleniferous areas in the western U.S. was 4.5 mg/kg, with a maximum of 80 mg/kg; 50 percent of the samples contained 1 to 6 mg/kg of selenium (Rosenfeld and Beath, 1964). Seleniferous soils also occur in part of China, the Amazon Basin, Columbia, Venezuela, Australia, Russia, and other countries. Selenium concentrations ranged from 1 to 80 mg/kg in seleniferous soils in a study published in 1945, and up to 1,250 mg/kg in seleniferous Ireland soils in a study published in 1962 (McNeal and Balistrieri, 1989; Reilly, 2006).

Soils containing as much as 100 mg/kg total selenium were reported in the northern plains and along the eastern front of the Rocky Mountains in surveys between 1933 and 1949 in the western U.S. (Sarquis and Mickey, 1980). Soil total selenium as selenite and selenate ranged from 0.6 mg/kg to 26 mg/kg in reclaimed lands at a uranium site in southeastern Wyoming, and resulted in increased food chain transfer and water contamination (Sharmasarkar and Vance, 2002). Grasses, forbs, and shrubs in the area accumulated selenium at 11 mg/kg dry weight up to as high as 1,800 mg/kg, which may be potentially toxic.

Selenium in the upper 15 cm of soil from a Wyoming grassland community receiving wastewater from a uranium mine ranged from 2.6 to 4.2 mg/kg in 1998; the mean concentration was five times higher than a reference site (Ramirez and Rogers, 2002). In the top 15 cm of soil at the Kesterson, California upland grassland habitat, average total selenium ranged from 0.5 to 8 mg/kg, while water extractable selenium ranged from 0.01 to 0.7 mg/kg and varied by a factor greater than 100 among soil samples (Wu, 2004).

The release of selenium to U.S. soil has been summarized by ATSDR (2003) based on U.S. EPA's TRI report (U.S. EPA, 2001). An estimated total of 157,521 pounds of elemental selenium and 7,209,933 pounds of selenium compounds were discharged to land from manufacturing and processing facilities in the U.S. in 2000. In addition, 40,246 pounds of selenium and 27,699 pounds of selenium compounds were injected underground (ATSDR, 2003).

A soil content of less than 0.005 mg/kg is considered as selenium deficiency for livestock (Frost, 1972). A soil content of greater than 2 mg/kg is considered potentially dangerous if there are alkaline conditions and limited humic materials in the soil while the rainfall is less than 50 cm per year. These are the possible factors that contribute to increased selenium uptake by plants (ATSDR, 1996). Vegetation grown in poorly drained, highly organic soils in Ireland containing more than 1,200 mg/kg selenium contained toxic concentrations of selenium (Sarquis and Mickey, 1980). Soils of Hawaii containing 6 to 15 mg/kg selenium did not produce seleniferous vegetation, while soils of South Dakota and Kansas containing selenium at less than 1 mg/kg produced toxic seleniferous vegetation (Lakin, 1973).

The average selenium content of 18 soil samples in the seleniferous Enshi area in China was about 9.68 mg/kg with a range from 0.08 to 45.5 mg/kg, and five different local grown vegetables had selenium exceeding 100 mg/kg, at 128 mg/kg in *Aster*, 152 mg/kg in *Astragalus*, 738 mg/kg in *Musci*, 814 mg/kg in garlic leaves, and 891 mg/kg in daikon leaves (Yang *et al.*, 1981, 1982a). Enshi soil contained selenium at 787 mg/kg and soluble selenium at 35.4 mg/kg (Mei, 1985). The selenium content of soil samples in Enshi City ranged from about 12.8 to 45.5 mg/kg in Luojiawan, about 7.1 to 23.6 mg/kg in Xinton, and about 0.4 mg/kg in Yutangba (Chen and Mei, 1980). In the studies of Yang *et al.* (1983, 1989a,b) of selenosis in three sites with low, medium, and high blood selenium levels from the Enshi area, the mean ± standard deviation soil selenium levels were about 0.42 ± 0.04 mg/kg (n = 5) with a range from 0.37 to 0.48 mg/kg, 3.09 ± 1.77 mg/kg (n = 6) with a range from 0.73 to 5.66 mg/kg, and 9.54 ± 1.88 mg/kg (n = 6) with a range from 0.704 to 12.08 mg/kg, respectively (Yang *et al.*, 1989b).

The reported selenium content of soil samples at 30-cm depth in Ziyang County, Shaanxi Province, a seleniferous area with selenium poisoning in China, ranged from about 2.2 to 27.9 mg/kg in the Shuanan selenosis area, and about 0.21 to 18.3 mg/kg in the rest of the Ziyang County without selenosis (Chen and Mei, 1980). The average Ziyang soil contained selenium at about 0.49 mg/kg with a high of 15.7 mg/kg in the Shuang'an selenosis area (Mei, 1985). Another study showed total selenium in Shuang'an soil of 23.5 mg/kg, with soluble selenium at 0.056 mg/kg equivalent to 0.24 percent (Zhao *et al.*, 1993). Soils with higher levels of free calcium carbonate ($CaCO_3$) derived from limestone under alkaline condition appeared to accumulate more soluble selenium than soils with lower free calcium carbonate (Fang and Wu, 2004). The higher alkalinity of the soil samples also correlated with the higher selenium content. Zhao *et al.* (1993) reported that among the 22 soil samples collected in Ziyang, the three samples with the highest free calcium carbonate of 210, 163, 108 mg/kg and the highest pH of 8.12, 8.09, 7.83 contained the highest total selenium of 23.53, 17.05, 5.98 mg/kg, respectively. The samples with the lowest free calcium carbonate of 1.3 mg/kg with a pH of 5.83 contained a total selenium of 1.986 mg/kg. The sample with the lowest pH of 4.24 with free calcium carbonate of 7.3 mg/kg contained a total selenium of 1.701 mg/kg. The high levels of free calcium carbonate during a drought in 1959 to 1961 are postulated to be a major factor for the high incidence of selenosis in Shuang'an. Low free calcium carbonate may explain the low incidence in nearby Huigouhe and Mixiliang (Fang and Wu, 2004; Wang and Gao, 2001; Zhao *et al.*, 1993).

Food

The primary source of selenium intake in humans is food (Finley, 2005a). Selenium composition in human food is highly variable (Mayne, 2003). A British study estimated the approximate mean concentration of selenium in food to be 0.06 µg/g (Bennett, 1986). The dominant food sources of selenium in most diets are organ meats, fish, muscle meats, cereals, grains, and eggs (Holben and Smith, 1999). Dairy products, fruits, and vegetables tend to have the least amount of selenium. Variability in selenium concentrations in animal foods is less evident than in plant foods as some plants like certain nuts and certain mushrooms may contain particularly high levels of selenium.

The bioavailability of selenium is greater from selenium-enriched yeast, cereals, and grains than from these nuts and mushrooms (Thomson, 2004b).

Studies in rats and humans both *in vitro* and *in vivo* have shown a widely varied bioavailability of selenium in food (Reeves *et al.*, 2005; Finley, 2006). Bioavailability was about two percent in ewe milk, five percent in mushrooms, seven percent in cow and goat milk, 15 percent in meat products, 20 to 50 percent in seafood, and 60 to 80 percent in broccoli and buckwheat bran (Finley and Davis, 2001; Finley *et al.*, 2004; Muniz-Naveiro *et al.*, 2006; Navarro-Alarcon and Lopez-Martinez, 2000; Reeves *et al.*, 2005; Thomson, 1998). Selenomethionine is one of the most bioavailable form of selenium (Schrauzer, 2003).

The efficiency of human gastrointestinal digestion to dissolve yeast selenium proteins may be about 89 percent; however, its efficiency to convert them into free selenomethionine may be only about 34 percent (Reyes *et al.*, 2006). More than 95 percent of the selenium found as selenocystine, selenomethionine, and selenium-methylselenocysteine in radish enriched in sodium selenite was unaltered after a simulated gastrointestinal digestion; about 65 percent of selenium-methylselenocysteine remained in the potentially bioabsorbable fraction (Pedrero *et al.*, 2006).

Smith *et al.* (1936) surveyed 50 rural families residing in seleniferous areas of South Dakota and Nebraska with urinary selenium levels ranging from trace to 1,330 µg/L, and found that meat, milk, and eggs are the most important sources of selenium exposure. The chemical forms of selenium in the human diet have not been well characterized (Hawkes *et al.*, 2003). Much of the selenium in plants is in small selenoamino acid compounds, with 64 to 73 percent of the selenium in wheat and yeast recoverable in the methyl-selenium fraction derived from selenomethionine and methylated selenocysteine derivatives (Wolf and Zainal, 2002).

Higher plants do not appear to utilize selenium as functional amino acid residues within proteins (Terry *et al.*, 2000). The ability of a plant to absorb selenium is dependent on transport mechanisms similar to those for sulfur, and sulfate has an antagonistic effect on selenate uptake by plants. Sulfur inputs from fertilizers and manures as well as atmospheric deposition have a negative impact on the selenium status of wheat grains. Thus plants normally rich in sulfur like garlic and broccoli can accumulate selenium without toxicity (Finley, 2005a,b). Selenium is then stored in the plant's tissues as selenoamino acids. Selenomethionine incorporated into proteins is the major selenoamino acid in selenium accumulator plants like garlic as well as nonaccumulator plants like cereal grains and legumes.

The amount of selenium accumulated in tissues varies between plants and is directly related to the concentration found in the soil. In the U.S., certain plants not for food like *Astragalus* selectively accumulate selenium, reaching a selenium content as high as 0.9 percent of the dry weight of the plant, equivalent to 900 ppm (Lakin, 1973). In the seleniferous Ziyang area in China, the corn grown on soil containing a total selenium level of 23.53 mg/kg or water-soluble selenium of 56.38 µg/kg (with free calcium carbonate of 210 mg/kg and pH of 8.12 as discussed earlier in this document) had a selenium level of 27.5 mg/kg dry weight (Zhao *et al.*, 1993). The selenium content of each of the ten raw unprocessed dry tea leaf samples from Ziyang was over 1 mg/kg with

the highest at 2.56 mg/kg. In the seleniferous Enshi area in China, selenium content of fresh leafy vegetables was found to be 7.6 mg/kg (Yang *et al.*, 1989a).

When grown on nonseleniferous soils, crop plants typically have selenium concentrations ranging from 0.01 to 1 mg selenium/kg dry weight (Terry *et al.*, 2000). Most forage and crop plants do not contain more than 25 mg selenium/kg dry weight and do not accumulate selenium above a ceiling of 100 mg/kg when grown on seleniferous soils. These plants are referred to as nonaccumulators (Terry *et al.*, 2000).

Plants which hyperaccumulate selenium in their shoots when grown on seleniferous soils include some strains of *Astragalus, Stanleya, Morinda, Neptunia, Oonopsis,* and *Xylorhiza* that can accumulate hundreds to several thousand mg/kg dry weight of selenium (Terry *et al.*, 2000; Finley, 2005b). Of over 1,500 known *Astragalus* species, only about 25 are toxic due to their ability to concentrate selenium (Spallholz, 1994).

The selenium concentration can be as high as 68 mg/kg in moderately enriched garlic, or 1,355 mg/kg in highly enriched garlic (Fox *et al.*, 2005). As much as 80 percent of the selenium in accumulator plants is the selenoamino acid selenium-methylselenocysteine. Another major selenocompound in highly enriched garlic is γ-glutamyl-selenium-methylselenocysteine. Selenocystathionine, which is metabolized to selenomethionine through selenohomocysteine, is found in *Morinda reticulata* and *Neptunia amplexicaulis* grown in Queensland, Australia (Tinggi, 2003). Selenocysteine is also found in normal and moderately enriched garlic.

Edmonds and Morita (2000) reviewed the identification of selenium species in biological samples and speculated that: "The major difference between accumulator and nonaccumulator plants appears to be that in the former the selenoamino acids remain largely in the free state, but in the latter they are rapidly and almost completely incorporated into proteins. The ability to prevent selenoamino acids from being incorporated into proteins may be the reason why selenium accumulating plants can tolerate seleniferous soils and accumulate considerable quantities of selenium without suffering toxic damage."

Plants absorb selenium from soil primarily as selenates and selenites. The solubility and hence availability of selenates and selenites is affected by factors such as soil pH, redox potential, and iron concentration (Fordyce *et al.*, 2000). Plants translocate the absorbed selenium to the chloroplast, where it follows the sulfur assimilation pathway to be reduced to selenide both enzymatically and nonenzymatically (Finley, 2005b). Selenide then reacts with serine to form selenocysteine. Selenocysteine can be further metabolized to selenomethionine and methylated to other selenoamino acids such as selenium-methylmethionine. The chemical form of selenium in nonaccumulator plants such as cereal grains and forage crops is primarily selenomethionine (Schrauzer, 2000). A specific methyl transferase may result in the formation of selenium-methylselenocysteine, allowing the plant to accumulate large amount of selenium. Transfection of the gene for the specific transferase into a nonaccumulator plant will convert it into an accumulator (Fox *et al.*, 2005). Animals can metabolize both selenomethionine and selenium-methylselenocysteine from edible plants (Whanger, 2004).

Brewer's yeast is another source of selenium for human and animal diets, and selenomethionine is the major selenium compound in enriched yeast. Selenium

supplements derived from the yeast *Saccharomyces cerevisiae* cultured with inorganic selenium salts can yield a mixture of selenoamino acids and selenium salts, the exact composition being dependent on the production conditions (Schrauzer, 2000). Selenomethionine and other organic selenoamino acids can be produced by strains of *Saccharomyces cerevisiae*, *Candida albicans*, *Escherichia coli*, rumen bacteria, and marine algae grown in selenium-containing media. Marine algae such as *Chlorella vulgaris* and *Chaetoceros calcitrans* convert selenium predominantly into selenomethionine. Some of the organic selenium compounds produced by these marine algae are not incorporated into cellular proteins (Schrauzer, 2003).

Selenium concentrations in plants in the U.S. were estimated as about 0.01 to 0.04 mg/kg in grasses, about 0.03 to 0.88 mg/kg in clover and alfalfa, about 0.2 to 1.8 mg/kg in barley, and about 0.15 to 1 mg/kg in oats (McNeal and Balistrieri, 1989). The estimated selenium concentration in marine algae ranged from about 0.04 to 0.24 mg/kg, and in freshwater algae was less than 2 mg/kg (McNeal and Balistrieri, 1989). Shibata *et al.* (1992) estimated selenium concentrations in marine algae of about 0.05 to 0.5 mg/kg wet weight, and from 0.5 to more than 10 mg/kg wet weight in marine animals.

The U.S. Food and Drug Administration (FDA) Total Diet Studies, also referred to as Market Basket Programs, monitor toxic and nutrient elements in the U.S. food supply (Capar and Cunningham, 2000; U.S. FDA, 2000; Egan *et al.*, 2002). For the 1981/1982 study 27 market basket samples consisting of about 120 individual food items were collected from U.S. retail markets. Food items were blended into homogeneous composites for chemical analysis by food group (Gartrell *et al.*, 1986a,b; U.S. FDA, 1982). About 51.8 percent of daily selenium intake was estimated to be from ingestion of grains and cereals, about 36.4 percent was from meat, fish, and poultry, and about 9.7 percent was from dairy products based on data from the Fiscal Year 1979 Total Diet Study (U.S. FDA, 1982).

Selenium was detected in 3,654 of 6,679 food samples analyzed in the U.S. FDA Total Diet Study survey from March 1991 to January 1999 (U.S. FDA, 2000; Capar and Cunningham, 2000). ATSDR (2003) reported on selenium content in over 300 types of food items on the basis of 65 articles published after 1960 and analyzed by Schubert *et al.* (1987). Selenium was detected in about 50 percent of the foods, with about 17 percent of these at trace levels. The highest mean selenium concentrations were reported in canned tuna in oil at 69 µg/100 g, fried beef liver at 65 µg/100 g, dry roasted mixed nuts without peanuts at 53 µg/100 g, pan-cooked pork chop at 46 µg/100 g, pan-cooked haddock at 39.7 µg/100 g, boiled shrimp at 38 µg/100 g, pan-cooked pork bacon at 38 µg/100 g, baked pork roast at 34 µg/100 g, roasted turkey breast at 34 µg/100 g, yellow mustard at 33 µg/100 g, whole white bread at 32 µg/100 g, plain bagel at 31.1 µg/100 g, strained egg yolk junior baby food at 29.3 µg/100 g, baked ham at 29 µg/100 g, strained squash junior baby food at 28.5 µg/100 g, and cracked wheat bread at 28.5 µg/100 g.

An analysis of diets in the U.S. (Schubert *et al.*, 1987) revealed that five foods, including beef, white bread, pork or ham, chicken, and eggs, contributed about 50 percent of the total selenium intake, and that 80 percent of the total dietary selenium was provided by only 22 core food items. Seafood, beef, pork, and chicken had the highest selenium content among the frequently consumed foods from Lubbock, Texas that were analyzed between 1970 and 1993 (Zhang *et al.*, 1993). ATSDR (2003) reported the major dietary

sources of selenium as fish, liver, red meat, shellfish, eggs, garlic, and certain types of grain (dependent on the soil content of selenium). Brazil nuts had an average selenium content of 1,470 µg/100 g in 72 samples, mainly as selenomethionine. Swordfish had an average selenium content of 284 µg/100 g in four samples. Beef kidney had an average selenium content of 170 µg/100 g in four samples. In poultry and meat, selenium concentrations range between 13 and 52 µg/100 g. The selenium content of fruits and vegetables was low compared to cereal grains and meats. However, certain plants of the *Liliaceae* family such as onion and garlic, and the *Cruciferae* family such as cabbage and broccoli are capable of storing higher amounts of selenium, as these plants are rich in sulfur (ATSDR, 2003).

The U.S. Department of Agriculture (USDA) Continuing Survey of Food Intakes by Individuals (CSFII) data for 1994 to 1996 provided national population estimates for dietary sources of selenium among U.S. adults. The top three dietary sources among U.S. adults were yeast breads, beef, and poultry, using single 24-hour dietary recalls from a nationally representative sample of 10,019 adults aged 19 years or older, with a total of 6,419 foods assigned to 112 food groups (Cotton *et al.*, 2004).

Table 3, adapted from Schubert *et al.* (1987) and Holden (1991), lists the major contributors of selenium in the U.S. diet. Animal products such as meat, eggs, fish, and dairy products have a relatively high selenium content. Various type of grains, the major staple foods worldwide, are a major dietary source of selenium. Holden (1991) conducted a U.S. nationwide study of the selenium content of retail white bread. From the dietary studies of Schubert *et al.* (1987), it is estimated that white bread contributes about 14 percent of the daily selenium intake as shown in Table 3. Mean selenium concentrations in white bread ranged from 17 µg/100 g in Los Angeles to 46 µg/100 g in Boston, with an overall mean of 29 µg/100 g for the nine sites sampled. The source of wheat may explain the regional differences in the selenium content of white bread.

Table 3. Major Contributors of Selenium in the U.S. Diet

Food	Mean (µg/100 g)	Percent of daily intake
beef, cooked	26	17
bread, white	32	14
pork, ham, cooked	35	9
chicken, cooked	21	6
eggs, cooked	25	5

The dairy food group, which includes standard or selenium fortified infant formula, was the most significant source of selenium for infants, with grains being the second most important source based on the U.S. FDA Total Diet Studies from 1991 to 1996 for 14 age-gender groups in the U.S. population (Egan *et al.*, 2002). The NRC (2000) lists an average selenium concentration in human milk in the U.S. and Canada as 18 µg/L (Debski *et al.*, 1987; Levander *et al.*, 1987; Mannan and Picciano, 1987; Shearer and Hadjimarkos, 1975). Mother's milk is the only source of selenium for exclusively breast-fed infants. Selenium in human milk occurs mainly as various forms of glutathione

peroxidase proteins (Bhattacharya *et al.*, 1988), and as selenocystamine, selenocystine, and selenomethionine (Dorea, 2002; Michalke and Schramel, 1997). Selenium concentrations in human milk of well-nourished women from selenium sufficient areas worldwide range from about 15 to 20 µg/L, while that in unsupplemented cow milk-based infant formulas range from about 2 to 13 µg/L. Either sodium selenite or selenate has been used as an ingredient of the selenium-fortified infant formula, while selenite has been the common additive (Carver, 2003; Dorea, 2002).

In the selenium-deficiency Jilin Province, China, the selenium concentration in various foods was estimated as follows: 0.4 to 1 µg/100 g in Chinese cabbage, 0.1 to 1.6 µg/100 g in radish, 0.7 to 1.2 µg/100 g in eggplant, mean ± standard deviation of 0.683 ± 0.478 µg/100 g in vegetables, 1.643 ± 0.551 µg/100 g in grains, and 18.12 ± 9.436 µg/100 g in meat and eggs, with a range from 11 to 14 µg/100 g in meat and 20 to 35 µg/100 g in eggs (Ma and Zhang, 2000). In these areas where soils are low in bioavailable selenium, selenium deficiencies in food crops may have adverse health impacts for humans. In selenosis areas, consumption of food which has accumulated high selenium levels represents the major selenium exposure source (Yang *et al.*, 1989a,b, 1995; Hira *et al.*, 2004).

Low selenium soils in Finland have provided evidence that supplementation of commercial fertilizers with sodium selenate affects positively not only the nutritive value of the whole food chain from soil to plants, animals and humans, but also the quantity of plant yields, even though higher plants have been considered not to require selenium (Hartikainen, 2005). When added at low concentrations, selenium may exert a beneficial effect on plant growth. As in humans and animals, selenium may improve the capacity of plants to counteract oxidative stress caused by oxygen radicals produced by internal metabolic or external factors. At proper levels, selenium delays some of the effects of senescence and may improve utilization of short-wavelength light by plants. However, high selenium levels are toxic and may trigger pro-oxidative reactions. Thus, fertilizer supplementation with proper selenium concentrations can be considered an effective and readily controlled way to increase the average daily selenium intake (Hartikainen, 2005).

Selenium Intake

Selenium intake in humans varies according to geographic location, due to factors such as the selenium content in soils and biotas, bioavailability and retention, and interactions with other minerals. Most of the selenium intake is through food (NRC, 2000).

Dietary intake studies and estimates

Dietary assessment methods used to estimate nutrient intakes have been reviewed by the Federation of American Societies for Experimental Biology (FASEB) (1995), West and van Staveren (1997), and Nelson and Bingham (1997). To determine the nutrient intake of a person or population group, the methods must include evaluation of the type and amount of food consumed as well as the nutrient content of that food. Methods to determine the type of food consumed include retrospective studies such as dietary questionnaires (e.g., 24-hour dietary recall, diet histories and food frequency

questionnaires), where the subjects recall their recent or typical food consumption, and prospective studies such as diet records, where the subjects keep records of all food and beverages consumed at the time of their consumption (Nelson and Bingham, 1997; West and van Staveren, 1997). Twenty-four hour dietary recall can provide a reasonable estimate of the mean intake of a nutrient in a population; however, values in either the upper or lower percentiles of the distribution are considered particularly unreliable (FASEB, 1995). Diet records are considered the "gold standard" of dietary intake measurements because of their lower measurement error (Rosner and Gore, 2001).

Nutrient content of the food is either determined directly by chemical analysis or indirectly using food composition tables. When nutrient composition is measured directly, this may be done using the duplicate portion technique, the aliquot sampling technique, or by equivalent composite (West and van Staveren, 1997). Selenium intake estimates based on food composition tables are considered to have low accuracy (NRC, 2000) because the selenium content of foods varies widely (Levander, 1986, 1987). A dietary selenium score calculated from a food frequency questionnaire failed to predict individual selenium intake because of the highly variable selenium composition of the various foods (Hunter *et al.*, 1990).

One of the most recent estimates of selenium intake in the U.S. population comes from the Third National Health and Nutrition Examination Survey (NHANES) III database as reported in NRC (2000). The NHANES data provide information on the health and nutritional status of the civilian, noninstitutionalized population of the U.S. residing in the 50 states and the District of Columbia. The U.S. Department of Health and Human Services (DHHS, 1997, 2002) reviewed the NHANES III data from 1988 to 1994, which included selenium consumption data in 27,266 individuals of varying age groups. Methodology for estimating selenium intake included a 24-hour dietary recall questionnaire for all individuals, coupled with estimation of selenium content of those foods based on food composition data from the University of Minnesota Nutrition Coordinating Center foods database (McDowell, 2003). For all individuals, the median and mean selenium consumption rates for diet plus supplements were 108 and 116 µg/day, respectively. The 95th and 99th percentile selenium intake values, including supplements, were198 and 250 µg/day, respectively. The median and mean selenium intakes for infants zero to six months of age were 34.2 and 35.3 µg/day, respectively, and for seven- to 12-month-old infants, were 46.9 and 49.7 µg/day (NRC, 2000).

Strenghs of this study include the relatively recent data, a very large sample size, the percentile intake data available, broken down by various age and sex groups, and estimates of supplement intake. Study weaknesses include the low accuracy of intake estimation methodology, specifically the 24-hour dietary recall, reliance on food composition tables, and the fact that the "all individuals" percentiles include values for infants and young children, which likely lowers the mean for adult consumers.

Rather than collecting food consumption data from individuals as in NHANES III, the Total Diet Study conducted each year by the U.S. FDA analyzes nutrient content of "core" foods in the U.S. food supply based on food consumption data generated from the National Food Consumption Survey (NFCS) and NHANES (FASEB, 1995). These data can then be used to estimate intakes for select nutrients in the U.S. population. Using food consumption data collected in the late 1970s and combining those data with

selenium analysis of 234 core foods from ten years of the Total Diet Study from 1982 to 1991, Pennington and Schoen (1996a) reported selenium intake estimates for a few discrete age-gender groups. The median and mean selenium intakes for males and females for the age groups of 14 to 16 years, 25 to 30 years, and 60 to 65 years over this period ranged from 61 to 106 µg/day and 64 to 111 µg/day, respectively. The median and mean selenium intakes for six- to 11-month-old infants were 15 and 18 µg/day, respectively. For the age-sex groups reported (including six- to 11-month-old infants and two-year-old children), mean selenium intakes for that time period ranged from 116 percent (60- to 65-year-old females) to 228 percent (two-year-old children) of the Recommended Dietary Allowance. Although exact age comparisons are not possible, in general, selenium intake estimates in Pennington and Schoen (1996b) were approximately 30 to 35 percent lower than those reported for NHANES III (NRC, 2000). For infants aged six to 12 months, the selenium intake estimate was nearly 70 percent lower than the intake estimate generated from NHANES III data. As estimates in the Total Diet Survey are based on only 200 to 300 core foods, they do not represent the true diversity of U.S. food consumption (FASEB, 1995) and, thus, are not as useful for conducting a selenium exposure assessment as are more precise methods.

Welsh *et al.* (1981) measured selenium intakes of 11 male and 11 female subjects, aged 14 to 64 years, for a total of six days each, using what is considered to be a more accurate method than that used for the NHANES III or Total Diet surveys. Duplicate portions of all food and beverages consumed by each subject for the 132 daily diet composites were analyzed for selenium. The median and mean selenium intake values were 74 and 81 µg/day, respectively, and the 95th percentile consumption level was approximately 150 µg/day. The authors suggested that, because a small number of high-selenium consumers skewed the data upwards, the median was a better estimate of central tendency than the mean. When the authors used a selenium data file to indirectly calculate selenium intakes for these subjects, they found that the median and mean selenium intake levels were higher, at 95 and 108 µg/day, respectively, than when direct chemical measurement was used. Interestingly, the median value using the indirect method was identical to the median selenium intake for the NHANES III study, which also used food tables to estimate selenium levels in the diet.

Strengths of this study included the most accurate dietary assessment methodology. Study weaknesses included the small sample size, and a relatively homogeneous study population from a specific geographic area. Although a homogeneous study population can bias data, the authors suggested that the relatively high socioeconomic status of their subjects might have led to an overestimation of selenium intake for the general population in this area but was not likely to have underestimated it. In addition, while locale can significantly impact selenium intake, these effects are buffered in the U.S. because of the food distribution system (NRC, 2000).

A study designed to find subjects with unusually high selenium intakes was conducted in a seleniferous region of South Dakota (Longnecker *et al.*, 1991). This survey also used the duplicate portion technique combined with analysis of food homogenates to determine selenium intake levels. Although the mean selenium intake in this population was 239 µg/day with a range from 68 to 724 µg/day, the subjects included ranchers who consumed beef from areas where selenium toxicity in livestock had previously occurred.

Thus, the selenium intake patterns of this study population cannot be considered representative of typical or even extreme California consumers.

Non-Hispanic blacks had lower serum selenium concentrations than non-Hispanic-whites ($p \leq 0.01$) using multiple regression methods adjusted for education and income covariates in data from the NHANES III from 1986 to 1994 with 13,113 U.S. adults and NHANES from 1999 to 2002 with 7,246 U.S. adults; this suggests the importance of ethnicity-specific nutrition interventions (Kant and Graubard, 2007).

Lawrence and Chapman (2007) estimated dietary intake of selenium from fish in an area of British Columbia which has high levels of selenium in rivers and streams resulting from mining activities. Selenium intake levels of over 600 µg /day by First Nations fishers were considered possible from consuming fish taken from the most polluted area. No selenium-associated toxicity has been observed in these populations.

Intake from food in the United States

The USDA obtained the three-day estimated dietary intake of 36,255 individuals in a 1977 to 1978 Nationwide Food Consumption Survey. The selenium intake was obtained by multiplying the food consumption by the estimated mean selenium content of core foods. An average selenium intake of 71 µg/day was obtained with a range of 49 to 81 µg/day. The median calculated intake was 87 µg/day with a range of 79 to 104 µg/day (Schubert *et al.*, 1987). In a study in South Dakota (Longnecker *et al.*, 1991), where selenium soil levels in soil are known to be high, the median daily selenium intake was estimated to be 146 µg/day, ranging from 60 to 160 µg/day (Tinggi, 2003).

Based on dietary data collected by four standardized 24-hour dietary recalls, the mean ± standard deviation dietary selenium intake of U.S. participants was 153 ± 78 µg/day for men and 109 ± 37 µg/day for women (Zhou *et al.*, 2003). In another U.S. study using the 24-hour diet recall methodology, the daily selenium intake of persons from arsenic endemic areas in the Edison community near Bakersfield, California was about 61.8 µg/day (Valentine *et al.*, 1994).

Based on the 1981/1982 Total Diet Study conducted by the U.S. FDA Market Basket Programs, the average selenium dietary intake for 16- to 19-year-old males (who generally consume more food than other age groups) was estimated to be about 139 µg/day. Dietary intake was about 22 µg/day for six-month-old infants, and about 54 µg/day for two-year olds (Gartrell *et al.*, 1986a,b). U.S. FDA (1982) estimated the average selenium intake of 152.3 µg/day in the U.S. based on the 1979 data. Using the Total Diet Study data from 1982 to1986, Pennington *et al.* (1989) estimated selenium intake to be 20 µg/day for infants six- to 11-months of age and 120 µg/day for males 25- to 30-year-old. The U.S. selenium intake from food is also reported as about 61.8 µg/day (Valentine *et al.*, 1994), 81 µg/day (Welsh *et al.*, 1981), 98 µg/day (Reilly, 1996), 134 µg/day (Watkinson, 1974), 152 µg/day (U.S. FDA, 1982), or 168 µg/day (Schrauzer *et al.*, 1977). The estimated selenium dietary intake in the U.S. has also been reported to be 60 to 160 µg/day (Tinggi, 2003), 61 to 106 µg/day (Pennington and Schoen, 1996a), 74 to 90 µg/day (Levander and Morris, 1984), 79 to 104 µg/day (Schubert *et al.*, 1987), or 99 to 102 µg/day (Thompson and Robinson, 1980). The mean selenium intake of eight adults in California was estimated to be 127 µg/day (Schrauzer and White, 1978).

As part of the Market Basket Programs, the U.S. FDA analyzed food items purchased in different regions of the U.S. from 1982 to 1991 based on NHANES III (U.S. DHHS, 1997) and calculated dietary selenium intake (NRC, 2000; Pennington and Schoen, 1996a,b). They reported median selenium intakes of 106 µg/day from food, or 108 µg/day from food and supplements for all individuals based on dietary recall and food tables. The mean selenium consumption from diet plus supplements was 116 µg/day.

In this NHANES III data, the highest selenium exposure through ingestion was noted in young adult males, a result of their higher food consumption. The 50th percentile ± standard error estimated selenium intake of 19- to 30-year-old males was about 154 ± 3.6 µg/day from food and 156.1 µg/day from food and supplements. U.S. DHHS (2002) as well as NRC (2000) reviewed the NHANES III data from 1988 to 1994 and estimated the average dietary selenium intake for all ages and both genders in the U.S. to be about 114 µg/day and the average dietary plus supplement selenium intake for all ages and both genders to be about 116 µg/day.

The NHANES III study also determined plasma selenium in 17,630 subjects in the U.S. The plasma or serum selenium concentration is often used to assess selenium nutritional status. Based on the criterion that a plasma selenium concentration of 8 µg/dL or greater in a healthy subject indicates that plasma selenoproteins are optimized and the subject is selenium replete, the results of the NHANES III study indicated that more than 99 percent of the subjects studied had selenium at or more than the Recommended Dietary Allowance of 55 µg/day for adults (NRC, 2000). Thus the diets of most Americans provide adequate amounts of selenium (Bialostosky *et al.*, 2002). Since most estimates of selenium intake in the U.S. are 80 µg/day or greater, routine selenium supplementation is not recommended in the U.S. (Burk, 2002).

Intake from water

The intake from water is limited since most of the drinking water in the U.S. contains low levels of selenium, most commonly as selenate (Se^{+6}) or to a lesser degree as selenite (Se^{+4}) (NRC, 2000). In a WHO (2005) review, selenium in drinking water was not considered to make a significant contribution to total selenium intake for most of the population. For example, selenium was 11 percent or less than the intake from foods in about 77 percent of the drinking water systems studied. For those populations receiving selenium concentrations near 50 µg/L, about 23 percent of the drinking water systems studied, the drinking water can provide about half of the total dietary selenium intake. For those population receiving selenium concentrations greater than 100 µg/L, about 0.002 percent of the drinking water systems studied, the selenium in drinking water can provide more than half of the total dietary intake (WHO, 2005).

The intake from bottled water is also limited since most of the bottled water in the U.S. contains low levels of selenium. However, some samples of 25 brands of bottled waters randomly collected from three cities in eastern Alabama, both purified and spring water, had selenium exceeding the U.S. EPA and European Union drinking water standard of 50 µg/L (Ikem *et al.*, 2002).

In isolated situations, contamination of water wells may lead to excessive selenium exposure to a small number of individuals. Irrigation practices, mining residues, or

selenium-containing rocks are possible sources of well water contamination (Valentine, 1997; Valentine *et al.*, 1980). Such high selenium water supplies appear to be very limited (NRC, 2000).

Intake from supplements

Dietary supplements can be a major source of selenium oral exposure for both adults and infants. Selenium as selenomethionine had nearly twice the bioavailability of selenium as selenite based on a supplement study in a low selenium area of China (Xia *et al.*, 2005). In the U.S., human food is not fortified with selenium because there is little or no selenium deficiency (Hatfield, 2001). The exception is infant formula that is designed to be the sole source of nutrients for an infant.

The American Society for Nutritional Sciences (ASNS) sets specific recommendations for infant formula selenium content at 10 to 35 µg/L, which assumes an intake of 100 kcal/kg-day (Raiten *et al.*, 1998). The maximum allowable selenium content in commercially available baby formulas recommended by ASNS is 5 µg/kg-day, the same as the Reference Dose of U.S. EPA (2009) or the Minimal Risk Level of the ATSDR (2003). We found that 2.1 or 2.8 µg selenium/150 mL or 5 fl oz or 100 cal, which is equivalent to 14 or 18.7 µg selenium/L, is commonly listed as the selenium content for a standard milk or soy based formula, based on an internet search for data on baby formulas. A hypoallergenic formula for severely allergic infants fortified with sodium selenite contains 3.73 µg selenium/100 kcal, 15.7 µg selenium/100 g, or 24.9 µg/L. A special pediatric nutritional drink for children age one to 10 years is fortified with sodium selenite and contains 7.5 µg selenium/8 fl oz, or 31.3 µg/L.

NRC (2000) identified an estimated average human milk intake of 0.6 L/day (Dewey *et al.*, 1984) for infants seven through 12 months of age, and 0.78 L/day (Allen *et al.*, 1991; Butte *et al.*, 1984; Heinig *et al.*, 1993; NRC, 2000) for infants zero through six months of age. NRC (2004) recommended an Adequate Intake for total water intake for infants seven to 12 months of age as 0.8 L/day with the source assumed to be human milk and complementary foods and beverages, and for infants zero to six months of age as 0.7 L/day with the source assumed to be human milk. Therefore, the selenium intake of a fully standard formula-fed infant can be estimated as 8.4 µg/day (14 µg/L × 0.6 L/day), 9.8 µg/day (14 µg/L × 0.7 L/day), 10.9 µg/day (14 µg/L × 0.78 L/day), 11.2 µg/day (18.7 µg/L × 0.6 L/day), 13.1 µg/day (18.7 µg/L × 0.7 L/day), or 14.6 µg/day (18.7 µg/L × 0.78 L/day). Infants taking a hypoallergenic formula fortified with sodium selenite may have a selenium intake of 14.9 µg/day (24.9 µg/L × 0.6 L/day), 17.4 µg/day (24.9 µg/L × 0.7 L/day), or 19.4 µg/day (24.9 µg/L × 0.78 L/day).

The U.S. Dietary Reference Intakes are recommended at 15 to 20 µg selenium/day for infants, 20 to 30 µg/day for children, 40 to 55 µg/day for adults, 60 µg/day for pregnant women, or 70 µg/day for lactating women (Levander, 2001; NRC, 2000). However, 200 µg selenium/day for adults or 40 µg/day for pregnant women is commonly suggested by the supplement industry, based on an internet search for data on selenium supplements. Dietary supplements commonly exist in liquid inorganic selenate or selenite form, or tablets or capsules of sodium selenite on a yeast culture base, or tablets or capsules of food grade organic selenomethionine (Schrauzer, 2000, 2001). The amounts in these

supplements are usually 100 to 200 µg/tablet, although some products are available at 10 to 25 µg/tablet (Goodman *et al.*, 1990). Hendler (2001) listed available supplies at 50, 100, or 200 µg/capsule; extended release tablets at 200 µg/tablet; and 50, 100, 126, 150, or 200 µg/tablets. A guide to vitamins and minerals recommends that not more than 200 µg/day should be taken in any form of selenium supplement (Hendler, 1990).

A daily dose of 200 µg/day from selenium supplements is nearly four times the Recommended Dietary Allowance of 55 µg/day (Levander, 1987, 1989b, 2001; NRC, 2000) and one-half of the Tolerable Upper Intake Level of 400 µg/day (NRC, 2000). Because some people consume a much more selenium-rich diet than the average, we surmise that some people who consume 200 µg/day selenium supplements may frequently exceed the Tolerable Upper Intake Level. In the 1986 U.S. National Health Interview Survey, nine percent of all participating adults reported use of supplements containing selenium (Moss *et al.*, 1989).

Intake of infants

NRC (2000) recommends a selenium Adequate Intake of 15 µg/day for infants aged zero to six months, and 20 µg/day for infants aged seven to12 months. WHO (1996b, 2002) recommends a selenium Recommended Nutrient Intake of 6 µg/day for infants aged zero to six months assuming a body weight of six kg. Reifen and Zlotkin (1993) as well as Litov and Combs (1991) recommended a selenium intake for preterm and term infants of 1.3 to 3 µg/kg-day. Based on the 1981/1982 Total Diet Study, the average selenium dietary intake was estimated to be about 22 µg/day for six-month-old infants (Gartrell *et al.*, 1986a,b). Using the Total Diet Study data from 1982 to1986, Pennington *et al.* (1989) estimated the selenium intake to be 20 µg/day for infants 6-11 months of age. Egan *et al.* (2002) reported the range of dietary intake of selenium from the U.S. FDA Total Diet Studies from 1991 to 1996 as about 13 to 18 µg/person-day for six- to 11-month-old infants in the U.S.

Based on a selenium concentration in milk of 18 µg/L and an assumed breast milk volume of 0.78 L/day (18 µg/L × 0.78 L/day = 15 µg/day (rounded)), NRC (2000) recommended a selenium Adequate Intake of 15 µg/day for infants aged zero to six months. Pennington *et al.* (1989) estimated the U.S. dietary intake of selenium as 20 µg/day for 6-11-month-old infants based on the U.S. FDA 1985-1986 total diet study.

The mean dietary selenium intake of a group of 23 lactating and 13 nonlactating women living on the U.S. east coast assessed from 37 weeks of gestation through six months postpartum was 80 ± 37 µg/day, and the intake of their babies was from 8.8 to 11.4 µg/day (Levander *et al.*, 1987; WHO, 2002). Another U.S. study showed the intake of infants as 12.3 µg/day (Shearer and Hadjimarkos, 1987; WHO, 2002).

A 1982 study reported the mean selenium intake of formula-fed infants in the U.S. as 5.9 µg/day with a range from 4.2 to 8.1 µg/day (WHO, 2002). Lonnerdal (1997) reported the intake of formula-fed infants in the U.S. as from 11.7 to 18.3 µg/day (WHO, 2002). Gropper *et al.* (1990) investigated selenium intakes in 57 eight- to twelve-month-old infants consuming either cow's milk or whey-predominant milk-based formula as part of a mixed diet for at least three months. A three-day dietary record and a 24-hour dietary recall was used to determine food consumption, which was then combined with USDA

food composition data to estimate selenium intakes. The mean ± standard deviation selenium intake in 26 cow's milk-fed infants was 34 ± 13 µg/day, while the mean selenium intake in 31 formula-fed infants was statistically lower at 22 ± 11 µg/day. Nineteen percent of the formula-fed infants had selenium intakes less than the Adequate Intake of 15 µg/day recommended by the NRC (2000).

METABOLISM AND PHARMACOKINETICS

Absorption

Oral absorption of selenium depends on the chemical form (e.g., organic or inorganic), the physical state of the compound (e.g., solid or solution), and the dosing regimen (ATSDR, 1996, 2003). Selenium compounds are readily absorbed from the gastrointestinal tract of humans and animals. In a study using ligated digestive tract segments in mature OSU brown rats, no absorption of selenite or selenomethionine occurred from the stomach, whereas the greatest absorption occurred from the duodenum with slightly smaller amounts from the jejunum or ileum (Whanger *et al.*, 1976). The intestinal absorption is pH dependent, and may be influenced by the presence of sulfhydryl-containing compounds as well as other food components, including proteins and heavy metals, and by nutritional status (ATSDR, 1996, 2003; Daniels, 1996; Fairweather-Tait, 1997; Hawkes *et al.*, 2003; Whanger *et al.*, 1976; Willhite *et al.*, 1992). The degree of absorption in humans appears to be independent of the exposure level (ATSDR, 1996, 2003).

Overall absorption of selenium in experimental animals and humans appears to be around 80 percent (Reilly, 1996, 2006). Sodium selenite is absorbed from the intestine by passive diffusion. The absorption of sodium selenate by the intestinal brush border is an active transport, competing with the corresponding sulfur compounds in rat, rabbit, sheep, and pig (Arduser *et al.*, 1985; Wolffram, 1995; Wolffram *et al.*, 1988). Absorption of organic selenium compounds from food is more efficient than absorption of inorganics (Vendeland *et al.*, 1994), e.g., uptake of more than 90 percent of selenomethionine compared to about 60 percent of selenite (Reilly, 1996, 2006). However, the rate-limiting step for the bioavailability of selenium is conversion into a metabolically active form instead of the rate of absorption (Contempre *et al.*, 1996).

Thomson and Stewart (1973) estimated that the intestinal absorption of selenium following a single oral dose containing no more than 5 µg selenium in rats was 91 to 93 percent for [^{75}Se]sodium selenite and 95 to 98 percent for [^{75}Se]selenomethionine. More than 90 percent of an oral dose of [^{75}Se]sodium selenite was absorbed through the gastrointestinal tract in rats, mice, and dogs (Bopp *et al.*, 1982; Brown *et al.*, 1972). Vendeland *et al.* (1992a) compared the intestinal absorption of [^{75}Se]labeled selenite, selenate, and selenomethionine in ligated segments of the rat duodenum, jejunum, and ileum. Selenomethionine was rapidly absorbed from all segments, and selenate was readily absorbed from the ileum. The absorption of selenite was less efficient, leading the authors to suggest that selenite may react with thiol groups on membrane proteins and be reduced to elemental selenium, a form unavailable for absorption. In a subsequent study, Vendeland *et al.* (1992b) showed that selenite absorption may be enhanced in the

presence of glutathione, but that it was critical to maintain a pH of six or below to prevent the spontaneous breakdown of selenotrisulfides formed from selenite and sulfhydryl amino acids. The authors noted that at higher pH selenite may be reduced or otherwise bound to proteins of the brush border membrane of the gut, and thus become unavailable for absorption. The chemical reactivity of selenite in the gut may account for some of the variable results seen in studies of selenite absorption in humans (Finley, 1999). Also, in an *in vitro* experiment, selenomethionine and selenate were found to be significantly more diffusible than selenocystine and selenite under the simulated gastrointestinal conditions (Shen *et al.*, 1997).

Apparent absorption of dietary selenium was about 57 percent in 10 healthy men with a mean age of 28 years from a low selenium area in northern China, determined for three consecutive days each in the summer, fall, and winter of 1983, and the spring of 1984 while consuming self-selected diets (Luo *et al.*, 1985). After six weeks of either sodium selenite or selenomethionine supplementation, selenium levels plateaued in erythrocytes till the end of the experiment at eight weeks (Luo *et al.*, 1987). Depending on the subjects and the study, selenium reaches a plateau in plasma after 11 weeks to six months of selenite, selenate, or selenomethionine supplementation (Aaseth *et al.*, 1998; Alfthan *et al.*, 1991; Kumpulainen *et al.*, 1985). Following about two months of selenium yeast intake, selenium in whole blood reaches a plateau (Schrauzer and White, 1978).

The kinetic behavior of selenate and selenomethionine is analogous to that of sulfate and methionine, respectively. Selenomethionine, like methionine, is absorbed in the gut via the sodium (Na^+)-dependent neutral amino acid transport system (Wolffram *et al.*, 1989). In humans, selenite and selenomethionine absorption were reported as 84 percent and 98 percent, respectively, in kinetic modeling experiments with a dose of 200 µg selenium (Sunde, 1997). Selenium absorption was approximately 80 percent of intake in women in early and late pregnancy as well as nonpregnant controls while all women were fed with a defined diet providing selenium at about 150 µg/day for 20 days and measured for selenium balance during the last 12 days; however, pregnant women conserved more selenium by progressively decreasing urinary excretion (King, 2001; Swanson *et al.*, 1983). Net selenium retentions of the women in early and late pregnancy were 10 and 23 µg/day, respectively. Pregnant women accumulated selenium at approximately 3.5 to 5 µg/day. Increased maternal retention of selenium during pregnancy at a mean ± standard deviation of 34 ± 2 µg in pregnant women versus 11 ± 2 µg in nonpregnant controls was shown in women consuming selenium at 150 µg/day for 20 days (Swanson *et al.*, 1983). Absorption, distribution and excretion of selenium, mainly from beef and rice, were studied in 11 healthy North American men, aged 20 to 45 years, continuously fed conventional foods naturally low in selenium at 14 µg/day or high in selenium at 297 µg/day for 17 weeks. The study included a 21-day baseline period and a 99-day intervention period, while subjects were confined in a metabolic research unit. All three parameters were similar for high and low exposure to selenomethionine, but were different from sodium selenite (Hawkes and Turek, 2001; Hawkes *et al.*, 2001, 2003).

Selenium absorption from wheat and garlic was higher than from fish, with low inter-individual variation (Fox *et al.*, 2005). Form of selenium and food constituents appear to be key determinants of post-absorptive metabolism. Absorption of selenium was measured as luminal loss using a fecal monitoring technique over an eight-day period,

after meals with wheat, garlic, and cod fish intrinsically labeled with [^{77}Se] or [^{82}Se] stable isotopes. The meals were fed in random order to 14 adults in the United Kingdom, with a minimum washout period of six weeks between the test meals. Absorption was significantly higher ($p < 0.001$) from wheat (81.0 ± 3.0 percent standard deviation) and garlic (78.4 ± 13.7 percent) than from fish (56.1 ± 4.3 percent). Lowest plasma concentration of the isotope was observed after the fish meal at seven, 24, and 48 hours post-ingestion, with a peak at 24 hours, whereas wheat produced the highest plasma concentration at all three time points and peaked at seven hours.

In several of the earlier human studies, the γ-emitting [^{75}Se] was used for quantitative monitoring of selenium absorption and incorporation into proteins. The long half-life, relative strength of the γ-energies, and the tendency of [^{75}Se] to be retained and to concentrate in some tissues, has made this technique obsolete. The stable isotopes [^{74}Se] and [^{82}Se] are now used (Finley *et al.*, 1995) and analyzed by hydride generation followed by inductively coupled plasma mass spectrometry. Following a single oral dose of 200 µg [^{74}Se]L-selenomethionine given to human volunteers, 46 percent of the amount leaving the liver appeared to enter the intestines, according to the kinetic model (Swanson *et al.*, 1991). Turnover times in the liver-pancreas system ranged from 1.6 to 3.1 days, and in peripheral tissues ranged from 61 to 86 days.

There is general agreement that over 65 percent of orally administered doses of selenate, selenomethionine, or selenium incorporated into proteins (i.e., in yeast and broccoli) are absorbed in humans. In a New Zealand study, Stewart *et al.* (1978) administered [^{75}Se]sodium selenite to three normal women of ages 20 to 33, or [^{75}Se]selenomethionine to four normal women aged 22 to 34, consuming a normal *ad libitum* diet of 24 µg selenium/day for 14 days. They found that an average of 79 percent was absorbed through the intestines, with a range from 76 to 83 percent. The intestinal absorption of 200 µg [^{74}Se]L-selenomethionine by female subjects was 95 to 97 percent of the administered dose in the study of Swanson *et al.* (1991).

Thomson and Robinson (1986) administered 1 mg of selenium as sodium selenite or sodium selenate in a 5 mL solution to healthy female subjects in New Zealand. Selenium levels in feces, urine, and blood were measured for several days before and after dosing. Selenium absorption, calculated as the difference between the total dose and fecal excretion, was estimated to be 62 ± 14 percent for sodium selenite and 94 ± 4 percent for sodium selenate. Sodium selenate or selenomethionine mixed in brewer's yeast, given as tablets at 200 µg selenium/day for 32 weeks, elevated blood and urinary concentrations of selenium, indicating these compounds were readily absorbed in 36 New Zealand women aged 18 to 23 (Robinson *et al.*, 1997).

Finley (1999) examined selenium absorption in 27 healthy American males, aged 18 to 45 years, and found that [^{74}Se]sodium selenite absorption was highly variable. The absorption of [^{74}Se]sodium selenate and [^{82}Se]labeled broccoli depended on the previous dietary intake of selenium in the volunteer subjects. Subjects receiving selenium at 33 µg/day for 85 days absorbed 66 to 68 percent of selenium from [^{74}Se]sodium selenate and [^{82}Se]labeled broccoli, versus 74 to 76 percent for individuals fed selenium at 226 µg/day for 85 days. This difference was significant and indicated that more selenium isotope was absorbed by subjects fed the higher selenium diet.

Selenium in shrimp, a possible good source of selenium, was absorbed and retained by 12 healthy young subjects (nine females and three males) in Denmark consuming 100 g of shrimp/day for six weeks in addition to their habitual diet (Bugel *et al.*, 2001). Blood samples were collected at commencement of the study, and after two, four, and six weeks. The selenium intake increased from 39.4 ± 15.3 µg/day to 127 ± 5.5 µg/day with the addition of shrimp to the diet. The apparent absorption of selenium from shrimp was 83 ± 4 percent. Fecal and urinary selenium excretion was 32.5 ± 17 µg/day and 21.2 ± 9 µg/day, respectively, and the total selenium retention was 3.1 ± 1.1 mg. Plasma selenium concentrations were 95.2 ± 9.7 µg/L and 101.5 ± 9.7 µg/L before and after six weeks of shrimp intake, respectively ($p < 0.05$). Despite the high absorption and retention, plasma selenium concentrations were only moderately affected by an increase in selenium intake of about 100 µg/day in the chemical forms found in shrimp.

The absorption, retention, and appearance in milk and blood of selenomethionine or selenite was studied in lactating, nonlactating and never pregnant women with similar selenium status at the start of the study (Moser-Veillon *et al.*, 1992). All groups retained significantly more selenium from selenomethionine than from selenite. Milk contained more selenium from selenomethionine than from selenite. Lactating women retained more selenium from selenite than did the other two groups, suggesting that milk losses may be partially compensated by enhanced retention of dietary selenium as selenite.

Absorption and retention of inorganic selenium added to a milk-based infant formula were determined in healthy infants in Switzerland (Van Dael *et al.*, 2002). Two batches of labeled formula containing 10 µg selenium as [^{76}Se]selenate or [^{74}Se]selenite in 500 mL formula, were fed during the first day followed by unlabeled formula. Selenium isotopes were determined in feces collected for 72 hours after intake and in three consecutive 24-hour urine collections. Mean absorption was 97.1 percent for [^{76}Se]selenate and 73.4 percent for [^{74}Se]selenite; mean difference was 23.7 ±6.8 percent standard deviation ($p < 0.001$), with a range of 13.8 - 35.7 percent. Mean urinary excretion as percent of ingested dose was 36.4 percent for [^{76}Se]selenate and 9.7 percent for [^{74}Se]selenite; mean difference was 26.7 ±5.9 percent ($p < 0.001$), with a range of 13.9 to 36.5 percent. Mean selenium retention from [^{76}Se]selenate and [^{74}Se]selenite was not significantly different, 60.7 versus 63.7 percent, respectively.

Based on the results discussed above, the absorption of inorganic sodium selenite, sodium selenate, and selenomethionine ranges about 62 to 93 percent, 66 to 97 percent, and 95 to 98 percent, respectively. A schematic diagram summarizing the absorption and disposition of selenium is shown in Figure 1, adapted from ATSDR (1996, 2003), Robinson *et al.* (1997), and Finley (2005a). The absorption estimate of Robinson *et al.* (1997) shown in Figure 1 falls in the ranges given above.

According to a review by Francesconi and Pannier (2004), the long-held view of trimethylselenonium ion as the major human urinary metabolite of selenium compounds is not justified. Instead, selenosugars are considered as the major metabolites in nonruminant mammalian urine (Gammelgaard *et al.*, 2003; Gammelgaard and Bendahl, 2004; Juresa *et al.*, 2007; Kobayashi *et al.*, 2002; Ogra *et al.*, 2002; Suzuki and Ogra, 2002; Suzuki *et al.*, 2005, 2006a,b,c,d,e,f, 2007). An overview of selenium metabolites is provided in the section entitled "Excretion" in this document.

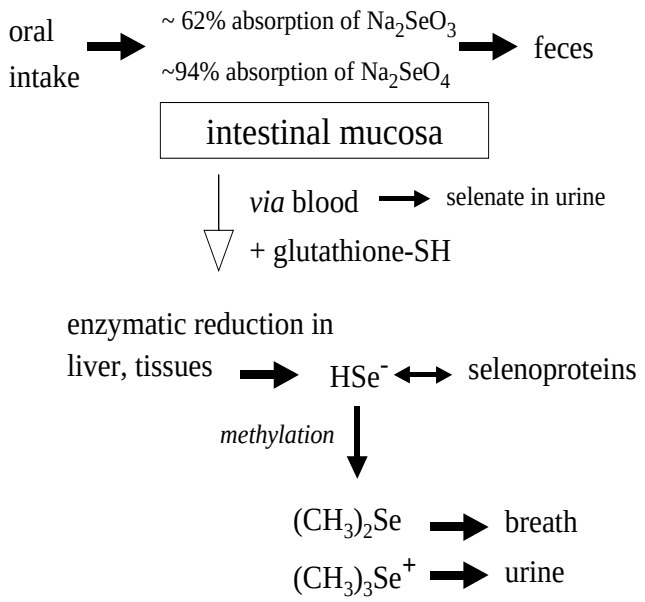

Figure 1. Absorption and Disposition of Sodium Selenite and Sodium Selenate

Metabolism

The metabolic disposition of orally-absorbed selenium depends on the chemical form, and can follow several different pathways (Whanger and Butler, 1988; Sunde, 1990). Inorganic selenite and selenate salts enter a reductive pathway that results in the formation of selenide or a closely related species. Organic selenium compounds, mainly from food, can be transformed to selenomethionine, selenocysteine, or selenium-methylselenocysteine, then to selenide (Finley, 2005a,b). All forms of ingested selenium can be metabolized to selenide, although the rate of transformation varies. The rate-limiting step for the bioavailability of selenium is the conversion into a metabolically active form (Contempre *et al.*, 1996). Selenide can then be methylated and excreted, or used for the synthesis of selenium-containing proteins (Lobinski *et al.*, 2000). Absorbed selenium is carried throughout the body in the blood and distributed to all tissues (ATSDR, 2003). Liver, kidney, nail, muscle, skeleton, plasma, gonad, and semen tend to have higher concentrations of selenium following oral exposure. As a result of metabolism, selenium is present in the mammalian body mainly in forms with covalent carbon-selenium bonds, particularly SelP as the principal selenoprotein in plasma, and selenoenzymes (ATSDR, 2003).

Selenite (H_2SeO_3) metabolic pathways have been studied *in vitro* as described below. Once in an intracellular environment, selenite reacts nonenzymatically with the abundant reduced glutathione (GSH) to form the fairly unstable glutathione selenotrisulfide or selenodiglutathione (GSSeSG) with a stoichiometry ratio of one to four between selenite and glutathione (Ganther, 1968, 1971, 1999). Glutathione selenotrisulfide is then converted to reactive glutathione selenopersulfide (GSSeH) by glutathione reduction (Ganther, 1971). Glutathione selenopersulfide either decays spontaneously and

nonenzymatically to elemental selenium (Se^0) and glutathione as terminal products, or is enzymatically converted under anaerobic conditions by glutathione reductase to hydrogen selenide (H_2Se) in the presence of glutathione (Hsieh and Ganther, 1975). Hydrogen selenide can be metabolized through two selenophosphate synthetases with ATP and transformed into selenophosphate ($H_2SePO_3^-$), which can be used for the biosynthesis of selenocysteine (Small-Howard and Berry, 2005). Both selenocysteine and selenophosphate are required for selenoprotein biosynthesis (Brenneisen *et al.*, 2005).

Selenide can be methylated to methylselenol (CH_3SeH), dimethyl selenide [$(CH_3)_2Se$], trimethylselenonium ion [$(CH_3)_3Se^+$], or selenosugars such as 1-β-methylseleno-N-acetyl-d-galactosamine in the presence of selenium methyltransferase (Berry *et al.*, 1993; Francesconi and Pannier, 2004; Gammelgaard and Bendahl, 2004; Ganther, 1966, 1999; Guimaraes *et al.*, 1996; Ip *et al.*, 1991; Mozier *et al.*, 1988; Suzuki *et al.*, 2005). Dimethyl selenide can be excreted through the urine or exhaled by breath, or can be converted into selenocysteine in mammals. Selenosugars can be excreted through the urine. Hydrogen selenide is the metabolite released from the red blood cells. Trimethylselenonium ion can be excreted through the urine, but probably is not exhaled by breath as suggested by some earlier researchers. The same goes for methylselenol which is probably not excreted through urine. Selenide is the form required for the formation of selenocysteine or selenomethionine. However, mammals cannot synthesize selenomethionine. Selenomethionine conversion to selenocysteine uses the transsulfuration pathway of the sulfuramino acids (Berry *et al.*, 1993; Ganther, 1966, 1999; Guimaraes *et al.*, 1996; Ip *et al.*, 1991; Mozier *et al.*, 1988). Selenomethionine can be transformed to methylselenol by selenocysteine γ-lyase then to hydrogen selenide (Suzuki, 2005). Selenocysteine can be transformed to hydrogen selenide by selenocysteine β-lyase (Brenneisen *et al.*, 2005; Ganther, 1999; Suzuki, 2005). Selenium-methylselenocysteine and selenite follow similar metabolic pathways (Martin and Hurlbut, 1976).

Selenate (H_2SeO_4) is converted to selenide but not as readily as selenite (ATSDR, 2003). Ganther (1986) has suggested that absorbed selenate may be enzymatically activated with ATP by ATP sulfurylase to form adenosine-5'-selenophosphate, then reduced to selenite (H_2SeO_3) through nonenzymatically reactions with reduced glutathione (GSH) to form oxidized glutathione or glutathione disulfide (GSSG). The activation and reduction scheme shown in Figure 2, adapted from ATSDR (1996, 2003), has been demonstrated in microorganisms such as the yeast *Saccharomyces cerevisiae* (Dilworth and Bandurski, 1977). The results found in microorganisms indicate the ability of mammals, lacking a sulfate reductase system, to incorporate selenium from selenate into selenoamino acids.

A comprehensive study of selenite and selenate metabolism *in vivo* in rats has been conducted by Shiobara *et al.* (1999). Shiobara *et al.* (1999) showed that sodium selenate was not directly reduced by glutathione or dithiothreitol, but could be slowly reduced *in vitro* in either a liver homogenate or supernatant fraction. This contrasted with sodium selenite, which was reduced by glutathione and then taken up by red blood cells and transferred to the liver in a protein-bound form. The pathways of selenite reduction are described by Ganther (1986) and Sunde (1990) and shown below in Figure 3, adapted from ATSDR (1996, 2003). Selenite (H_2SeO_3) is reduced to elemental selenium (Se^0) through the unstable selenotrisulfide (GSSeSG), to selenopersulfide (GSSeH), to

hydrogen selenide (H_2Se), to methylselenol (CH_3SeH), and to dimethyl selenide [$(CH_3)_2Se$]. Endogenous reducing agents other than glutathione, such as ascorbic acid, may also be cofactors in this reaction. Both selenite and selenate generate reduced and methylated metabolites that appear in urine *in vivo* (Shiobara *et al.*, 1999).

1. H_2SeO_4 + ATP $\rightarrow$ PPi + adenosine-5'-selenophosphate
   (catalyzed by ATP sulfurylase, Mg^{+2})
2. adenosine-5'-selenophosphate + GSH (reduced glutathione) $\rightarrow$ $GSSeO_3$ + AMP
3. $GSSeO_3$ + GSH $\rightarrow$ H_2SeO_3 + GSSG (nonenzymatic reaction)

Figure 2. Reduction of Selenate to Selenite

1. H_2SeO_3 + 4 GSH (reduced glutathione) $\rightarrow$ GSSeSG + GSSG + 3 H_2O
2. GSSeSG + NADPH + GSH $\rightarrow$ GSSeH + NADP + GSSG
   (catalyzed by GSH reductase)
3. GSSeH (unstable) $\rightarrow$ GSH + Se^0 (reaction is nonenzymatic decomposition)
4. GSSeH + NADPH $\rightarrow$ H_2Se + NADP
   (catalyzed by GSH reductase)
5. H_2Se + 2 S-adenosylmethionine $\rightarrow$ S-adenosylhomocysteine + $(CH_3)_2Se$
   (catalyzed by thiol-Se-methyltransferase)

Figure 3. Reduction of Selenite to Dimethyl Selenide

Selenomethionine in yeast and plants can be converted to selenocysteine which can then be enzymatically metabolized to hydrogen selenide in the presence of lyase (Ganther and Lawrence, 1997). Selenocysteine in meat and dairy products can be converted to hydrogen selenide with lyase. Hydrogen selenide can then be converted to methylated selenide. Yeast and plants like garlic also contain selenium-alkylselenocysteine or selenocystathione which can be metabolized to selenol in the presence of lyase. Selenol can then be converted to methylated selenide. The hydrogen selenide generated from selenite, selenate, selenomethionine, selenocysteine, and selenium-alkylselenocysteine may be activated by ATP to form selenophosphate ($H_2SePO_3^-$) (Lacourciere, 1999). The selenium atom of selenophosphate is then incorporated into selenoamino acids like L-selenocysteine for the formation of selenoproteins.

Alternatively, the selenide can be methylated to form the excretory products dimethyl selenide, trimethylselenonium ion, and selenosugars (Gammelgaard and Bendahl, 2004; Ganther, 1986; Suzuki, 2005). Methylation of the selenide by thiol methyltransferase forms methylselenol and dimethyl selenide, and further methylation by thioether methyltransferase forms trimethylselenonium ion (Ganther and Lawrence, 1997). The general pathways of selenide metabolism are described by Ganther (1986) and Sunde (1990) and shown in Figure 4, as adapted from ATSDR (1996, 2003).

1. [Se]methionine proteins $\rightarrow$ [Se]methionine $\rightarrow$ [Se]methionine proteins via t-RNA^{Met}
2. [Se]methionine proteins $\rightarrow$ [Se]methionine $\rightarrow$ CH_3SeH $\rightarrow$ H_2Se $\rightarrow$ HSe^-
3. [Se]cysteine proteins $\rightarrow$ [Se]cysteine $\rightarrow$ CH_3SeH $\rightarrow$ H_2Se $\rightarrow$ HSe^-
4. selenate $\rightarrow$ selenite $\rightarrow$ H_2Se $\rightarrow$ HSe^-
5. HSe^- $\rightarrow$ seleniun-containing proteins
6. HSe^- $\rightarrow$ methylated excretion products
7. HSe^- $\rightarrow$ incorporation into selenoproteins via t-RNA^{Sec}, UGA codon, serine, ATP, and various selenium gene products

Figure 4. Functional Pathways of Selenide Metabolism

Mammals do not synthesize selenomethionine (Sunde, 1997). The exogenous selenomethionine entering the blood is either degraded to selenocysteine, hydrogen selenide, and dimethyl selenide, or incorporated into tissue proteins via the normal ribosomal processes utilizing the tRNA for methionine (Martin and Hurlbut, 1976). Organs and tissues engaged in continuous protein synthesis that rapidly incorporate selenomethionine are the enteric mucosa, liver, kidney, skin and its appendages, skeletal muscle, and erythroblasts. Accordingly, the selenium content of these tissues reflects dietary intake of selenomethionine. It should be noted that a significant fraction of selenium in blood is selenomethionine incorporated into protein.

As noted above, the hydrogen selenide formed from the reduction of selenate and selenite or from the degradation of selenomethionine and selenocysteine can be utilized for the synthesis of selenoproteins. The term "selenoproteins" now refers to macromolecules containing selenocysteine which are the ribosome-mediated synthesized proteins specified by the genetic code UGA codon for selenocysteine and its associated cofactors. UGA codes for translational termination, for tryptophan in mitochondria, and for selenocysteine in the nuclear genome (Papp *et al.*, 2007). The observations of specific incorporation of selenium, mainly selenocysteine, into macromolecules led Sunde (1990) to define several classes of selenium-containing proteins differentiated by the selenium precursor and the mechanism used for selenium incorporation. Selenoprotein synthesis *in vivo* is selenium-dependent, and there is a hierarchy of selenoprotein expression as well as selenium distribution in mammals when selenium is limiting (Berry, 2005b; Driscoll and Copeland, 2003; Schomburg *et al.*, 2006).

Mammalian selenium-containing proteins can be divided into three groups: 1) specific selenoproteins containing selenium in the form of genetically encoded selenocysteine, 2) specific selenium binding proteins in which the selenium moiety is externally bound to the polypeptide, and 3) proteins containing nonspecifically incorporated selenium such as selenomethionine-containing proteins (Behne and Kyriakopoulos, 2001; Brenneisen *et al.*, 2005; Hatfield, 2001; Kyriakopoulos and Behne, 2002; Papp *et al.*, 2007). The term selenoproteins refers only to selenocysteine-containing proteins, and does not include selenium binding proteins or selenomethionine-containing proteins. Selenoproteins represent an essential prerequisite for regular development and a long and healthy life

(Flohe, 2005, 2007; Gromer *et al.*, 2005; Schomburg *et al.*, 2004). One important selenoprotein is glutathione peroxidase (GPx), which is present in blood plasma and erythrocyte fractions, in milk, in the gastrointestinal system, and in testes. SelP, another important selenoprotein, accounts for more than 50 percent of the selenium content in human plasma (Mostert, 2000).

The diverse role of selenium within selenium-containing proteins expressed in various tissues and organs has been reviewed and summarized (Arteel and Sies, 2001; Brenneisen *et al.*, 2005; Brigelius-Flohe, 2006; Edmonds and Morita, 2000; Gladyshev and Hatfield, 1999; Hatfield, 2001; Hatfield and Gladyshev, 2002; Holben and Smith, 1999; Jacob *et al.*, 2003; Kohrle, 1999; Kohrle *et al.*, 2000; Papp *et al.*, 2007; Schomburg *et al.*, 2004; Sunde, 1990).

Using SelP as an example, Schomburg *et al.* (2004) summarized the central known steps and unresolved issues in selenium metabolism: "Step 1: Dietary-derived selenium-containing compounds are taken up, and selenium is liberated, activated by phosphorylation and charged onto its tRNA by incompletely defined pathways. Step 2: Specific posttranscriptional processes give rise to two different $tRNA^{[Ser]Sec}$ isoforms that are used with unequal efficiencies for the synthesis (Step 3) of the different selenocysteine (Sec)-containing proteins. Step 4: Additional factors might be required to assemble specific selenosomes for efficient translation of particular selenoproteins. Step 5: Two individual selenocysteine-insertion elements are likely necessary to decipher multiple UGA codons in SelP mRNA for successful readthrough and consecutive insertion of up to 10 selenocysteine residues per protein. Step 6: After synthesis, SelP is released into the extracellular space and found in plasma or locally bound to the cell surface from where it might be remobilized and taken up for selenium retrieval (SelP cycle)."

Excretion

Human and animal studies indicate that the major route of selenium excretion in monogastrics after oral exposures is primarily via urine. Excretion through feces and expired air constitute other elimination pathways; excretion through sweat is a minor pathway (ATSDR, 1996, 2003; Tiwary *et al.*, 2005). The metabolic elimination of selenium compounds involves a series of S-adenosylmethionine-dependent methylations yielding dimethyl selenide which is exhaled in breath, and selenosugars as well as trimethylselenonium ion which are excreted in urine (Suzuki *et al.*, 2005, 2006a,b,c,d,e,f, 2007). Water-soluble selenium compounds including selenite, selenate, selenomethionine, selenocysteine, and selenocholine have also been detected in urine.

Multigastric ruminants such as sheep, lambs, and ewes eliminate most of the ingested selenium as a nonbioavailable reduced or elemental form in feces, and to a lesser degree, via urine (Jacobsson, 1966; Krishnamurti *et al.*, 1989; McClure and Mahan, 1988). Urinary excretion of selenium was reviewed by Robberecht and Deelstra (1984), Oster and Prellwitz (1990), Sanz Alaejos and Diaz Romero (1993), and Francesconi and Pannier (2004). Selenium homeostasis is regulated via excretion by the kidneys but not controlled by the gut. Decreased selenium intake is followed by decreased urinary excretion of metabolites, indicating the role of the renal system in conservation, allowing

the body to adapt in a timely fashion to low dietary intakes. As selenium intake increases, urinary excretion increases. Females appear to excrete daily higher amounts of selenium in urine per kg of body weight than males (Rodriguez Rodriguez *et al.*, 1995). Children under 10 years of age appear to have daily urinary selenium excretion per kg of body weight higher than older persons, and daily urinary selenium excretion appear to increase up to 30 years of age. Similar age-dependent excretion is observed in laboratory rats (Suzuki *et al.*, 2000) as discussed later in this section.

Selenium excretion is influenced by the forms of the element ingested (Robinson *et al.*, 1985; Yang *et al.*, 1989a,b). Selenium is excreted into urine mainly after being transformed to methylated metabolites. Mono-, di- and trimethylated selenium compounds are excreted into urine in response to a supply within the required to low-toxic range. Tri- and dimethylated selenium compounds increase with excessive supply at a toxic dose, whereas monomethylated selenosugars are bioavailable for recovery from urine back to tissues where and when in need (Juresa *et al.*, 2007).

Basal urinary levels of 28.7 ± 8.3 (mean ± standard deviation) µg of selenium/g creatinine were found in U.S. children five to 18 years of age (n = 66) (Tsongas and Ferguson, 1977). Lower levels (stated as µg of selenium/g of creatinine) were measured in German populations at 16 ± 4.6 µg/g (n = 18), and Scottish populations at 24 ± 8.5 µg/g (n = 16). Higher levels were reported in two Japanese populations at 60 ± 25 µg/g (n = 21), or 95 ± 12 µg/g (n = 8). Even higher levels were reported in three Venezuelan populations at 152 µg/g (n = 1,055), 224 µg/g (n = 111) from Caracas, or 636 µg/g (n = 50) from a seleniferous area. These differences are most likely due to variations in dietary intake of selenium and are similar to the range of tissue levels of selenium found in these populations. In a rural Colorado community exposed to drinking water containing 50 to 125 µg selenium/L, mean selenium levels of 24-hour urinary samples from exposed residents at 158 µg/L (n = 85) were about twice that from unexposed controls at 80 µg/L.

Excretion of sodium selenite and sodium selenate by human subjects was compared by Thomson and Robinson (1986). The fraction of an absorbed dose of sodium selenate excreted over a five-day period was found to be 66 ± 11 percent (mean ± standard deviation) versus 35 ± 3 percent for sodium selenite. Finley (1999) measured the urinary excretion of selenium in 27 healthy American males that received oral doses of [^{74}Se]sodium selenate or [^{82}Se]labeled broccoli. The rate of urinary excretion of selenium, administered as [^{74}Se]selenate, was at least twice that of selenium from [^{82}Se]labeled broccoli. One day after dosing, 18 percent of the dose was excreted in the urine for [^{74}Se]selenate and three percent for [^{82}Se]labeled broccoli. The gradual incorporation of labeled selenium into plasma proteins could be followed over a 20-day period. Six species including selenomethionine and selenocystamine were identified as organic selenium metabolites in a human urine sample, estimated to be present at approximately 11 and 40 ppb, respectively (Cao *et al.*, 2001).

Selenium elimination was shown to be triphasic following a single oral administration of [^{75}Se]sodium selenite to three New Zealand women aged 20, 25, and 33 years with a mean weight of 57 kg (Thomson and Stewart, 1974) or [^{75}Se]selenomethionine in four New Zealand women aged 22, 23, 26, and 34 years with a mean weight of 60 kg (Griffiths *et al.*, 1976). Selenium elimination starts with an initial rapid excretion and a

slower secondary phase resembling a first order reaction, then ends with a much longer third phase (Thomson and Stewart, 1974; Griffiths *et al.*, 1976).

For selenite, the first phase lasted about one week, elimination of selenium during this phase was fast with a half-life of approximately one day (Thomson and Stewart, 1974). The second phase lasted about a week; elimination was slower with a half-life of about eight to nine days. The third phase lasted much longer, with an elimination half-life of approximately 115 to 116 days. Urinary excretion accounted for about 14 to 20 percent of absorbed [^{75}Se] in the first week. After initially rapid decreased radioactivity, whole body retention of [^{75}Se] diminished exponentially with a half-time of 96 to 144 days. Fecal elimination of nonabsorbed selenium and urinary excretion of absorbed but unutilized selenium corresponded to the first two elimination phases.

The terminal half-life of selenomethionine elimination is longer than that of sodium selenite (Thomson and Stewart, 1974). The long-term fate of an oral dose [^{75}Se]selenomethionine, including urinary and fecal excretion, respiratory losses, whole body retention of [^{75}Se], and [^{75}Se] turnover in whole body, plasma and erythrocyte during a period of 33 to 34 weeks, was studied in four women. No radioactivity was detected in expired air. Intestinal absorption of [^{75}Se]selenomethionine by the four subjects was 95.5 to 97.3 percent of the administered dose. Urinary excretion accounted for six to nine percent of absorbed [^{75}Se] in the first two weeks. The half-life for the three elimination phases was estimated to be approximately 0.4 to two, five to 19, and 207 to 290 days, respectively (Griffiths *et al.*, 1976). Comparing the metabolism of [^{75}Se]selenomethionine with the metabolism of [^{75}Se]sodium selenite in two of the same women in these two studies, [^{75}Se] from [^{75}Se]selenomethionine was more completely absorbed, had a greater retention and smaller endogenous urinary and fecal losses than [^{75}Se]selenite, and these differences persisted throughout the experimental period of approximately 44 weeks (Griffiths *et al.*, 1976; Thomson and Stewart, 1974). Selenium elimination was also triphasic in sheep administered selenite intramuscularly or intravenously (Blodgett and Bevill, 1987a).

The ionized form of trimethyl selenide [$(CH_3)_3Se$], namely trimethylselenonium ion [$(CH_3)_3Se^+$] as shown in Figure 1, was initially identified as an urinary metabolite of sodium selenite administered to rats in drinking water (Byard, 1969; Palmer *et al.*, 1969, 1970) with subsequent reports of its detection in human urine (Gammelgaard *et al.*, 2000, 2005; Ganther *et al.*, 1987; Kuehnelt *et al.*, 2006; Sun *et al.*, 1987). However, the basal excretion of trimethylselenonium ion was about 2 µg/day in human subjects receiving 200 µg/day of sodium selenate mixed with yeast and about 1.5 µg/day in subjects given 200 µg/day of selenomethionine in New Zealand subjects (Robinson *et al.*, 1997). In addition, many of the earlier studies had deficiencies with analytical methods and instrumentation (Francesconi and Pannier, 2004). Later studies indicate that trimethylselenonium ion does not appear to be the major metabolite of selenium excretion. Trimethylselenonium ion may be metabolized by liver enzymes that require homocysteine as a cofactor. A nonlinear relationship was found between urine trimethylselenonium ion and urine selenium, suggesting a complex relationship of this metabolite to body reservoirs of selenium (Janghorbani *et al.*, 1999a,b,c).

In a series of three metabolism studies in China, [^{74}Se]selenite was infused for five hours into Chinese male adults living in deficient, adequate, or excessive selenium areas to

collect daily blood and 24-hour urine samples for the next seven days in the first study (Janghorbani *et al.*, 1999b). When the urine excretion was normalized internally within each group, a sharp increase in the slope of this relationship was found when long-term intake increased to adequate amounts. The slope reached a plateau when the daily intake exceeded the adequate group. Even though there was a positive correlation of selenium intake with the urinary excretion, this relationship was not linear over the entire range of deficient, adequate, or excessive selenium intake (Janghorbani *et al.*, 1999a). In the second study, the relationship between either urine selenium or trimethylselenonium ion to the estimated chronic selenium intake of approximately 480 µg/day in 10 seleniferous Enshi men was again not linear over the range of intake (Janghorbani *et al.*, 1999b). However, these studies (Janghorbani *et al.*, 1999a,b,c) measured trimethylselenonium ion without realizing that it is not the major selenium metabolite in urine (Ogra *et al.*, 2002).

In the third study, these 10 men were transferred to Lichuan County where their daily selenium intake was approximately 30 µg/day for 63 days (Janghorbani *et al.*, 1999c). The subjects received an infusion of 106 µg selenium on the day before consuming foods low in selenium and a second infusion of 113 µg selenium 63 days later. Blood and 24-hour urine samples were collected each day for seven days and on days 22, 43, and 62 following the first infusion, and again daily for the seven days after the second infusion. Plasma total selenium concentration increased for seven days after each of the two infusions and urine selenium decreased exponentially following both infusions. The excretion of trimethylselenonium ion followed the same pattern as the total urinary selenium. However, there was not a significant difference in selenite retention between the two infusion periods. Regardless of the chemical form of selenium present in various organs, its catabolism leading to excretion in urine followed the same pathway as that of selenite. Labeled selenium was incorporated predominantly in the plasma SelP fraction and the half-life of selenium in this fraction was determined to be 1.9 to 2.9 days. A two-month dietary restriction of selenium of the subjects from the excess area did not result in a reduction of urinary excretion of infused selenite. The authors suspected that a longer depletion period would be required in these subjects to obtain more significant changes.

For over 30 years since the first discovery by Byard (1969) and Palmer *et al.* (1969), trimethylselenonium ion has been viewed as a major urinary metabolite of selenium (Ganther *et al.*, 1987). However, after critically assessing the approximately 60 reports published from 1969 to 2004 on a total of 16 identified selenium metabolites in urine of humans or rats in terms of the rigor of the data on which structures have been proposed, the analytical methods used, and the validity of the ensuing structural assignments, Francesconi and Pannier (2004) concluded that the more recent work describing selenosugars as major urinary metabolites (Gammelgaard *et al.*, 2003; Gammelgaard and Bendahl, 2004; Kobayashi *et al.*, 2002; Ogra *et al.*, 2002) is more convincing. Once selenium is absorbed by the body, it is transformed and excreted mostly into the urine with selenosugar 1 as the major metabolite, selenosugar 3 and trimethylselenonium ion as minor metabolites, and selenosugar 2 as trace metabolite in humans (Gammelgaard and Bendahl, 2004) and in adult rats (Suzuki *et al.*, 2005), within the normal nutritional selenium intake range through the intermediate selenide or its equivalent. With excessive selenium intakes or increased body burden of selenium, trimethylselenonium ion starts to increase after selenosugar has plateaued in young rats (Suzuki *et al.*, 2005). However, the increase does not occur in adults even though adults are more sensitive to higher

doses, suggesting that trimethylselenonium ion cannot be a simple biomarker of an excessive or toxic dose of selenium (Suzuki *et al.*, 2006a). In addition, the recent work by Kuehnelt *et al.* (2006, 2007) refutes the trimethylselenonium ion biomarker hypothesis. There is marked and consistent individual variability in the amount of trimethylselenonium ion produced by human adults at background selenium levels without supplemental intake, at a modest one-time intake of 200 µg (Kuehnelt *et al.*, 2006) as well as at intakes up to 8,000 µg/day for four weeks (Kuehnelt *et al.*, 2007).

The results of Kuehnelt *et al.* (2005, 2006, 2007) support that selenosugar 1, instead of trimethylselenonium ion, is the major urinary metabolite after increased selenium intake. Kuehnelt *et al.* (2007) confirmed trimethylselenonium ion not to be the preferred excretion metabolite with high selenium exposure based on investigation of five cancer patients receiving high doses of L-selenomethionine. Doses, espressed as Se, were 4,000 µg twice per day, totaling 8,000 µg/day for seven days, then 4,000 µg/day for 21 days as an adjunct to their normal cancer chemotherapy. Urine samples were collected at day zero from all five patients, and at two to three other times ranging from one to 33 days. The background selenium concentrations ranged from 12 to 55 µg/L and increased to 870 to 4,420 µg/L for the five patients during the study. All five patients had appreciable levels of selenosugars in their background urine sample, and the concentrations increased dramatically after selenium intake. Trimethylselenonium ion, on the other hand, was generally present as only a trace metabolite in background urine, and, although the concentration of trimethylselenonium ion increased following selenium exposure, it became a less significant proportion relative to selenosugars.

About 80 percent of excreted selenium was present as selenosugar 1 when a human ingested either selenite or selenomethionine (Kuehnelt *et al.*, 2005). Kuehnelt *et al.* (2005) performed three experiments in duplicate to obtain quantitative information on human metabolism of selenium. Speciation analysis was conducted on samples of urine from one volunteer over a 48-hour period after ingestion of 1.0 mg selenium as sodium selenite, L-selenomethionine, or D,L-selenomethionine. Normal background urine in 22 samples from the volunteer contained 8 to 30 µg/L total selenium with about 30 to 70 percent speciated. The major species in background urine were selenosugar 1 and selenosugar 3. Selenium was rapidly excreted after ingestion of the compounds as demonstrated by peak concentrations of 250 to 400 µg/L within five to nine hours, returning to close to background levels within 48 hours. By 48 hours 25 to 40 percent of the ingested selenium, depending on the species ingested, had been accounted for in the urine. In all experiments, the major metabolite was selenosugar 1, constituting about 80 percent of the selenium excreted over the first 24 hours after L-selenomethionine or selenite and about 65 percent after D,L-selenomethionine. Selenite was not present at significant levels, at less than 1 µg/L in any of the samples. Selenomethionine was present in only trace amounts (about 1 µg/L) following ingestion of L-selenomethionine, less than 0.5 percent of the total selenium. However, selenomethionine constituted about 20 percent of the excreted selenium in the first 24 hours after ingestion of D,L-selenomethionine, presumably because the D form was not efficiently metabolized. Trimethylselenonium ion could not be detected at the detection limit of 1 µg/L in urine after ingestion of selenite or selenomethionine (Kuehnelt *et al.*, 2005).

Kuehnelt *et al.* (2006) found trimethylselenonium ion as a trace metabolite at 0.02 to 0.28 µg/L in urine from five of seven volunteers who ingested 200 µg selenium as selenite. This was equivalent to just one to five percent of the sum of selenosugars and trimethylselenonium ion. However, it was a significant metabolite in one person, up to 4.6 µg selenium/L or 22 percent, and it was the major identified metabolite in another person, up to 15 µg selenium/L or 53 percent. This marked individual variability in formation of trimethylselenonium ion was maintained in a duplicate investigation of urine from the same seven people (Kuehnelt *et al.*, 2006). This result dispels recent doubts about the possible previous misidentification of trimethylselenonium ion with a cationic selenosugar.

A double-blind placebo-controlled study evaluated urinary excretion of selenium during pregnancy and postpartum to investigate the influence of pregnancy and lactation on selenium metabolism in women of low environmental selenium intake (Thomson *et al.*, 2001). Thirty-five women in the earliest stages of pregnancy and 17 nonpregnant women were recruited in New Zealand. Eighteen pregnant women received 50 µg selenium as L-selenomethionine daily, while the others received a placebo daily during pregnancy and 12 months postpartum. The nonpregnant women received the supplement, serving as a positive control. Blood samples and 24-hour urine samples were collected monthly during pregnancy and at three, six, and 12 months postpartum for analysis of selenium and iodine. Selenium content in plasma and urinary excretion of selenium fell during pregnancy; however, total excretion of selenium was greater during pregnancy than postpartum.

Concentrations of D- and L-selenomethionine were measured in food supplements and in urine of six Belgium subjects with limits of detection and quantification of 4 and 12 picograms (pg), respectively (Devos *et al.*, 2002). At least 90 percent of the selenium food supplements on the market contained L-selenomethionine. Data for urine samples after intake of L-selenomethionine or D,L-selenomethionine corresponding to 100 µg selenium/day indicate that the D-enantiomer is not metabolized. The identification of selenomethionine, trimethylselenonium ion, and selenoadenosylmethionine in a human urine sample suggests that selenomethionine might follow the metabolic route of its sulfur analog in the human body (Wrobel *et al.*, 2003, 2005).

Other pathways of selenium excretion include losses from enteric secretions, sloughing of cells of the intestinal mucosa, skin, and hair, and exhalation of dimethyl selenide in the breath. The rate of gastrointestinal excretion of selenium is not precisely known. Feldmann *et al.* (1996) analyzed volatile metal and metalloid compounds in human breath, finding dimethyl selenide in the intraoral air of all six subjects tested, at 0.08 to 0.98 µg/m^3. Based on a breathing volume of 10 to 20 m^3/day and an average dietary intake of 39 µg selenium/day for adults in Germany, it was estimated that 11 to 23 percent of the daily selenium dose may be excreted in the expired air.

The following animal metabolism studies provide evidence of selenium elimination. Thomson and Stewart (1973) found that the urinary selenium excretion rate in rats was greater following oral ingestion of [^{75}Se]sodium selenite than of [^{75}Se]selenomethionine. However, Shiobara *et al.* (1998) found that excretion of selenium increased with the dose, but was similar for both selenomethionine and selenite. Male Wistar rats were fed a selenium-deficient diet at less than 0.03 µg selenium/g for three weeks, then either a

selenium-adequate (0.2 µg/g) or selenium-excess diet (2.0 µg/g) with either L-selenomethionine or selenite for up to 12 weeks. The rats attained constant levels of selenium in hair, serum, and urine by two weeks after the change of dietary selenium concentration irrespective of the chemical forms. Selenite gave lower constant levels in hair and serum than L-selenomethionine, both in a dose-dependent manner. The urine selenium concentration was dependent on the dose but not on the chemical species. The different metabolic characteristics may be because selenomethionine is either utilized through the regulated pathway similar to selenite or utilized as intact selenomethionine without being distinguished from methionine.

A study by Shiobara *et al.* (1999) may help clarify the differences between selenite and selenate excretion. Sodium selenite or sodium selenate were administered intravenously to rats at 0.3 mg selenium/kg and selenium species were analyzed in the blood stream, liver and urine. Selenate was not taken up by red blood cells and disappeared from the blood stream much faster than selenite. With the selenate group, the concentration of selenium in urine was highest at zero to six hours and the chemical species of selenium was selenate at zero to six hours; thereafter a monomethyl selenol (CH_3SeH)-related compound and trimethylselenonium ions appeared. By contrast, in the selenite group, the urine concentration of selenium peaked at six to 12 hours, and the chemical species of selenium were the monomethyl selenol-related compound and trimethylselenonium ion. Approximately 20 percent of the selenate was excreted unchanged into the urine and the rest was taken up by the liver.

Selenate and selenite are transformed reductively into selenide which is used for the synthesis of selenosugars and selenoproteins rather than for methylation to methylselenol and then to trimethylselenonium ion (Suzuki *et al.*, 2006d). Although parenteral selenate is utilized less efficiently by the body, it is utilized in the liver in a similar manner to selenite (Kobayashi *et al.*, 2001). In dose relation experiments, [^{82}Se]selenate or [^{82}Se]selenite was injected intravenously into male Wistar rats of eight weeks of age, three rats per group, at single doses of 10, 25, 50, 100 or 200 µg/kg for the selenate group, and 2, 5, 10, 25 and 50 µg/kg for the selenite group. The animals were sacrificed one or 24 hours later, and the concentrations and distributions of [^{82}Se] in the liver, kidneys, serum, and urine remaining in the bladder or 24-hour urine were determined. In time course experiments, [^{82}Se]selenate or [^{82}Se]selenite was injected at doses of 50 and 10 µg/kg, respectively, and the animals were sacrificed 5, 15, 30, 60 and 180 minutes later. The results suggested that selenate is directly taken up by the liver about half as efficiently as is selenite, the latter being taken up by the liver after being metabolized to selenide in red blood cells. Although selenate and selenite were metabolized differently in the blood stream, they were apparently metabolized similarly in the liver. A portion of the selenate was also excreted directly into the urine.

Selenide is the common intermediate for the inorganic selenite and selenate and organic selenocysteine and selenomethionine sources, and is the checkpoint metabolite between utilization for the selenoprotein synthesis and methylation for the excretion of selenium (Kobayashi *et al.*, 2002; Ogra *et al.*, 2002). Selenite was taken up by red blood cells within several minutes after intravenous administration of [^{82}Se]-selenite and [^{82}Se]-selenate to rats, reduced to selenide by glutathione, and then transported to the plasma, bound selectively to albumin and transferred to the liver. Selenate was either taken up

directly by the liver or excreted into the urine. Two selenosugar metabolites as selenium-methyl-N-acetyl-selenohexosamine were detected in the liver, and one metabolite was methylated to the other *in vivo* and *in vitro* (Suzuki and Ogra, 2002).

Selenosugar 1, or 1-β-methylseleno-N-acetyl-D-galactosamine or 2-acetamide-1,2-dideoxy-β-D-glucopyranosyl methyl selenide or methyl-2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside, was identified as a major urinary metabolite of selenium in rats by Suzuki and coworkers (Ogra *et al.*, 2002). Two other monomethylated selenosugars related to selenosugar 1 were also found as urinary selenium metabolites within the required to low toxic range consumed by human volunteers as selenized yeast (Kobayashi *et al.*, 2002). Kobayashi *et al.* (2002) identified the first one as selenosugar 2, or 1- β-methylseleno-N-acetyl-D-glucosamine or methyl-2-acetamido-2-deoxy-1-seleno-β-D-glucopyranoside. Selenosugar 2 is an analog of selenosugar 1 and its glucosamine isomer. This urinary metabolite was also detected in the liver and rat urine. A second metabolite that increased with inhibition of methylation was again a selenosugar conjugated with glutathione instead of a methyl group and was assumed to be a precursor for methylation to the first metabolite. A metabolic pathway for the urinary excretion of selenium, i.e., from the glutathione-S-conjugated selenosugar to the methylated one, was proposed by Kobayashi *et al.* (2002). Selenosugar 3 or methyl-2-amino-2-deoxy-1-seleno-β-D-galactopyranoside was found in human urine before and after ingestion of the selenium supplement (Bendahl and Gammelgaard, 2004). Selenosugar 3 is a deacylated analog of selenosugar 1. The type of selenium compounds ingested does not seem to influence these human urinary metabolites.

Selenite in drinking water fed *ad libitum* to male Wistar rats of either adult 36 weeks of age or young five weeks of age showed dose-related changes in the two urinary selenium metabolites (Suzuki *et al.*, 2005). In young rats, selenosugar 1 was the major urinary metabolite and plateaued with a dose higher than 2.0 µg selenium/mL water or g diet; trimethylselenonium ion increased with a dose higher than 2.0 µg selenium/mL drinking water. In adult rats, trimethylselenonium increased marginally, although the rats suffered greatly from the selenium toxicity, indicating that trimethylselenonium cannot be a biomarker of selenium toxicity. The results suggest that sources of the N-acetyl-D-galactosamine sugar moiety of selenosugar are more abundant in adult rats than in young rats. Chondroitin 4-sulfate did not affect the ratio of the two urinary metabolites, suggesting that the sugar source is of endogenous origin and that it increases with age.

Juresa *et al.* (2007) investigated the biological availability and metabolism of selenosugar 1 or selenosugar 2 administered in the drinking water for 48 hours to eight-week-old male Wistar rats, three per group. Sodium selenite and trimethylselenonium ion were included as positive and negative controls, respectively. The selenium species found in background urine were selenosugar 1 as the major metabolite and trimethylselenonium ion as the minor metabolite. Rats orally exposed to selenite in drinking water excreted large quantities of selenosugar 1 at about 47 percent and trimethylselenonium ion at about 21 percent, with less than one percent of selenosugar 3. This is consistent with efficient uptake and biotransformation of selenite. Trimethylselenonium ion was absorbed from the stomach in the exposed rats, but was not biologically available and was excreted largely unchanged, without significant increase of other selenium metabolites. Rats exposed to selenosugar 1 or 2 excreted significant quantities of

trimethylselenonium ion, suggesting that the sugars were at least partly biologically available and biotransformed. Rats exposed to selenite accumulated selenium in the liver, kidney, small intestine and blood, whereas no accumulation was observed for the other treatments except for small increases in selenium concentrations of small intestine from the two selenosugar-exposed groups.

Differences in the distribution and metabolism among organs between [^{76}Se]selenite and [^{77}Se]selenomethionine were compared in twelve-week-old male Wistar rats depleted of endogenous natural-abundance selenium. The rats were fed the stable isotope [^{82}Se]selenite and then administered [^{76}Se]selenite and [^{77}Se]selenomethionine simultaneously (Suzuki *et al.*, 2006e). Selenite was distributed more efficiently than selenomethionine in organs and body fluids, except the pancreas. Intact selenomethionine was taken up by organs and selenium of selenomethionine origin was distributed selectively in the pancreas and mostly bound to a protein together with intact selenomethionine. Selenosugars 1 and 2 but not trimethylselenonium ion were detected in the liver. Selenosugar 1 and trimethylselenonium ion were detected in kidneys (Suzuki *et al.*, 2006e). In selenium-deficient rats, intact [^{76}Se]selenosugar was distributed to organs and used for selenoprotein synthesis (Suzuki *et al.*, 2006b). Approximately 80 percent of the [^{76}Se]selenosugar given intravenously to rats depleted of endogenous natural-abundance selenium with a single stable isotope ([^{82}Se]) and then made Se-deficient was recovered intact in urine within one hour. Oxidation to methylseleninic acid and/or hydrolysis of the selenoacetal group to methylselenol were proposed to transform selenosugar for reuse. Formation of trimethylselenonium ion depends on the selenium sources, the dose, the capacity to demethylate methylselenol to selenide and metabolic balance of the transformation between the two, and further transformation to selenosugar and trimethylselenonium ion (Suzuki *et al.*, 2006a). Production of selenosugars and trimethylselenonium ion appears to depend on the availability of methylselenol or selenide, depending on the selenium source, relative activities of methyltransferase and demethylase, availability of the cofactor S-adenosylmethionine, and availability of the sugar moiety of selenosugar (Suzuki *et al.*, 2006a,b,c,d,e,f).

Selenium in expired air is associated with metabolism that occurs in the liver and may not correlate with clinical toxicosis signs or selenium concentrations in the blood or serum. Tiwary *et al.* (2005) determined the selenium concentration in expired air from 38 sheep given selenium via an intragastric tube as a single dose of 0, 1, 2, 3, or 4 mg/kg as sodium selenite or selenomethionine in water. The selenium in expired air at four, eight, and 16 hours after administration was greater in sheep receiving selenium as selenomethionine than as sodium selenite. The selenium concentration in expired air was higher and was elevated longer at the higher doses than at the lower doses. Clinical signs consistent with selenium intoxication, apparent 10 to 12 hours after treatment, were seen in groups given sodium selenite but not in groups given the equivalent amount of selenium as selenomethionine. However, a distinct garlic-like odor was evident in the breath of all sheep receiving 2 to 4 mg of selenium/kg. The intensity of odor in the breath did not correlate with clinical signs in affected animals receiving sodium selenite treatment.

Selenium distribution

Humans

Upon acute administration of selenium to humans, the highest selenium concentrations generally occur in either liver or kidney, with somewhat lower levels in the heart, lung, spleen, pancreas, and adrenals, and even lower levels in muscle and brain (Burk *et al.*, 1972; Hopkins *et al.*, 1966; Thomson and Stewart, 1973). Although selenium concentration in the brain is lower than most other organs with a normal selenium dietary supply, it is protected from selenium loss during dietary deficiency because selenium can be taken from other organs and transported to the brain.

Upon chronic oral administration, the selenium of the diet and the duration of feeding affect the tissue distribution (Bopp *et al.*, 1982). The distribution of selenium in tissues depends on the type and the oxidation state of the selenium and most studies report similar distribution for organic and inorganic compounds (ATSDR, 1996, 2003). The distribution of selenium in human tissues was analyzed by Schroeder *et al.* (1970) and by Oster *et al.* (1988) and summarized by Sunde (1997). The selenium concentration in endocrine systems including thyroid, adrenals, pituitary, testes, and ovary is higher than that in other organs (Behne *et al.*, 1988; Kohrle *et al.*, 2005). During nutritional deficiency, selenium is retained in endocrine tissues, reproductive organs, and the brain to ensure continuation of the most critical life functions. The total body pool of selenium has been estimated to be between 5 mg to 15 mg in adults (SCF, 2000). Table 4, mainly adapted from Sunde (1997), lists tissue concentrations and body burden of selenium in humans and rats.

The thyroid tends to have the highest selenium concentration among the organs, as shown in a Canadian study of selenium in human blood and tissues (Dickson and Tomlinson, 1967). The thyroid of a 52-year-old male contained selenium at 1,240 µg/kg, and the thyroid from a 2.5-year-old male child contained 640 µg/kg. Another study on selenium in human thyroids found the lowest value of 505 ± 51 µg/kg from the central left lobe and the highest of 1,495 ± 204 µg/kg from the central right lobe of a male adult in Venezuela (Murillo *et al.*, 2005).

The mean concentration of non-mercury-bound selenium from a normal population of 133 adults in Germany without any known occupational mercury burden was 576 µg/kg in kidney cortex, 545 µg/kg in pituitary gland, 363 µg/kg in thyroid gland, 308 µg/kg in liver, 205 µg/kg in spleen, and 111 µg/kg in cerebral cortex (Drasch *et al.*, 2000). The different distribution of selenium in the body confirms that there is a controlled hierarchy in the selenium supply of different organs, which tries to prevent a selenium deficiency in organs with essential selenoenzymes like the thyroid gland even with a suboptimal selenium supply.

Table 4. Tissue Concentrations and Body Burden of Selenium

Tissue or organ	Selenium levels in tissues (concentrations and total amount)				
	Human*			Rat**	
	A (mg/kg)	B (mg)	C (mg/kg)	D (mg/kg)	E (µg)
thymus	0	0	—	0.23	0.06
fatty tissue	0.04	0.504	—	0.04	0.99
blood plasma	0.13	0.435	0.07	0.52	5.16
brain	0.13	0.221	0.11	0.13	0.27
lungs	0.15	0.15	0.13	0.38	0.67
muscle	0.24	6.984	0.11	0.12	16.61
heart	0.28	0.092	0.16	0.37	0.39
erythrocytes	0.29[a]	0.67	—	0.54	4.57
pancreas	0.3	0.03	—	0.41	0.36
testes	0.3	0.009	0.27	0.92	1.84
spleen	0.34	0.044	0.23	0.45	0.56
skeleton	0.42	4.662	—	0.15	1.73
liver	0.54	0.864	0.29	0.78	10.66
kidneys	1.09	0.294	0.77	1.5	3.28
adrenals	—	—	—	0.7	0.05
Total		14.96			47.2

*Column A is from estimates by Schroeder *et al.* (1970) for a 70-kg male, accounting for 92.6 percent of body weight. The erythrocyte([a]) value assumes a 40 percent hematocrit. Column B is concentration multiplied by organ weight. Column C is data collected by Oster and Prellwitz (1989) for West German adults who have a lower selenium intake than North Americans.
******Rat data are from Behne *et al.* (1994), estimated for a 309 g male rat fed sodium selenite at 0.25 mg selenium/kg. The total amount in Column E accounts for 72.8 percent of body weight but not skin and hair.

Transfer of selenium across the human placental has been reported (Hansen, 1991; Hawkes *et al.*, 1994; Jandial *et al.*, 1976; Korpela *et al.*, 1984; Plantin and Meurling, 1980; Willhite, 1993). Premature and full-term babies generally have lower blood selenium levels at birth than their mothers or other healthy adults, and preterm babies have lower blood selenium than their term counterparts (Gathwala and Yadav, 2002). Plasma selenium concentrations of the majority of healthy infants and children fall in the range of 50 to 150 µg/L (Litov and Combs, 1991). The plasma selenium levels in cord blood of 82 full-term, appropriate for gestational age babies were 54.17 ± 1.34 ppb, which was significantly lower than 70.63 ± 1.62 ppb seen in their mothers at delivery in Rohtak, India. Anemic mothers with a hemoglobulin level less than 8 g/dL had a plasma selenium level of 60.74 ± 4.57 ppb, which was lower than those with a hemoglobulin level greater than 8 g/dL of 74.19 ± 2.17 ppb. Maternal age, parity, literacy and socioeconomic status did not affect plasma selenium levels (Gathwala *et al.*, 2000).

Selenium status in the human body, mainly estimated by measurement of selenium in blood, plasma, or serum, can be used to quantify exposure to selenium. However, once adequate selenium intake is achieved, the blood selenium level does not continue to rise with increases in dietary selenium, based on animal studies (Burk *et al.*, 1968; Scott and Thomson, 1971).

Thomson *et al.* (2005) evaluated two cross-sectional studies and three intervention studies in New Zealand and suggested that plasma selenium concentrations of 65 to 70 µg/L may be adequate for optimum function of iodothyronine deiodinases, which are associated with dietary selenium intake at about 30 to 35 µg/day, compared to plasma selenium concentrations of about 85 to 90 µg/L for maximum activities of GPxs, which are associated with dietary selenium intake at about 45 to 55 µg/day.

Niskar *et al.* (2003) of the Centers for Disease Control and Prevention reported serum selenium levels in the U.S. population of 1.58 µmole/L as the mean and 1.56 µmole/L as the median, from 18,597 persons in the NHANES III survey. Mean serum selenium levels differed by age group, gender, race ethnicity, economic status indicated by Poverty Income Ratio (PIR), and geographic region. Niskar *et al.* (2003) found that the serum selenium increased with increasing PIR. However, Kant and Graubard (2007) of the National Cancer Institute did not find PIR to be an independent predictor of serum selenium using the NHANES III data.

Incorporation of selenomethionine into connective tissue proteins has been evaluated by studying selenium levels in toenails. Human nail clippings have been used in some epidemiological studies as a routine bioindicator of selenium exposure. However, the most detailed exposure assessments explained only about 50 percent of the variation in toenail selenium concentrations (Longnecker *et al.*, 1996; Slotnick and Nriagu, 2006). Slotnick and Nriagu (2006) reviewed the use of human nails to measure exposure to selenium in the context of the biomarker validation framework, laying the framework for future studies measuring elemental composition of nails.

Experimental animals

Beilstein and Whanger (1988) administered selenium to rats for seven weeks as either sodium selenite or selenomethionine with [^{75}Se] radiotracer of the same chemical form. Selenomethionine was the predominant selenium form in tissues of rats given selenomethionine. Selenocysteine was the predominant selenium form in tissues of rats given selenite. All tissues of the rats fed selenomethionine maintained a ratio of selenium to [^{75}Se] greater than the dietary ratio; however, all tissues of the rats fed selenite were equilibrated with the dietary ratio. Deposition of dietary selenium and [^{75}Se] was higher in most tissues of rats fed selenomethionine than selenite. Muscle [^{75}Se] was the largest single tissue pool of [^{75}Se] in both groups, accounting for one-third of recovered [^{75}Se] in the rats fed selenite, and one-half of recovered [^{75}Se] in the rats fed selenomethionine. Tissue GPx activities were similar between the two dietary groups. The proportion of selenium as GPx in tissues was highest in erythrocytes of the rats fed selenite at 81 percent and lowest in testes and epididymides of the rats fed selenomethionine at 0.9 percent. The proportion of selenium present in cytosolic GPx was consistently higher in tissues of rats fed selenite. Erythrocytes of the rats fed selenomethionine had more [^{75}Se]

associated with hemoglobin, and muscle cytosol of the rats fed selenite had more [^{75}Se] associated with the GPx protein. The proportion of [^{75}Se] as selenomethionine was higher in tissues of rats fed selenomethionine, with the highest in muscle and hemoglobin at about 70 percent, and lowest in testes at about 16 percent.

Chen *et al.* (1994b) studied tissue distribution of selenate, selenite, selenocystine, and selenomethionine in female ICR mice injected intraperitoneally with 0 or 5 mg selenium/kg, at 3, 6, 9, 12, 15, 18, or 24 hours after injection. Selenium from selenomethionine accumulated in all tissues examined, mainly in the heart, kidney, spleen, liver, and brain, to a greater extent than selenite or selenocystine. Selenium from selenate accumulated in all tissues examined, mainly in the skeletal muscle and lung, to a greater extent than selenite. Selenium from selenite and selenocystine accumulated in tissues to about the same extent, except in kidney selenocystine accumulated more. The maximum selenium concentrations from all four compounds occurred three to nine hours after injection. Secondary peaks of maximum selenium accumulation were seen in the muscle, heart, and brain of selenomethionine-injected mice; in the brain of selenocystine-injected mice; in the lung, heart, and brain of selenate-injected mice; and in the muscle, kidney, spleen, and liver of selenite-injected mice. The selenium concentrations in most tissues were still above the control values 24 hours after the injection.

Nishimura *et al.* (1991) found that the whole body retention of [^{75}Se]sodium selenite after a single oral administration in rats varied by age. In adult rats, five percent of the dose was retained at 36 days after administration; six-day-old and 14-day-old rats at administration had higher retention rates than the adults. The liver showed a larger burden than the other organs, followed by the kidneys, gastrointestinal tract, and testes.

Testes, mainly spermatozoa, contain the highest concentration of selenium in the mammalian reproductive system (Behne *et al.*, 1986). Administration of [^{75}Se] in the form of sodium selenite to mice (Gunn *et al.*, 1967) or rats (Brown and Burk, 1973; Calvin and Cooper, 1979; Calvin *et al.*, 1981, 1987; Gunn and Gould, 1970; Hawkes *et al.*, 1985) resulted in selenium accumulation in the testis where it was incorporated into developing spermatozoa. Selenium retention in the testis was especially significant in rats fed a selenium-deficient diet (Behne *et al.*, 1982; Behne and Hofer-Bosse, 1984; Brown and Burk, 1973; Wallace *et al.*, 1983a,b; Wu *et al.*, 1969, 1979).

Selenite accumulates in the anterior pituitary in rats after a single ip injection and is mostly lost from the gland within 60 days (Thorlacius-Ussing and Jensen, 1988). Rats were injected ip with 5 mg/kg [^{75}Se]sodium selenite and whole body counted after two, 24, and 48 hours, and 4, 10, 20, 30, 40, 60, and 80 days, before sacrifice on day 80. The maximum selenium content was observed in anterior pituitary, thyroid gland, testis, adrenals, liver, kidney, and blood two hours after injection, at which time the anterior pituitary contained 2.9 µg/g wet weight. The loss of selenite from the anterior pituitary resembled that seen in other organs. The selenium content in pituitaries from untreated rats was estimated to be 2.2 ± 0.1 µg dry weight and 0.48 ± 0.03 µg/g wet weight.

Smith and Picciano (1987) reported that lactating rats fed sodium selenite at 0.5 µg selenium/g of diet for 18 days had 16, 26, 29, and 38 percent less selenium in the plasma, kidney, liver, and heart, respectively, than rats fed selenomethionine. The bioavailability of selenomethionine and selenium yeast was greater than that of selenite in both lactating

dams and their nursing pups. The greater availability of organic selenium to pups may be because of the greater concentration of selenium in the milk of dams fed organic selenium. When supranutritional L-selenocystine was consumed, comparing to L-selenomethionine at 2.0 µg selenium/g of diet as-fed basis for 18 days by 13-week-old Sprague-Dawley virgin, pregnant, and lactating rats, selenium concentrations were 67 percent less in muscle, and 18 to 41 percent less in liver, spleen, lung, heart, and uterus regardless of reproductive state (Taylor *et al.*, 2005). Different selenium distribution patterns were found in female rats reared fed *ad libitum* either L-selenomethionine or L-selenocystine diets at four to seven times the daily selenium requirement, without significant adverse effects in mothers, fetuses, or pups. Selenium distribution from selenocystine was similar to that from inorganic selenium salts. Lactating rats consuming selenomethionine had the greatest selenium concentration in the brain, with pregnant rats being intermediate, and virgin rats having the least ($p < 0.02$). Selenium concentrations in the heart, liver, lung, muscle, spleen, plasma, placenta, uterus, and fetus were greatest ($p < 0.001$) in rats consuming selenomethionine. When fed as selenomethionine, the selenium was equally available for thioredoxin reductase synthesis as the selenium in selenocystine. Regardless of reproductive state, selenomethionine provided greater selenium load than selenocystine.

SELENIUM ESSENTIALITY AND DEFICIENCY

Molecular Basis of Essential Action

The essentiality of selenium for mammals was first reported by Schwarz and Foltz (1957), who showed that traces of dietary selenium prevent liver necrosis in rats fed a diet deficient in vitamin E (Schwarz and Pathak, 1975). The molecular basis for the essential role of selenium in higher vertebrates became apparent in the early 1970s when Rotruck *et al.* (1972, 1973) showed selenium as a component of GPx (Diplock, 1976; Schomburg *et al.*, 2004). The essentiality of selenium is due to the requirement for selenocysteine.

Selenium biological effects occur mostly after dietary selenium is incorporated into selenoproteins as selenocysteine (Sec) (Gromer *et al.*, 2005). The selenocysteine incorporation is regulated using the genetic code UGA codon and selenocysteinyl-$tRNA^{Sec}$ (Chambers *et al.*, 1986; Zinoni *et al.*, 1986, 1987; Lee *et al.*, 1989; Low and Berry, 1996; Sunde, 1997; Mehta *et al.*, 2004; Berry, 2005a; Papp *et al.*, 2007). More remains to be determined, such as which sulfur in cysteine is replaced by selenium in a number of selenoproteins (Longtin, 2004; Stadtman, 1996).

Mammalian selenium-containing proteins can be divided into three groups: 1) specific selenoproteins containing genetically encoded selenocysteine, 2) specific selenium binding proteins in which selenium is externally bound to the polypeptide, and 3) proteins containing nonspecifically incorporated selenium such as selenomethionine-containing proteins (Behne and Kyriakopoulos, 2001; Brenneisen *et al.*, 2005; Hatfield, 2001; Kyriakopoulos and Behne, 2002; Papp *et al.*, 2007). Selenoproteins refer only to selenocysteine-containing proteins, and do not include selenium binding proteins or selenomethionine-containing proteins. Selenoproteins are required for development and

a long and healthy life (Flohe, 2005, 2007; Gromer *et al.*, 2005; Schomburg *et al.*, 2004). The diverse role of selenium within selenium-containing proteins in various tissues has been reviewed and summarized (Sunde, 1990; Gladyshev and Hatfield, 1999; Holben and Smith, 1999; Kohrle, 1999; Edmonds and Morita, 2000; Kohrle *et al.*, 2000; Arteel and Sies, 2001; Hatfield, 2001; Hatfield and Gladyshev, 2002; Jacob *et al.*, 2003; Schomburg *et al.*, 2004; Brenneisen *et al.*, 2005; Brigelius-Flohe, 2006; Papp *et al.*, 2007).

The functions of major mammalian selenoproteins that have been characterized or hypothesized are listed in Table 5, modified from Kohrle (2004) and Papp *et al.* (2007).

Table 5. Functions of Major Mammalian Selenoproteins

Selenoproteins	Characterized or Hypothetical Functions
glutathione peroxidase family, GPx1, GPx2, GPx3, GPx4, GPx6	antioxidant device, modulation of lipoxygenases, redox signal transduction, oxidoreductase, H_2O_2 reduction
iodothyronine deiodinase family, DI1, DI2, DI3	catalyses activation and inactivation of thyroxine T_4, triiodothyronine T_3, and reverse triiodothyronine rT_3, degradation of T_4 and T_3, oxidoreductase
thioredoxin reductase family, TR1, TR2, TR3	multiple roles in redox regulation, drug metabolism, signal transduction, oxidoreductase
selenophosphate synthetase 2, SPS2	catalyses conversion of selenide to selenophosphate production for selenocysteine synthesis, oxidoreductase
selenoprotein R, SelR, MsrB, methionine sulfoxide reductase B	*R*-methionine sulfoxide reductase, antioxidant, potential link to neurologic diseases and cataract
selenoprotein P, SelP	selenium transport and storage, antioxidant, neurotoxicity prevention
selenoprotein W, SelW	antioxidant, potential link to muscle and neurologic diseases
selenoprotein N, SelN	hypothetical cell proliferation and regeneration, potential protein folding, link to early-onset muscle disease
selenoprotein 15, Sep15, Sel15	potential protein folding, potential tumor suppression
selenoprotein M, SelM	potential protein folding, potential link to Alzheimer's disease
selenoprotein S, SelS	potential link to type 2 diabetes, inflammation, and cardiovascular diseases
selenoprotein K, SelK	antioxidant, possible link to heart disease
selenoprotein I, SelI	CDP-alcohol phosphatidyltransferase
selenoprotein Y, SelY	function related to DI2

Well-characterized selenoprotein enzymes are the families of glutathione peroxidases (GPxs), thioredoxin reductases (TRs), and iodothyronine deiodinases (DIs). These enzymes are capable of modifying cell function by acting as antioxidants and modifying redox status and thyroid hormone metabolism. Selenoproteins are also involved in cell growth, apoptosis and modifying the action of cell signaling systems and transcription factors. Restoration of normal selenoprotein levels may be, apart from direct supranutritional effects, one explanation for the effects of selenium supplements.

Essential Role in Animal Nutrition

Deficiency

In 1957, it was discovered that selenium was the key component in a yeast preparation that protected rats against fatal liver necrosis induced by a vitamin E and cystine deficient diet (Schwarz and Folz, 1957, 1958; Schwarz and Mertz, 1957; Schwarz and Pathak, 1975). This observation stimulated much interest in the use of selenium as a dietary supplement to optimize nutrition (Scott, 1987). It was shown that the element was also effective for controlling exudative diathesis in poultry, a fact of commercial significance (Patterson *et al.*, 1957). The essential role of selenium in animal health was recognized and the U.S. FDA approved the use of selenium as a dietary supplement in animal feed (U.S. FDA, 1987, 2001, 2003, 2004). Selenium-enriched yeast is approved for use in diets for poultry (U.S. FDA, 2001), swine (U.S. FDA, 2003), and cattle (U.S. FDA, 2004), and sodium selenate and sodium selenite are approved for swine diets (U.S. FDA, 1987). In a complete diet for livestock, the fortification cannot exceed the equivalent of 0.3 ppm selenium (U.S. FDA, 2004). NRC (1988) has recommended a dietary level of 0.15 ppm selenium for reproducing sows.

Selenium deficiency in livestock has been recognized in many countries (Allaway, 1969; Lakin, 1973; NRC, 1971). The livestock and poultry diseases or conditions listed in Table 6 are reported to respond to selenium treatment. Selenium deficiency causes depression of growth and decreased efficiency of feed utilization in general (NRC, 1971). These findings are of economic importance to Canada, New Zealand and the U.S. that had large tracts of selenium-deficient agricultural soil, where significant livestock losses were attributable to selenium deficiency. Much of the U.S. east of the Mississippi River and along the west coast produces grains deficient in selenium (NRC, 1971, 1983).

Table 6. Selenium-Responsive Conditions in Farm Animals

Condition	Species	Tissue affected
white muscle disease	cattle, sheep, poultry, pig	skeletal and heart muscle
ill thrift	cattle, poultry, sheep	muscle mass
nutritional muscular dystrophy	chicken, turkey, salmon	skeletal and heart muscle
rigid lamb syndrome	sheep	limbs and spine
fatal myopathy	guinea pig	muscle
exudative diathesis	poultry	capillary walls
pancreatic degeneration	poultry	pancreas
liver necrosis	pig	liver

White muscle disease, a nutritional muscular dystrophy, is a degenerative disease of the striated muscles with extensive calcification caused by low selenium diet inducing low SelW concentration (Reilly, 1996; Rederstorff *et al.*, 2006). It gets its name from a characteristic lightening of the color of muscle, which is accompanied by deposits of

calcium. When heart muscles are affected, sudden exertion can cause heart failure and death. White muscle disease is probably the most widely recognized and economically important selenium deficiency condition in livestock. It affects many different species, primarily lambs and calves, but also horses, goats, poultry, rabbits, deer, and rats.

Rigid lamb syndrome or stiff lamb disease is a myopathy in lambs characterized by the involvement of the muscles of limbs causing the animal to become stiff and have difficulty getting up and down and walking. It is a nutritional muscular dystrophy indicated by alteration of cardiac and skeletal muscle fibers with extensive calcification caused by a combination of selenium and vitamin E deficiency (Rederstorff *et al.*, 2006). A fatal myopathy in guinea pigs affects muscle and lipid peroxidation with low GPx causing by a combination of selenium and vitamin E deficiency.

A nutritional muscular dystrophy caused by low selenium diet that affects chicken, turkey, salmon, and other species is characterized by alteration of cardiac and skeletal muscle fibers (Rederstorff *et al.*, 2006). Exudative diathesis is a disease of selenium deficiency in poultry (Reilly, 1996). It usually occurs in three- to six-week-old birds and shows initially as an edema of the musculature of the breast, wings, and neck which can develop into subcutaneous hemorrhages. The growth rate of the birds is slowed, leg weakness develops, and death eventually follows. Exudative diathesis occurs in commercial flocks feeding on low selenium grain.

Other selenium-responsive conditions are liver necrosis in pigs, pancreatic atrophy in poultry, myopathy of the gizzard and heart in turkeys, ill thrift in sheep, cattle, and lambs, impaired reproduction in livestock, reduced production and hatchability of fertile eggs, and poor viability of newly hatched chicks (NRC, 1971; Reilly, 1996).

Taconic® SVJ 109 mice fed a selenium-deficient diet for three generations showed a decline of selenium content in the liver, muscle, and brain with the slowest depletion in the brain (Hooven *et al.*, 2006). Selenium was not detectable in any of these tissues in the third generation with a detection limit of 0.004 µg/g. Many selenium-responsive genes were identified in the muscle of the third generation mice fed the deficient diet with distilled water or distilled water with either 0.1 ppm or 1 ppm sodium selenate.

In the diabetic rat, selenium has been reported to restore glycaemic control and prevent or alleviate the adverse effects that diabetes has on cardiac, renal and platelet function (McNeill *et al.*, 1991; Berg *et al.*, 1995; Becker *et al.*, 1996; Battell *et al.*, 1998; Stapleton, 2000; Beckett and Arthur, 2005). Selenium has been reported to act as an insulin-mimetic agent with regard to regulation of insulin-mediated enzymes and their reactions *in vitro* (Hei *et al.*, 1998; Stapleton *et al.*, 1997).

Following supplementation of ruminants grazing pastures deficient in selenium, milk production and growth responses are likely to occur in cattle, while in sheep improvements in growth and fertility are most likely (Wichtel, 1998a). Fertility in ewes and calving in cows are improved by selenium supplementation. In sheep, in particular, reproductive disorders generally occur in association with other selenium-responsive conditions such as white muscle disease and ill thrift. A general mechanism for the improved health of livestock and poultry in response to selenium supplementation is an improved immune response to pathogens. Selenium administered to calves improves their ability to form antibodies in response to *Leptospirosis pomona* vaccine. It is

believed that adequate levels of GPx are required to produce the oxygen derived radicals in neutrophils to kill ingested foreign organisms (Reilly, 1996).

Selenium in excess of the amounts required for selenoprotein synthesis is toxic in general (Flohe, 2005). Excess selenium that is not incorporated specifically into selenoproteins can cause cataracts, presumably via redox cycling. Selenium deficiency is also implicated in the development of cataracts. However, clinical evidence for a protective role of selenium in the development of cataract, macula degeneration, retinitis pigmentosa or any other ocular disease is not available.

In agricultural practice, selenium is most commonly added to fertilizers as sodium selenate, rather than selenite, which is used more often as a direct dietary supplement for animals (Reilly, 1996). Selenate is much more readily available to plants than selenite and can bring about a more rapid increase in the selenium content of plants. Sodium selenate is weakly adsorbed on soil colloids, in contrast to selenite, which is tightly bound and is thus less easily taken up by plant roots. Selenium soil supplements are widely used in New Zealand, Finland, and Scotland.

Thyroid effects

The thyroid gland is rich in selenium and expresses a variety of selenoproteins that are involved in antioxidative defense and metabolism of thyroid hormones. Selenium is essential for normal thyroid function and thyroid hormone homeostasis. In dietary selenium deficiency, synthesis of selenoproteins is impaired, but the thyroid conserves selenium to ensure these critical functions and maintain adequate thyroid hormonal metabolism (Bermano *et al.*, 1995; Dhingra *et al.*, 2004). In rats and humans, the thyroid gland contains more selenium per gram of tissue than any other organ and is considered as one of the most critical organs (Dickson and Tomlinson, 1967).

The thyroid gland and thyroid hormone metabolism is positioned at thetop of the hierarchy of selenium-dependent tissues and selenium-dependent reactions (Schomburg *et al.*, 2006). Low levels of serum selenium or SelP in the absence of other challenges do not necessarily interfere with regular functioning of the thyroid hormones axis. The DI isozymes are preferentially supplied and selenium-dependent enzymes in the thyroid are even less dependent on serum levels of selenium or SelP than in brain. However, SelP-knockout mice display decreased serum selenium levels and manifest growth defects and neurological abnormalities partly reminiscent of thyroid gland dysfunction or profound hypothyroidism.

There is differential regulation of the mRNAs of the selenoenzymes GPx1, GPx4, and DI1 and subsequent selenoprotein synthesis within and between liver, thyroid, and heart of rats (Bermano *et al.*, 1995). During selenium depletion, the levels of selenoenzyme mRNA did not necessarily parallel the changes in enzyme activity, suggesting a distinct mechanism for regulating mRNA levels. Severe selenium deficiency in rats caused almost total loss of GPx1 activity and mRNA in liver and heart; DI1 activity decreased by 95 percent in liver and its mRNA by 50 percent; GPx4 activity reduced by 75 percent in the liver and 60 percent in the heart but mRNA levels were unchanged. In the thyroid, DI1 activity increased by 15 percent and mRNA by 95 percent, GPx4 activity was unaffected but its mRNA increased by 52 percent.

Thyroidal type I 5´-iodothyronine deiodinase DI1 shows marked species difference in its expression. In man, mice, guinea pigs, and rats, DI1 makes an important contribution to thyroidal triiodothyronine T_3 production (Beech *et al.*, 1993). DI1 was present in the livers of all species studied, but could not be detected in thyroids from cattle, pigs, sheep, goats, rabbits, deer, or llamas (Beech *et al.*, 1993). Selenium depletion causes imbalances in thyroid hormone metabolism in cattle and sheep (Arthur *et al.*, 1988; Wichtel, 1998a,b). In selenium-deficient weanling rats, hepatic DI1 activity was reduced to 11 percent of the activity found in selenium-replete groups, while thyroid DI1 activity increased by 42 percent (Beech *et al.*, 1993). Hepatic and thyroid GPx1 activities were also reduced by selenium deficiency to about 0.6 and 70 percent, respectively, of the values found in the selenium-replete animals. Selenium supplementation at up to 1 ppm enhances selenoenzyme activities. However, excess dietary selenium does not elevate the activities of selenoenzymes and thyroid hormones; rather, it has adverse effects and would not be considered as an indicator of selenoenzyme activities.

Immune system effects

Selenium can help confer resistance of animals to a variety of infections. Although the mechanisms are not fully understood, dietary selenium is essential for an optimum immune response, for example, the antioxidant GPxs are likely to protect neutrophils from oxygen-derived radicals that are produced to kill ingested foreign organisms. As such, there is much potential for selenium to influence the immune system (Arthur *et al.*, 2003). Selenium is also able to modify the immune response in patients with autoimmune thyroiditis (Beckett and Arthur, 2005).

Finch and Turner (1996) have reviewed the effects of selenium on the immune responses of domestic animals. Deficiency can compromise the immune system and result in a decline in livestock production and performance. Selenium deficiency results in decreased antibody production, compromised neutrophil functions, decreased lymphocytic response, and greater susceptibility to natural infections; these indicators of immune function are restored by selenium supplementation (Erskine *et al.*, 1987; Weiss *et al.*, 1990; Cebra *et al.*, 2003). Selenium deficiency reduces epidermal Langerhans cell numbers, an effect that compromises cutaneous immunity (Rafferty *et al.*, 2003). Selenium supplementation decreases skin damage and tumor formation induced by ultraviolet radiation in mice, which may reflect the role of selenium in protection from oxidative damage as well as immune function.

Kiremidjian-Schumacher *et al.* (1994) and Kiremidjian-Schumacher and Roy (1998) showed using a mouse model system that selenium enhances the capacity of lymphocytes to respond to stimulation with mitogen or alloantigen, to proliferate, and to differentiate into cytotoxic effector cells. Supplementation with selenium resulted in a significant increase in the tumor cytotoxicity of mouse cytotoxic lymphocytes, lymphokine-activated killer cells and macrophages, and human cytotoxic lymphocytes and natural killer cells. Selenium also appears to abrogate the age-related deficiency of lymphocytes from an aged host to respond to stimulation by proliferation and differentiation into cytotoxic effector cells. These effects occurred in the absence of changes in the endogenous interleukin levels and were related to the ability of selenium to enhance the expression of

the interleukin-2 receptor on the surface of activated lymphocytes and natural killer cells. This resulted in enhanced proliferation and clonal expansion of cytotoxic precursor cells. The molecular mechanism that mediates the effects of selenium on immune cell function did not appear to be related to antioxidant properties or to gene activation by selenium.

Developmental and reproductive effects

A nutritional deficiency in selenium affects fertility and fetal development in animals (Hatfield, 2001). Of the nine biological trace elements, zinc, copper, and selenium are important in reproduction in male and female rats (Bedwal and Bahuguna, 1994). Selenium deficiency also exacerbates the adverse effects of metal exposure and affects bioavailability of metals such as mercury (Heinz and Hoffman, 1998), manganese (Gochfeld, 1997), lead and cadmium (Burger and Gochfeld, 1992).

Selenium, functionally incorporated into selenoproteins (Foster and Sumar, 1997), is critical for mammalian reproduction, especially for spermatogenesis (Flohe *et al.*, 2000). Selenium deficiency has been associated with decreased male and female fertility in both experimental animals and livestock (Hidiroglou, 1982; Hansen and Deguchi, 1996). Excess selenium not incorporated into selenoproteins accounts for selenium toxicity (Hansen and Deguchi, 1996).

The male reproductive system is more affected by chronic selenium deficiency than the female system (Maiorino *et al.*, 1999). Low sperm production and poor sperm quality are consistent features of selenium-deficient animals (Watanabe and Endo, 1991; Hill *et al.*, 1996). Selenium is essential for normal spermatozoa development and it influences sperm motility and function. The pivotal link between selenium, sperm quality and male fertility is GPx4 since the enzyme is essential in production of the spermatozoa midpiece (Wu *et al.*, 1969, 1973, 1979; Sunde, 1990; Bedwal and Bahuguna, 1994; Beckett and Arthur, 2005). The selenium increases in male gonads during pubertal development.

GPx4 in testes is also likely to protect the developing sperm cell against damage by converting toxic peroxides to harmless alcohols. Ursini *et al.* (1999) showed that GPx4 is the most abundant component of the mitochondrial capsule, accounting for about 50 percent of its material. In mature sperm, GPx4 appeared to lose its enzymatic activity by crosslinking to an inactive form (Strauss, 1999; Ursini *et al.* (1999). Thus GPx4 may play dual roles; first as a detoxifying enzyme and later as a protective structure. Selenium appeared to be protective in rodent testes against physical trauma (Avlan *et al.*, 2005), and antagonized the toxicity of cadmium (Ganther, 1980; Rana and Boora, 1992; Lu *et al.*, 1994b), lead (Han *et al.*, 1996), and arsenate (Holmberg and Ferm, 1969).

Selenium deficiency in females has been linked to decreased fertility, reduced implantation, abortions, and retention of the placenta (Hatfield, 2001; Hidiroglou, 1982; Maiorino *et al.*, 1999); which may be comparable to selenium toxicity in female reproductive systems as discussed later in this document (Parshad, 1999; Rosenfeld and Beath, 1954; Wahlstrom and Olson, 1959). Selenium deficiency has been linked to reproductive disorders in cattle and sheep (Wichtel, 1998a van Niekerk *et al.*, 1996), especially early embryonic mortality. The selenium requirements of a pregnant and lactating mother are increased as a result of selenium transport to the fetus via the placenta and to the infant via breast milk (Bedwal and Bahuguna, 1994).

Antagonism between selenium and methylmercury has been observed to varying degrees under experimental conditions (Chang, 1983; Ganther *et al.*, 1972; Ridlington and Whanger, 1981; Svensson *et al.*, 1992). Pregnant female ICR mice, maintained on torula-based diets containing selenium at 0.02, 0.05, or 0.4 mg/kg diet, were given methylmercury at 0, 5, or 9 mg/kg total mercury on the 12th to 14th days of gestation. The neurobehavioral function of the offspring was evaluated with respect to reflex and motor development, thermal preference, and open-field activity. Selenium deficiency per se as well as exposure to methylmercury exerted additive or synergistic effects in the neurobehavioral tests. The group of mice given the lowest amount of selenium and the highest dose of methylmercury was the most affected. Thus, the neurobehavioral outcome of *in utero* methylmercury exposure and selenium deficiency converged. Although the dietary selenium did not affect mercury concentration in the fetal brain, the selenium concentration and the activity of GPx were severely depressed by methylmercury in the neural tissue. The functional selenium deficiency induced by methylmercury exposure apparently partly accounts for the neurobehavioral toxicity of methylmercury (Watanabe *et al.*, 1999). Selenium deficiency has been correlated with decreased birth weight following methylmercury exposure in mice. However, selenium status did not appear to affect the incidence of cleft palate (Nishikido *et al.*, 1988).

Low dietary levels of selenium, below about 0.1 to 0.5 µg/g, cause nutritional deficiencies in wildlife (Ohlendorf, 1999). A decrease in egg-laying and incubation success associated with selenium deficiency has been noted in birds (Grabek *et al.*, 1991).

Essential Role in Human Nutrition

The public perception of selenium has changed significantly over the last decade or so (Gromer *et al.*, 2005). Originally known mainly for its high toxicity, it is presently recognized as an essential trace element and investigated for possible influence on many health conditions and disease processes. Numerous studies on selenium health effects are underway, and Table 3-8 of the ATSDR Toxicological Profile of Selenium lists 127 on-going projects (ATSDR, 2003). Animal data, epidemiological data, and intervention trials have shown a potential role for selenium compounds in both prevention of specific cancers and antitumorigenic effects in post-initiation phases of cancer (Patrick, 2004). Selenium enriched yeast, L-selenomethionine, and other organoselenium compounds are considered to have an increased therapeutic ratio compared with some of the historically studied forms such as selenite. Cancer in prostate, colon, stomach, intestine, lung, mammary gland, and oral cavity has been studied. Some clinical and preclinical studies suggest that selenium supplementation may be beneficial in a large number of clinical conditions. However, its mode of action is unresolved in these cases.

The essential functions of selenium for human and animal health are mediated through selenoproteins that are involved in antioxidant defense, redox regulation, inflammation, immune responses, thyroid hormone production, DNA synthesis, fertility, and reproduction (Bansal and Kaur, 2005; Rayman, 2000, 2004). Selenium may inhibit viral expression and delay the progression of AIDS in HIV positive patients. Furthermore, selenium appears to reduce the risk of heart disease and other cardiovascular and muscle disorders, to ameliorate diabetes, to slow the aging process, and to have roles in

mammalian development and male reproduction and immune function. Selenium may be involved in the detoxification of some metals and other xenobiotics (Hatfield, 2001; Hendler, 2001). Selenium deficiency is associated with diseases like arthritis, cancer, cardiovascular disease, depression, myocarditis related to *Coxsackievirus* and HIV, thyroid dysfunction, as well as a host of inflammatory tissue responses (Hatfield, 2001; Rossoff, 2002; Rayman, 2000, 2005).

According to results in the Women's Health and Aging Studies I and II in Baltimore, Maryland, older American women who have lower serum selenium and carotenoids are at a higher risk of death adjusting for age, education, smoking, BMI, poor appetite, and chronic diseases; and vice versa (Ray *et al.*, 2006; Walston *et al.*, 2006). Among the 619 participants in the Study I from 1992 to1998, those with the lowest selenium levels had a significantly higher risk of total mortality over five years (hazard ratio 1.54, 95 percent confidence interval 1.03 to 2.32). Participants with the highest serum levels of α-carotene, total carotenoids, and selenium were significantly less likely to be in the highest tertile of serum inflammatory cytokine interleukin-6 at baseline ($p < 0.0001$). Interleukin-6 is produced due to oxidative stress and was linked to poor health outcomes in older adults (Walston *et al.*, 2006).

In the French EVA study of 1,389 men and women aged from 59 to 71 years at the baseline, cholesterol levels, use of lipid-lowering drugs, and education and income levels were positively associated with selenium (Berr *et al.*, 1998). These findings support the hypothesis that lipid peroxidation and low selenium status are involved in the early phases of atherosclerosis. Mortality rates were significantly higher in individuals with low selenium, controlled for confounding factors (relative risk 1.54, 95 percent confidence interval (CI) 1.25 to 1.88), especially for cancer-related mortality (adjusted relative risk 1.79, 95 percent CI 1.32 to 2.44) (Akbaraly *et al.*, 2005). Cognitive decline was associated with decreases of plasma selenium over time (Akbaraly *et al.*, 2007). Among subjects who had a decrease in plasma selenium, the greater the decrease, the higher the probability of cognitive decline. Among subjects who had an increase in their plasma selenium levels, cognitive decline was greater in subjects with the smallest selenium increase. Arnaud *et al.* (2007) followed 751 subjects from the EVA study for nine years with clinical examinations and lifestyle questionnaires every two years. After controlling all potential associated factors, age of subjects ($p < 0.01$), obesity ($p = 0.02$) and occurrence of cardiovascular disease during follow-up ($p = 0.03$) increased the longitudinal decline in plasma selenium; gender, education, smoking, alcohol intakes, dyslipidemia, diabetes, hypertension had no effect ($p > 0.05$). It may be postulated that obesity and occurrence of cardiovascular events are the main factors associated with plasma selenium fall during aging. The respective roles played by nutritional and metabolism changes in the mechanism of these associations still need to be explored.

Deficiency

Adequate dietary intake of selenium is essential for human health because selenium is incorporated into selenocysteinyl residues of selenoproteins which are required for biological functions (Schomburg *et al.*, 2004). The value of selenium supplementation in animal nutrition led to widespread acceptance of the view that inadequate dietary

selenium could be a human health problem (WHO, 1996b). The scientific evidence for the assessment of deficiency or adequacy of selenium status and the selenium requirement has been reviewed (Thomson, 2004a).

Patients with critical illness may have increased requirements for selenium. Selenium deficiency, in combination with iodine deficiency, may be associated with endemic myxedematous cretinism which results in mental retardation (Vanderpas *et al.*, 1990). Chronic selenium deficiency may occur in individuals with malabsorption and long-term selenium-deficient parenteral nutrition (Coppinger and Diamond, 2001). Patients with severe gastrointestinal disorders may not have adequate selenium absorption resulting in lower selenium status. Vegetarians and the elderly may have low intakes and are at risk of selenium deficiency (Hardy and Hardy, 2004). Gender is also a factor influencing selenium status due to its role in selenoprotein expression and selenium distribution (Galan *et al.*, 2005a,b; Hardy and Hardy, 2004; Riese *et al.*, 2006). Prospective human studies have indicated that the anticancer potential of selenium may be greater in men than women, without identifying the gender-specific mechanisms (Hercberg *et al.*, 2007; Waters *et al.*, 2004; Wolters *et al.*, 2006). Studies of selenium-deficient populations in China suggest that children have the highest need for selenium next to pregnant women, compared to other age groups. Thus, children and pregnant women with their fetuses may be more susceptible to the effects of selenium deficiency (ATSDR, 2003; Chen *et al.*, 1980; Yang *et al.*, 1988a).

In Asia and Africa, insufficient selenium intake in humans due to the consumption of crops from low selenium soils (Li, 2007; Tan and Huang, 1991) is a necessary condition relevant to the initiation of either Keshan disease, a cardiomyopathy or a form of heart disease, or Kashin-Beck disease, an osteoarthropathy or a form of degenerative bone disease (Burke and Opeskin, 2002; Ge and Yang, 1993). Kashin-Beck osteoarthropathy may also be associated with iodine deficiency.

Keshan disease was described by Japanese investigators in 1935 and named after its place of occurrence in Heilongjiang Province in northeastern China. This name has been retained even though more recent epidemics have been detected in a diagonal low-selenium band crossing central China, extending from Harbin in the northeast to Kunming in the southwest, covering 14 of the 22 provinces of China (Wang and Gao, 2001). Keshan disease occurs generally in hilly regions with heavily eroded soils. Women of childbearing age and children aged two to 10 years are most susceptible to Keshan disease. The disease is more common among rural than industrial populations. New cases have been reported in families within three months after moving from nonaffected areas into affected areas, probably because the farmers only eat the crops and vegetables grown locally. Several cases of cardiomyopathy in fetuses from affected mothers have been reported (Liu *et al.*, 2002; Yang *et al.*, 1982a,b, 1984; Yang, 1983).

The main clinical features of Keshan disease are cardiac insufficiency and cardiac arrhythmias. There is also cardiac enlargement and replacement of the myocardium by fibrous tissues. Multifocal necrosis and fibrous replacement of the myocardium characterized the histopathological features and myocytolysis was present in most cases. Ultrastructural observations on 12 postmortem cases in China revealed significant changes in many organelles of which mitochondria appeared to be most important in the development of myocardial lesions (Ge *et al.*, 1983; Ge and Yang, 1993).

Measurements of selenium intake show that Keshan disease populations have low selenium content in the local foods. Yang *et al.* (1987) estimated selenium intake to be 7.7 and 6.6 µg/day in endemic versus 19.1 and 13.3 µg/day in nonendemic areas for adult male and female subjects, respectively (Thomson, 2004a). Addition of sodium selenite to table salt or oral administration of sodium selenite have reduced the incidence of this disease and daily intake in supplemented populations has risen to about 80 µg/day.

The Chinese investigations suggest strongly that poor selenium status is closely linked with susceptibility to the disease (Keshan Disease Research Group for the Chinese Academy of Medical Sciences, 1979). The incidence of Keshan disease has decreased considerably since 1978, possibly due to selenium supplementation. This view is accepted by WHO (1996b) and the Food and Nutrition Board of the Institute of Medicine (NRC, 2000). The minimum dietary selenium requirement for prevention of endemic Keshan and Kashin-Beck disease in China was estimated to be around 17 µg/day. An intake of 40 µg/day, required to maintain the GPx3 activity at a plateau, was considered as the adequate dietary selenium requirement. WHO (1996b) calculated the basal selenium intake required to prevent pathologically and clinically relevant signs of dietary inadequacy of Keshan disease to be 19 µg/day, or 21 µg/day for men and 16 µg/day for women with a correction for body weight.

The precise relationship of selenium deficiency to myocardial pathology is not known. It has been suggested that a virus, activated by selenium deficiency, may cause myocarditis. Strains of enteroviruses, including a benign strain *Coxsackie* B4, have been isolated from blood and tissues of some patients suffering from Keshan disease. When this virus was injected into mice fed on a low selenium diet based on grain from a Keshan disease area, extensive damage to heart muscle resulted (Beck and Levander, 1998). This myocardial toxicity was reduced by dietary supplementation with selenium. GPx1 knockout mice fed with adequate selenium diet develop a cardiomyopathy resembling Keshan disease when infected with a *Coxsackie* virus (Beck and Levander, 2000; Ho *et al.*, 1997). It is possible that the cardiomyopathy in Keshan disease patients is related to decreased GPx1 activities with reduced protection against oxidative stress, secondary to dietary selenium deficiency, which allow virulence of *Coxsackie* (Moghadaszadeh and Beggs, 2006).

Another condition called Kashin-Beck disease is also associated with selenium deficiency. However, the role of selenium is not well established because selenium supplements apparently do not relieve the signs. The main feature of Kashin-Beck disease is short stature, caused by multiple focal necroses in the growth plate of the tubular bones. The main signs are an osteoarthropathy characterized by a chronic disabling degeneration and necrosis of joints and epiphyseal plate cartilages of the arms and legs. Advanced cases show joint enlargement and limb deformity. This leads to a secondary, sometimes severe osteoarthrosis. The condition progresses to shortening of the fingers and long bones and may result in growth retardation and stunting. In addition, iodine deficiency, grain contamination with mycotoxin-producing fungi, and water pollution with organic material and fulvic acid have been associated with the disease.

Kashin-Beck disease was first described from the Bajkal area of Russia by Kashin in 1848 and later in 1906 by Beck. In Asia, Kashin-Beck disease affects people in a crescent-shaped region through northern China, Tibet, Mongolia, Siberia, and north Korea. It occurs in areas that overlap the Keshan disease belt in China (Ge and Yang,

1993). Cases have also been reported in parts of Africa, including Zaire. Growth-stunting effects of selenium deficiency, in combination with iodine deficiency, may also be associated with an increased risk of cretinism in African (Vanderpas *et al.*, 1990) or Tibetan (Moreno-Reyes *et al.*, 2003) populations.

The associations between selenium, oxidative stress, and inflammation, respectively, might be related to the proposed cardiovascular protective property of selenium (Helmersson *et al.*, 2005). Serum selenium levels were correlated with an indicator of oxidative stress *in vivo* (8-iso-prostaglandin F2α) and an indicator of cyclooxygenase-mediated inflammation (prostaglandin F2α). The serum selenium was measured in 615 Swedish men at 50 years of age in a health investigation. The status of oxidative stress and inflammation was evaluated in a re-investigation 27 years later by quantification of urinary 8-iso-prostaglandin F2α and 15-keto-dihydro-prostaglandin F2α (a major metabolite of prostaglandin F2α). Men in the highest quartile of serum selenium at age 50 had decreased levels of 8-iso-prostaglandin F2α compared to all lower quartiles and decreased levels of prostaglandin F2α compared to all lower quartiles at follow-up. These associations were independent of BMI, diabetes, hyperlipidemia, hypertension, smoking, α-tocopherol and β-carotene at baseline. The serum selenium was not associated with high sensitive C-reactive protein, serum amyloid A protein or interleukin-6 at follow-up. Thus, high concentrations of serum selenium predicted reduced levels of oxidative stress and subclinical cyclooxygenase-mediated but not cytokine-mediated inflammation in a male population.

A cross-sectional and longitudinal population study in Belgium investigated the relationship between blood selenium and blood pressure (Nawrot *et al.*, 2007; Schomburg, 2007b). In a Flemish population with a known history of low blood selenium, 710 randomly recruited subjects with a mean age of 48.8 years, 51.8 percent women, blood pressure and blood selenium were evaluated. At baseline, systolic and diastolic blood pressure averaged 130 ± 17.3 standard deviation and 77 ± 9.2 mmHg. Blood selenium was 97.0 ± 19.0 µg/L. Of 385 participants with normal baseline blood pressure at less than 130 and less than 85 mmHg, over 5.2 years (range 3.4 to 8.4 years), 139 developed high to normal blood pressure at 130 to 139 and 85 to 90 mmHg or hypertension at equal to or greater than 140 and 90 mmHg. In multivariate-adjusted cross-sectional analyses of men, a 20 µg/L higher blood selenium was associated with lower blood pressure with effect sizes of 2.2 mmHg systolic [95 percent confidence interval (CI) -0.57 to -5.05, $p = 0.009$] and 1.5 mmHg diastolic (95 percent CI -0.56 to -2.44, $p = 0.017$). In prospective analyses of men, a 20 µg/L higher baseline blood selenium was associated with a 37 percent (95 percent CI -52 to -17, $p = 0.001$) lower risk of developing high to normal blood pressure or hypertension. None of these associations was significant in women. Therefore selenium deficiency may be a risk factor for the development of hypertension in men.

Selenium deficiency may contribute to hepatic injury in alcoholics, most likely due to lipoperoxidation secondary to decreased activity of GPx. Hepatic selenium content in livers taken at autopsy of 12 patients with alcoholic cirrhosis was about half that of 13 patients matched for age and gender dying from other causes, mostly cardiopulmonary diseases ($p < 0.005$, Student's t test) (Dworkin *et al.*, 1988). Low blood selenium is

present in renal failure patients undergoing hemodialysis and is even more evident in peritoneal dialysis cases (Dworkin *et al.*, 1987).

Patients with Balkan endemic nephropathy had significantly lower ($p < 0.05$) plasma selenium levels than healthy individuals, both from regions close to endemic areas, and from Belgrade, Yugoslavia (Mihailovic *et al.*, 1992b). Mean plasma selenium of Balkan endemic nephropathy patients was slightly but insignificantly higher in samples taken immediately after dialysis than in those taken before, suggesting that very little of the selenium present in plasma is dialyzable. GPx3 activities before and after hemodialysis in both Balkan endemic nephropathy and nonendemic chronic renal failure patients were not significantly different, but Balkan endemic nephropathy patients had lower enzyme activities than those with nonendemic chronic renal failure and healthy controls. In Balkan endemic nephropathy patients, a significant correlation between plasma selenium and GPx activity was found. The selenium contents in common cereals in endemic and nonendemic areas in Serbia were very low. Plasma selenium levels of both patients and healthy subjects were also low, reflecting low selenium intakes.

Patients on total parenteral nutrition for prolonged periods can be at risk of developing selenium deficiency if there is inadequate selenium in the infusion fluids. Six cases of cardiomyopathy due to total parenteral nutrition-related selenium deficiency were reported between 1980 and 1990 (NRC, 2000). Of the six patients, three responded to treatment with selenium supplements, but the other three died. Other signs of selenium deficiency associated with total parenteral nutrition are myalgias and myopathy, which clear up rapidly when selenium is added to the infusion fluid (NRC, 2000). A six-year-old child on total parenteral nutrition without selenium supplementation for 33.5 months developed intermittent leg muscle pain and tenderness, white finger nails, and increases in serum alanine aminotransferase, aspartate aminotransferase, and creatinine kinase with low serum and erythrocyte selenium concentrations (0.003 to 0.007 µg/g) as well as low urine excretion (none to 8.9 µg/day). After intravenous supplementation at 42 µg selenium/day as selenite, serum and whole blood selenium concentrations and whole blood GPx activity increased after only one month, and enzyme activities markedly improved; the fingernail bed abnormalities resolved after further therapy (Kien and Ganther, 1983). Similarly, a selenium-deficient patient with pain and tenderness in thigh muscles was responsive to selenium supplementation (van Rij *et al.*, 1979). Supplementary selenium in the form of selenite or selenomethionine is now administered to patients on total parenteral nutrition (NRC, 2000).

Antagonism between selenium and methylmercury has been observed due to the formation of nontoxic Hg-Se-S complexes due to higher affinity between mercury and selenium than that of mercury and sulfhydryl compounds under experimental conditions (Chang, 1983; Ganther *et al.*, 1972; Ridlington and Whanger, 1981; Svensson *et al.*, 1992). However, human studies on the interaction between selenium and mercury are limited. The molar ratio of selenium and mercury in human tissues from 133 adults was about one to one by the formation of biologically largely inert adducts similar to the binding complexes in experimental animals. A deficiency of non-mercury-bound selenium could not be seen in this normal population even at a higher mercury burden (Drasch *et al.*, 2000). This trapping of free mercury reduces the mercury toxicity,

however, the binding of selenium to mercury could reduce the availability selenium in tissues, and could result in a relative deficiency of selenium.

Selenoproteins play two important roles in protecting against mercury toxicity; first, selenoproteins may bind more mercury through their highly reactive selenol group; and second, their antioxidative properties help eliminate the ROS induced by mercury *in vivo*. An association between selenium and mercury was found in urine ($r = 0.625$, $p < 0.001$) but not in serum of 72 human subjects in China. The subjects included 37 mercury miners who had high exposure as evidenced by mean mercury concentrations of 38.5 ng/mL and 86.8 ng/mL in serum and urine, respectively; and 35 local residents with very low mercury exposure as evidenced by mean mercury concentrations of 0.91 ng/mL and 1.25 ng/mL in serum and urine, respectively (Chen *et al.*, 2006a). Expression of both SelP and GPx3 was greatly increased with elevated serum selenium concentrations in mercury miners. In addition, SelP bound more mercury at higher mercury exposures. Both GPx3 activity and malondialdehyde concentrations increased in serum of the mercury-exposed group.

Selenium is known to interact with cadmium, which stimulates the growth of prostate epithelial cells and promotes their malignant transformation. Determinations of selenium and cadmium in 129 prostates of deceased men aged 15 to 99 years in Germany revealed a stoichiometric excess of selenium over cadmium to decline with age (Drasch *et al.*, 2005). Whereas the selenium to cadmium ratios of the prostates of young men were invariably greater than one, the ratios were reduced to about one in elderly nonsmokers, which suggested to the authors formation of a one to one cadmium-selenium complex. They postulated that an associated physiological inactivation of selenium could account for the increase of prostate cancer risk with advancing age. The selenium to cadmium ratios dropped more steeply and consistently with age in smokers than in nonsmokers. In the prostates of some smokers, selenium to cadmium ratios reached values less than one. The authors suggested that an excessive accumulation of cadmium in the prostates of smokers along with suboptimal selenium intakes could explain why smokers develop more aggressive and lethal forms of prostate cancer than nonsmokers.

A hospital-based case-control study in 294 factory workers in Taiwan evaluated the association of serum lead, manganese, and selenium concentrations with hearing thresholds (Chuang *et al.*, 2007). Geometric means of serum lead, manganese, arsenic, and selenium for 121 case subjects with average hearing threshold over 25 decibels were 107.2, 5.5, 17.8, and 229.1 µg/L, respectively, compared to 38.9, 5.4, 15.5, and 234.4 µg/L, respectively, in 173 controls with normal hearing. Lead concentration was positively associated, and age and selenium inversely associated with hearing thresholds. The authors suggested that selenium may be a protective element for auditory function as well as an antagonist to lead ototoxicity.

Thyroid effects

In humans, the thyroid gland contains more selenium per gram of tissue than any other organ. It is considered as one of the most critical organs for selenium actions (Dickson and Tomlinson, 1967). The selenium content in other endocrine and reproductive tissues like adrenals, pituitary, testes, and ovary is also higher than in many other organs (Kohrle

et al., 2005). Nutritional selenium depletion results in sparing of levels in these tissues compared to other organs, whereas selenium repletion is followed by a rapid accumulation of selenium in endocrine tissues, reproductive organs, and the brain. This is interpreted as a position of thyroid high in the hierarchy to retain adequate amounts of selenium during dietary selenium deficiency to ensure the critical thyroid functions (Bermano *et al.*, 1995). In the thyroid, H_2O_2 is generated in response to thyroid stimulating hormone or thyrotropin or TSH stimulation for thyroglobulin iodination by the enzyme thyroid peroxidase to produce thyroxin T_4 which is then transformed to the active 3,5,3'-triiodothyronine T_3. Selenoproteins protect the thyroid from excessive accumulation of H_2O_2, and the thyroid gland expresses a variety of selenoproteins that are involved in antioxidative defense and metabolism of thyroid hormones. When prolonged, selenium deficiency impairs adequate thyroid hormonal metabolism.

Selenoproteins that participate in the protection of thyrocytes from damage by H_2O_2 include the species-specific DIs (Kohrle *et al.*, 2005). The DI1 is expressed in human, and provides a source of thyroidal 3,5,3'-triiodothyronine T_3 in case of selenium deficiency (Beech *et al.*, 1993). TR selenoproteins constitute the link between the selenium metabolism and regulation of transcription by redox sensitive ligand-modulated nuclear hormone receptors. Hormones and growth factors regulate the expression of selenoproteins and, conversely, selenium supply modulates hormone actions. During selenium deficiency, selenium is retained and expression of the three DIs is maintained at almost normal levels in the thyroid gland, the brain, and several other endocrine tissues. This guarantees adequate local and systemic levels of the active thyroid hormone T_3, ensuring that the effect of selenium deficiency on thyroid function in infants, children, and adolescents is relatively modest (Chanoine, 2003; Thomson *et al.*, 2005).

Thyroid hormone synthesis, metabolism and action require adequate availability of the essential trace elements iodine and selenium, which affect homeostasis of thyroid hormone-dependent metabolic pathways (Kohrle, 2005). While systemic selenium status and expression of selenoproteins like GPx or SelP is impaired in patients with cancer, disturbed gastrointestinal resorption, unbalanced nutrition or intensive care patients, selenium-dependent deiodinase function is still adequate. However, disease-associated alterations in proinflammatory cytokines, growth factors, hormones and pharmaceuticals modulate deiodinase isoenzyme expression independent from altered selenium status and might thus appear to provide causal relationships between systemic selenium status and altered thyroid hormone metabolism. Limited or inadequate supply of both trace elements, iodine and selenium, leads to complex rearrangements of thyroid hormone metabolism, enabling adaptation to unfavorable conditions.

In areas with severe selenium deficiency there is a higher incidence of thyroiditis due to a decreased activity of GPx activity within thyroid cells. Selenoenzymes also have modifying effects on the immune system. As a result, selenium deficiency contributes to the development of autoimmune thyroid diseases and repletion may improve inflammatory activity (Gartner *et al.*, 2002; Gartner and Gasnier, 2003; Angstwurm *et al.*, 2004; Dumitrescu *et al.*, 2005; Berry, 2005b).

In areas where combined selenium and iodine deficiencies are present such as endemic goiter areas in Central Africa, selenium deficiency may be responsible for the destruction of the thyroid gland in myxoedematous cretins but may also play a protective role by

mitigating fetal hypothyroidism from iodine deficiency (Chanoine, 2003). It is essential to take selenium deficiency into consideration for preventing goiters as shown in a study of goiter prevalence and serum selenium and urine iodine status among school-age children in the endemic Ankara region of Turkey (Cinaz *et al.*, 2004).

Immune system effects

Selenium deficiency is implicated in human immunodeficiency virus infection and acquired immune deficiency syndrome (Rayman, 2000, 2004; Thomson, 2004a). The role of selenium as an antiviral agent (Beck and Levander, 2000; Levander, 2000; Beck, 2001; Beck *et al.*, 2003), in HIV-1 and AIDS (Baum *et al.*, 2001), and on human immunity and aging (McKenzie *et al.*, 2001) has been reviewed. Selenium deficiency has been implicated as contributing to the development of cardiovascular disease, skeletal muscle myopathy, anemia, and increased cancer risk (Thomson, 2004a; Brenneisen *et al.*, 2005). Selenium deficiency may be associated with deranged immune function including oral candidiasis, impaired phagocytic function, and decreased CD4 T-cell numbers in man. Most of the effects in these conditions are probably related to the function of selenium in selenoprotein antioxidant enzyme systems. Replenishing selenium in deficiency conditions appears to have immune-stimulating effects, particularly in patients undergoing chemotherapy. However, increasing the levels of selenoprotein antioxidant enzymes such as GPx or TR appears to be only one of many ways in which selenium-based metabolites contribute to normal cellular growth and function.

Selenium supplementation appeared to decrease mortality in male patients suffering from sepsis and septic shock (Angstwurm *et al.*, 2007). In subgroup analyses, the mortality rate was significantly reduced in 82 patients with septic shock with disseminated intravascular coagulation ($p = 0.018$) as well as in the most critically ill 54 patients ($p = 0.040$) or in 83 patients with more than three organ dysfunctions ($p = 0.039$). Whole blood selenium concentrations and GPx3 activity were within the upper normal range during selenium treatment, whereas they remained significantly low in the placebo group. No side effects were observed due to high dose sodium selenite treatment (Angstwurm *et al.*, 2007; Berger and Shenkin, 2007; Chan, 2007; Cho and Kanna, 2007). The effectiveness in female patients was unclear (Schomburg, 2007a).

Selenium induced rapid apoptotic death in human prostate cancer cells but not in normal prostate epithelial cells (Ghosh, 2004). The apoptosis-inducing effect was alleviated by metabolites of arachidonate 5-lipoxygenase, indicating that 5-lipoxygenase might be a molecular target of the anticancer action of selenium. Sodium selenite-induced apoptosis in murine B lymphoma A20 cells is associated with differential inhibition of protein kinase c-delta or PKC-δ, nuclear factor kappaB or NFκB, and inhibitor of apoptosis protein or cIAP-2 through intracellular signaling processes. The ability of selenium to induce cell cycle arrest by affecting critical cell signaling molecules may indicate one possible mechanism of its anticarcinogenic potential (Gopee *et al.*, 2004).

Studies in mouse lymphocytes showing that selenium enhanced responses to mitogen stimulation and had other immune-stimulating effects (Kiremidjian-Schumacher *et al.*, 1994) were extended in a study of immune function in human volunteers (Kiremidjian-Schumacher and Roy, 1998). The effect of dietary supplementation with selenium at 200

µg/day for eight weeks was examined on the ability of human peripheral blood lymphocytes to respond to stimulation with alloantigen, develop into cytotoxic lymphocytes, destroy tumor cells, and on the activity of natural killer cells. Study participants were randomized for age, gender, weight, height, and nutritional habits and given sodium selenite or placebo tablets. All participants were selenium replete, as indicated by plasma selenium levels prior to supplementation. The selenium treatment resulted in a 118 percent increase in cytotoxic lymphocyte-mediated tumor cytotoxicity and an 82.3 percent increase in natural killer cell activity, compared to baseline values. This apparently was related to enhanced expression of receptors for the growth regulatory lymphokine interleukin-2, and a consequent increased rate of cell proliferation and differentiation into cytotoxic cells. The results indicated immuno-enhancing effects of selenium in humans at levels above the replete level produced by normal dietary intake.

McKenzie *et al.* (1998) reviewed the role of selenium in immune function. In addition to the role of selenium in T cell activation (Kiremidjian-Schumacher and Roy, 1998), selenium may help prevent oxidative stress-induced damage to immune cells, and may alter platelet aggregation by decreasing the ratio of thromboxane to leukotriene production in inflammation. McKenzie *et al.* (1998) concluded that selenium is needed for proper immune system functioning, and appears to be a key nutrient in counteracting development of susceptibility to viruses. They proposed that selenium supplements may help prevent prostate, gastrointestinal, lung, and colorectal cancer (Blot *et al.*, 1993,1995; Clark *et al.*, 1996b, 1998; Mark *et al.*, 2000; Jacobs *et al.*, 2004; Rayman, 2004; Zhuo *et al.*, 2004), may improve neutrophil and monocyte activities of the elderly and sperm motility in subfertile men, decrease risk of hepatoma development in hepatitis B-infected people, and decrease lipid peroxidation in the skin after ultraviolet light exposure. Their recommendation to increase selenium intake to optimize health was echoed in a review by Rayman (2000), who suggested that deficiency may be linked to adverse mood states, increased risk of miscarriage, and perhaps, an increased risk of cardiovascular disease, inflammation, and cancer. A particular concern was that inhabitants of European countries that have a lower daily selenium intake and a lower selenium blood level than North Americans may be at increased risk of selenium deficiency.

An essential role of selenium in host defenses was described in the studies in rats of Beck and Levander (1998, 2000). These investigators showed that selenium deficiency in rats increased the virulence of *Coxsackie* virus, which induced a cardiomyopathy reminiscent of the cardiomyopathy in victims of Keshan disease in China. This suggested a possible mechanism for the etiology of Keshan disease. Mechanisms of interaction between selenium and viral pathogenicity are unclear, but Taylor *et al.* (1997a,b; 2000) showed that a number of viruses, including HIV-1, have genes that may encode for selenoproteins which may be involved in viral replication. In addition to *Coxsackie* virus and Keshan disease, selenium deficiency is linked to hepatitis B, liver disease and cancer, and selenium is a potent chemoprotective agent against the mouse mammary tumor virus. Furthermore, hemorrhagic fever viruses may encode for selenoproteins, and these viruses are endemic in areas of Africa that are deficient in selenium.

Dworkin *et al.* (1986, 1994) documented low plasma selenium levels and GI malabsorption in AIDS patients. Both plasma selenium and GPx were significantly correlated with total lymphocyte counts ($r = 0.65$, $p < 0.001$). Cardiac selenium was also

decreased versus age and sex-matched controls ($p < 0.01$). Two cases had cardiomyopathy pathologically consistent with the cardiomyopathy described in Keshan disease. Baum *et al.* (1997, 2001) summarized evidence that decreased selenium levels are found in persons with HIV infection or AIDS. Blood plasma selenium levels appeared to be sensitive prognosticators of disease progression, with selenium-deficient HIV patients nearly 20 times more likely to die from HIV-related causes than those with adequate plasma levels, equal to or greater than 85 µg/L.

Developmental and reproductive effects

Selenium is essential in mammalian embryonic development, however, selenoprotein levels in several organs in adults can be substantially reduced by selenium deficiency without any apparent change in phenotype (Hatfield, 2001). Selenium is essential for normal spermatozoa development in human males, but both low and high sperm selenium concentrations have been reported to have negative effects on the number of spermatozoa, on sperm motility, and on fertility (Flohe *et al.*, 2001).

The optimal selenium dietary intake to support sperm growth as well as the optimal seminal or spermatozoal concentration has yet to be determined (Hansen and Deguchi, 1996). In one study, 46 men in Scotland were given L-selenomethionine at 100 µg selenium/day, including 16 men with a mean age of 32.6 years given selenium only, and 30 men with a mean age of 33.9 years given selenium in combination with 1 mg of vitamin A, 10 mg vitamin C, and 15 mg vitamin E for three months. Fifty-six percent had increased sperm motility and plasma selenium concentration. Five men, or 11 percent of the treated subjects, subsequently fathered a child, in contrast to none in the placebo group of 18 men, mean age 32.9 years (Scott *et al.*, 1998). In a Russian study, ejaculate quality was assessed by spermogram, enzyme activity of α-amylase and lactate dehydrogenase, concentrations of prostatic acid phosphatase, sperm-specific inhibitor tripsin and prostate-specific antigen before treatment and after three courses of selenium at 3.5 µg/kg-day for 30 days. These indicators of ejaculate fertility improved for both groups of Russians, including 31 subfertile men living in a healthy environment and 25 subfertile men exposed for a long time to low doses of hydrogen sulfide, compared to the control of 43 fertile men (Nikolaev *et al.*, 1999). However, no improvement in fertility was observed in Polish men supplemented with 200 µg selenium/day (Rayman, 2000).

Selenium deficiency appears not to have been associated with pregnancy or delivery problems in humans. In a French study, a reduction in plasma selenium during pregnancy with a simultaneous increased retention through decreased urinary excretion was reported. The fetus was reported to accumulate selenium in the liver at the end of pregnancy; however, the stored selenium depleted rapidly after birth. Infants, particularly premature ones or those with diseases, may have pronounced deficiency symptoms due to the usual marginal selenium dietary intake in France (Neve, 1990). Selenium deficiency might be linked to an increased risk of miscarriage, however, only sparse information exists and selenium has not been reported to influence female reproduction in any specific way. No human data relating selenium to female infertility were found (Hansen and Deguchi, 1996). In a British case-control study, women with recurrent pregnancy loss had lower selenium levels in their scalp hair than controls (Al Kunani *et al.*, 2001).

Antineoplastic Effects of Selenium

The role of selenium as an antineoplastic or cancer preventive agent has been repeatedly reviewed (Willett, 1986; WHO, 1996b; Combs and Lu, 2001; Klein, 2004; Meuillet *et al.*, 2004; Rayman, 2004, 2005; Taylor *et al.*, 2004; Whanger, 2004; Combs, 2005; Finley, 2005a; Lipinski, 2005; Nelson *et al.*, 2005; Trumbo, 2005). For the studies, selenium was administered mainly as sodium selenite, selenium-enriched yeast, selenomethionine, or organoselenium compounds, in doses three- to six-fold higher than the RDA of 55 µg/day (NRC, 2000). Cancer protective properties are presumed to be mediated through selenoprotein actions as direct or indirect antioxidants, regulating enzymes, and controlling apoptosis, angiogenesis, and the cell cycle. Evidence for selenium as a cancer preventive agent includes that from geographic, animal, prospective and intervention studies (Rayman, 2005).

Selenium treatments have appeared to show benefit in reducing the risk of cancer incidence and mortality in all cancers combined, and in liver, prostate, colorectal and lung cancers. Recent prospective studies on oesophageal, gastric-cardia and lung cancer have reinforced previous evidence, which appeared most promising for prostate cancer. The effect seemed to be strongest in those individuals with the lowest selenium status (Zhuo *et al.*, 2004, Rayman *et al*., 2009).

Finley (2005a) considered the evidence of efficacy for cancer prevention by selenocompounds as strong. A large Selenium and Vitamin E Cancer Prevention Trial (SELECT) has been conducted by the National Institute of Health to evaluate possible effects of selenium and vitamin E supplements on prostate cancer. Five years of treatments involving 35,533 men were initiated from 2001 through 2004, and ended in 2008. Preliminary reports from five years of observation were not positive (Lippman *et al*., 2009), but monitoring of the cohort is continuing, with further results anticipated in 2013. A subsequent "meta-analysis" of this and one other study concluded that, "Our findings indicate that antioxidant vitamins and selenium supplement did not reduce the incidence and mortality of prostate cancer, these data provide no support for the use of these supplements for the prevention of prostate cancer" (Jiang *et al*., 2010).

As the level of selenium that appeared to be required for optimal anticancer effect is higher than that previously understood to be required to maximize the activity of selenoenzymes, the question has been raised as to whether selenoproteins are involved in the anti-cancer process. However, recent evidence showing an association between selenium, reduction of DNA damage and oxidative stress, together with data showing an effect of selenoprotein genotype on cancer risk, implies that selenoproteins are indeed implicated. The likelihood of simultaneous and consecutive effects at different cancer stages still would allow an important role for selenium metabolites such as methylselenol.

Evidence for a role of selenium in reducing the risk of human cancers remains limited, based primarily on animal studies, inverse geographic correlations between human intake and site-specific cancer incidence, and an inverse or U-shaped association between serum selenium and subsequent risk of cancer. The relationship between selenium status in humans, genotoxic damage, and cancer risk is enigmatic because epidemiologic studies have failed to consistently link low selenium status with increased cancer risk in men and women or consistently show that selenium supplementation can reduce human cancer

incidence (Willett, 1986; Rayman *et al*., 2009). Overall, mechanisms that may be responsible for anti-cancer effects of selenium remain to be determined. Future research must include well-planned clinical trials, development of analytical tools for speciation of selenium in foods and biological tissues, better means of assessing selenium status in ways that are relevant to cancer prevention, and determination of the optimal dose of selenium that may be both safe and effective in reducing cancer risk (Combs, 2005).

Estimated Average Requirement of Selenium

Current dietary reference values for selenium intakes for different age groups have been published by a committee of the Food and Nutrition Board of the Institute of Medicine of the U.S. NRC, together with Health Canada (NRC, 2000). Table 7, adapted from NRC (2000), lists these established dietary reference values. In the U.S., the first human Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for selenium was established by the NRC Committee on Dietary Allowances in 1980, and the first Recommended Dietary Allowance (RDA) was proposed by the Committee in 1985. These values were discussed in detail in the Tenth Edition of the Recommended Dietary Allowances, published in 1989 (Levander, 1987, 1989b, 2001). The NRC 2000 Committee's dietary reference values are the latest version of these efforts.

Table 7. Selenium Dietary Reference Values for Human Intake

Population	Dietary reference values		
	Selenium (µg/day)		
	EAR*	RDA*	UL*
infants 0 to 6 months	**	**	45
infants 7 to 12 months	**	**	60
children 1 to 3 years	17	20	90
children 4 to 8 years	23	30	150
children 9 to 13 years	35	40	280
adolescents 14 to 18 years	45	55	400
adults	45	55	400
pregnant women	49	60	400
lactating women	59	70	400

*EAR: Estimated Average Requirement; RDA: Recommended Dietary Allowance; UL: Tolerable Upper Intake Level
**Adequate Intake (AI): 15 µg/day for infants aged zero to six months, 20 µg/day for infants aged seven to12 months.
Adapted from NRC (2000).

Precise terms introduced in the NRC (2000) document include the Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI) and Tolerable Upper Intake Level (UL). The EAR is the intake at which the risk of

inadequacy is 0.5, or 50 percent, to an individual. The RDA is the intake at which the risk of inadequacy is very small (two to three percent). EAR and RDA are considered the lower bounds of selenium intake that are compatible with health. The AI is used when the RDA is not known with sufficient precision.

The AI is set instead of a RDA if the scientific evidence is not sufficient to calculate an EAR. It is applicable to young infants, for whom human breast milk is the recommended sole source of food. The AI in this case is based on the nutrient intake of apparently healthy, full term infants who receive only human milk. The UL is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals in the specified life stage group. This term is similar in concept to that of the NOAEL. At the UL the risks of inadequacy and of excess are both close to zero; if the UL is exceeded, there is an increased likelihood of adverse effects.

TOXICOLOGY

Toxicological Effects in Animals

The earliest written report of selenium poisoning is thought to be by Marco Polo in 1295, when traveling the Silk Road in northwestern China. He wrote of a necrotic hoof disease of horses following consumption of certain plants (Quinn *et al.*, 2007; Reilly, 2006; Rosenfeld and Beath, 1964; Spallholz, 1994). This report was likely to have originated from the part of northwestern China where endemic selenosis has more recently been reported in animals and humans due to consumption of selenium-accumulating plants (Yang *et al.*, 1983). Rosenfeld and Beath (1964) quoted an 1857 report by T.C. Madison, an army surgeon, describing similar conditions in cavalry horses in South Dakota, resulting in the sloughing of hooves, mane, and tail. The symptoms resembled those later named blind staggers' disease in cattle and sheep due to acute or subacute or subchronic selenium poisoning (Mayo, 1891) or alkali disease in cattle, hogs and horses (Trelease and Beath, 1949; Rosenfeld and Beath, 1964) that was attributed to the ingestion of seleniferous hyperaccumulator plants (Spallholz, 1994). Selenosis is still responsible for loss of livestock in the western states where seleniferous soils occur (Quinn *et al.*, 2007).

Selenium toxicity was first identified in the U.S. in the 1930s in western regional range animals which consumed selenium accumulator plants of the genus *Astragalus*, *Xylorrhiza*, *Oonopsis*, and *Stanleya* in the Great Plains (Painter, 1941). A 1934 report from Wyoming confirmed that selenium as high as 1,000 ppm in vegetation was responsible for the disease of livestock (Lipp, 1922; Moxon, 1937; Orskov and Flyvbjerg, 2000; Rayman, 2000; Spallholz, 1994). It was also shown in the 1930s that the feeding of selenium-containing cereals to hens may cause malformations or death in the embryo or chicks without apparent injury to the mother hens (Klayman and Gunther, 1973; Rosenfeld and Beath, 1964). NRC (1976) considered growth inhibition as a major indicator of toxic effects caused by selenium-containing substances.

Rosenfeld and Beath (1964) described three clinical types of selenium intoxication in domesticated animals raised for human food: acute selenosis, subacute or subchronic selenosis, and chronic selenosis. Acute poisoning occurs when high selenium content

plants are consumed in large quantities within a short period (Mihajlovic, 1992; Raisbeck, 2000). Accidental acute poisoning occurs as a consequence of errors in formulation of a selenium supplemented diet. Plants that accumulate enough selenium to be acutely toxic are so unpalatable that animals usually will not eat them. The most characteristic sign of acute selenosis is garlic breath due to the pulmonary excretion of volatile selenium metabolites. The clinical signs of acute selenium toxicity include an elevated temperature, increased pulse rate, watery diarrhea, and prostration. Pulmonary edema and extensive tissue hemorrhage are common findings. Death results from circulatory failure. The primary targets of acute selenium toxicity in animals are the cardiovascular, gastrointestinal, and possibly hematopoietic systems. Other signs include lethargy, excessive salivation, vomiting, dyspnea, muscle tremors, and respiratory distress. Pathological findings include congestion of the liver and kidney, fatty degeneration and focal necrosis of the liver, endocarditis, and myocarditis.

Subacute or subchronic selenosis such as blind staggers' disease occurs as a consequence of exposure to large doses of selenium over a longer period of time and manifests with neurological signs, e.g., blindness, ataxia, disorientation, and respiratory distress (Mihajlovic, 1992; Raisbeck, 2000). This form of selenosis is most frequently observed in grazing animals that have consumed selenium-accumulator plants or feeding diets containing 10 to 20 ppm selenium for seven to eight weeks. Affected animals lose weight, have a poor coat, and develop a staggery gait with impaired vision. As the disease progresses, blindness, excessive salivation, lacrimation, abdominal pain, and inability to swallow gradually show up, and eventually complete paralysis, collapse, and death from respiratory failure occurs (Clark, 1995). Flaccid paralysis or tetraplegia with neurologic lesions may occur in swine two days to a month after surviving exposure to toxic amounts of selenium in feed; however, the signs of poliomyelomalacia are quite distinct from those described as blind staggers' disease by early reports.

Chronic selenosis such as alkali disease occurs when animals consume more than 5 ppm selenium ("moderate" levels) for over 30 days (Mihajlovic, 1992; Clark, 1995; Raisbeck, 2000). Characteristic signs of chronic selenosis include hair and hoof loss, a rough coat, and dullness. Other less specific effects on the immune system and reproduction are economically more important. Horses and cattle lose long hair from the mane and tails. Other clinical signs of chronic selenosis in horses, cattle, and swine are emaciation, hoof lesions, lack of vitality, and loss of appetite. In advanced cases, liver cirrhosis, atrophy of the heart and anemia occur. In swine, symmetrical poliomyelomalacia of various spinal cord segments has been seen. Sheep appear to be more tolerant and get a milder form of the disease. They lose appetite and have reduced weight gain. In growing chicks, reduced weight gain and feed intake, rough feathers, and nervousness have been observed. Effect observed after treatment of hens include reduced egg production, embryonic deformations and reduced hatchability (Mihajlovic, 1992; Raisbeck, 2000).

Skorupa (1998) described 12 examples in which elevated selenium exposure adversely affected biota. Oviparous vertebrates are the most sensitive organisms because they can efficiently transfer selenomethionine and other selenium compounds to the eggs. Toxic effects are manifested in the embryos when selenium compounds are assimilated from the yolk. Some adults in high selenium areas appear healthy but give birth to offsprings with elevated mortality and deformities. The deformities include overt spinal curvatures,

shortened jaw structures, missing or deformed fins and edema in fish (Lemly, 1997); or spinal, wing, and cranial deformities in birds (Spallholz and Hoffman, 2002). Excessive selenium in fish mainly accumulates in eggs and causes larval deformities. Skin lesions, cataracts, swollen gill filament lamellae, myocarditis, and liver and kidney necrosis in fish have also been reported (Miller *et al.*, 2007).

Acute toxicity

The toxic effects of selenium depend on dose, chemical species, route of administration, duration of exposure, and animal species. Table 8, adapted mainly from ATSDR (1996, 2003), summarizes the lethal doses in mg selenium/kg body weight from animal studies after acute oral exposure to water-soluble selenium compounds.

In four-week-old male ICR mice given selenocystine orally at 23.6 mg selenium/kg, liver damage was revealed by increases in aspartate aminotransferase and alanine aminotransferase, and kidney injuries were reported as increases in urea nitrogen and phosphorus and a decrease of plasma calcium (Sayato *et al.*, 1993). L-selenocystine is more toxic than D-selenocystine when given orally in rats, and cystine and methionine are less toxic than their selenium analogues in effects on liver succinoxidase (Klug *et al.*, 1949). Acute toxicity of waterborne selenite was reported to be greater than that of selenate in fish (Hamilton and Buhl, 1990; Niimi and LaHam, 1976; Takayanagi, 2001).

Table 8. Summary of Lethal Doses in Acute Animal Studies

Species and gender	Exposure route	Chemical species	LD_{50} Se mg/kg	Reference
rat, F	gavage	sodium selenite	4.8	Pletnikova, 1970
rat, M	gavage	sodium selenite	7	Cummins and Kimura, 1971
rat, M	gavage	sodium selenite	12.7	Vinson and Bose, 1987
mouse, M	gavage	sodium selenite	3.2	Pletnikova, 1970
mouse, M	oral	sodium selenite	16	Gao *et al.*, 2000
mouse, F	oral	sodium selenite	8.4	Plasterer *et al.*, 1985
guinea pig, F	gavage	sodium selenite	2.3	Pletnikova, 1970
rabbit, F	gavage	sodium selenite	1	Pletnikova, 1970
sheep, F	intramuscular	sodium selenite	0.7	Blodgett and Bevill, 1987b
lamb	oral	sodium selenate with antihelminthic	0.45	Hopper *et al.*, 1985
rat, M	gavage	selenium yeast	37.3	Vinson and Bose, 1987
mouse, M	gavage	D,L-selenocystine	35.9	Sayato *et al.*, 1993

Shibata *et al.* (1992) summarized the acute toxicity of selenium in animal studies based mainly on the NRC (1976) and WHO (1987) reports. They reported acute oral LD_{50}s for

sodium selenite of 7 mg/kg in rats and 2.3 mg/kg in rabbits. The minimum fatal dose, a dose killing 75 percent of the animals within 48 hours, in rats was 3.25 to 3.5 mg/kg with intraperitoneal (ip) injection and 3 mg/kg with intravenous (iv) injection. For sodium selenate, the minimum fatal dose in rats was 5.25 to 5.75 mg/kg with ip injection. Klug *et al.* (1949) reported a minimum fatal dose in rats through ip injection of 4.25 mg/kg for D,L-selenomethionine, 4.0 mg/kg for D,L-selenocystine, 1,600 mg/kg for dimethyl selenide, and 49.4 mg/kg for trimethylselenonium chloride [$(CH_3)_3Se^+Cl^-$], as well as 6,700 mg/kg for elemental selenium (Se^0) through the oral route.

Spallholz (1994) summarized the LD_{50} values in rats injected ip with various selenium compounds as 4.1 or 3.25 mg/kg for selenite (Se^{+4}) in two studies, 3.6 or 5.25 mg/kg for selenate (Se^{+6}) in two studies, 4.25 mg/kg for D,L-selenomethionine (Se^{-2}) in one study, and 4.0 mg/kg for D,L-selenocystine (Se^{-2}) in one study. The three diselenides (Se^{-2}) showed similar LD_{50} in rats as selenite and were several times more toxic than the four monoselenide (Se^{-2}) selenoethers tested. Selenoethers and dimethyl selenide were much less toxic than selenite. Selenium-methylselenocysteine, a major selenoether found in *Astragalus* plants, and selenomethionine, the major selenoether of nonaccumulator plants, were less toxic than selenite (Spallholz, 1994).

The LD_{50} in mice injected iv with selenomethionine was 8.8 mg/kg, which was 4.4-fold less toxic than selenite (Ammar and Couri, 1981). Sodium selenite was 43.3-fold more toxic than selenomethionine with intracerebroventricular injection. However, this toxicity differential has not been seen in oral studies. The formation of methylated selenides by animals appears to be one mechanism for selenium detoxification, considering that the toxicity of dimethyl selenide is about 1/500 to 1/1,000 of the toxicity of the nonmethylated Se^{-2} compounds (McNeal and Balistrieri, 1989). Dimethyl selenide [$(CH_3)_2Se$] and trimethylselenonium ion [$(CH_3)_3Se^+$], which are methylated excretory products of selenium metabolism in humans as well as endogenous metabolites of sodium selenite and sodium selenate, each had much lower potency than the inorganic salts. The ip LD_{50} of dimethyl selenide in mice and rats was 1,300 and 1,600 mg selenium/kg, respectively (McConnell and Portman, 1952). Convulsions were observed in treated mice, but not in rats. Olson (1986) reported that dimethyl selenide had a LD_{50} of 1,600 mg/kg and trimethylselenonium had a LD_{50} of 49 mg/kg in rats. Selenite, in contrast, has an LD_{50} of about 5 mg/kg.

The acute toxic effects of sodium selenite have been described for cattle (Shortridge *et al.*, 1971), calves (MacDonald *et al.*, 1981), pigs (Mahan and Moxon, 1984), lambs (Gabbedy and Dickson, 1969), horses (Miller and Williams, 1940), dogs (Heinrich and MacCanon, 1957), and laboratory rodents (O'Toole and Raisbeck, 1998). Muth and Binns (1964) reviewed earlier studies of sodium selenite and sodium selenate toxicity in domestic animals. Wichtel (1998b) discussed selenium toxicity in grazing ruminants.

Eye effects

Selenite can induce cataracts in young rats, rabbits, and guinea pigs with a narrow time window of susceptibility and a variety of phenomena that are not explained by unspecific selenium toxicity (Flohe, 2005). Selenium has been reported to cause an increase in calcium and phosphate in the lens; binding of selenium to lens proteins; impairment of

protein tyrosine phosphorylation and phosphatidylinositol-3-kinase activity; decreased total reducing capacity of the lens as indicated by a decrease of glutathione, NADPH, and total protein sulfhydryls; and an increase of oxidative damage markers such as malondialdehyde. These effects are observed at doses that greatly surpass those required for optimum production of selenoproteins, at several mg/kg instead of 1 µg/kg (Brune, 1999; Combs and Combs, 1986).

Bilateral nuclear cataract was produced within four to six days of a single subcutaneous (sc) injection of 19 to 30 µmole of sodium selenite/kg into suckling Sprague-Dawley or Wistar rats of 10 to 14 days of age(Shearer *et al.*, 1997). The critical maturation period of the lens is completed at approximately 16 days of age. Injections of 0.15 µmole per pup per day on days five to nine postpartum also produced cataract in young rats (Shearer *et al.*, 1983). A cataractogenic effect after sc injection of a single dose close to the LD_{50} was observed in male rats up to age 15 days, but the effect was not observed in adults (Ostadalova *et al.*, 1978, 1979, 1982, 1988; Ostadalova and Babicky, 1980, 1983, 1984).

Selenite cataract cannot be attributed exclusively to glutathione depletion and does not appear to be caused by sulfhydryl oxidation, suggesting that the cataract is not caused by disulfide linked aggregates (David and Shearer, 1984). A dose of 20 µmole/kg selenite, a strong sulfhydryl oxidant, caused a 44 percent decrease in lens glutathione by six days postinjection in suckling rats. There was no concurrent increase in either oxidized or protein-bound glutathione as indicated by measurements of five kinds of lenticular sulfur including reduced glutathione, oxidized glutathione or glutathione disulfide, protein-bound glutathione, reduced protein sulfhydryl, and oxidized protein sulfhydryl. Likewise, there was no evidence for increased oxidation of reduced protein sulfhydryl to oxidized protein sulfhydryl. Lens glutathione dropped more than 96 percent by four days postinjection of the glutathione synthesis inhibitor buthionine sulfoximine; however, no cataracts formed in the injected normal rats.

Shearer *et al.* (1997) reviewed the model of nuclear cataract in young rats produced by a single sc selenite injection and reported that selenium blocked lens epithelial cells of the germinative zone in S and/or G_2 phase and interfered with fiber cell formation by disrupting the meridional rows of epithelial cells. Alteration of biochemical processes such as epithelial metabolism, calcium accumulation, calpain protease induced proteolysis, crystallin precipitation, phase transition of protein aggregation onto the membrane, and cytoskeletal protein loss occurred during production of selenite cataract. Suppression of mitosis and nuclear fragmentation karyorrhexis at five hours after selenite injection were observed before the onset of cataract. DNA replication was reduced by 30 percent at six to 12 hours after treatment, followed by increased DNA synthesis and repair above control values (Shearer *et al.*, 1997). Vitamin E (Mathew *et al.*, 2003; Sneha *et al.*, 2003), iodide (Muranov *et al.*, 2004), ginkgo biloba (Thiagarajan *et al.*, 2002), and green tea (Gupta *et al.*, 2002) have been reported to attenuate the selenite cataract in rats, possibly through protection against selenium-induced oxidative stress.

Chandrasekher and Sailaja (2004) reported that a single sc injection of sodium selenite at 30 µmole/kg in 10-day-old Sprague-Dawley rat pups induced cataract. Protein tyrosine phosphorylation, an important event in the cell signal transduction process, in the lens was disrupted within two to three days, before the onset of cataract. Opacification of the lens started four to five days after treatment. With the progression of cataract, the

decrease in protein tyrosine phosphorylation correlated with the decrease in tyrosine kinase activity associated with the lens membrane fraction. Signaling events involving the protein tyrosine phosphorylation process and activation of phosphatidylinositol-3 kinase were altered during selenite cataract formation, which implicated defects in signal transduction mechanisms as contributing factors in the development of cataract. Differential gene expression, expression changes in mRNAs, release of cytochrome c, impaired mitochondrial function, and induction of apoptosis may also play roles (Belusko *et al.*, 2003; Nakajima *et al.*, 2002).

Babicky *et al.* (1985) administered selenium sc to male Wistar rats aged 5, 10, 15, 20, 30, 40, 50, or 60 days. Doses were 20 µmole/kg as sodium selenate, equivalent to 3.8 mg/kg-day; 20 or 40 µmole/kg as sodium selenite, equivalent to 3.5 or 6.9 mg/kg-day; or 40 µmole/kg as D,L-selenomethionine, equivalent to 7.8 mg/kg-day. Cataracts were produced only in the youngest rat groups. All substances caused deaths at 100 µmole/kg. D,L-Selenocystine was also cataractogenic at 10 µmole/kg, but not dimethyl selenide or trimethylselenonium ion. Within 24 hour of a single injection of selenite, glutathione concentration decreased to 40 percent of that in age-matched controls, but returned to 80 percent at the time nuclear opacity was observed. The effects of selenocompounds on the lens were attributed to disruption of glutathione metabolism as well as age-dependent differences in the structure and function of membranes or system barriers.

Maternal selenium is considered a cataractogen in suckling rodents (Bhuyan *et al.*, 1981). In chick embryo studies, eye malformations have been reported when sodium selenate was injected into the air sac prior to incubation (Franke *et al.*, 1936).

Liver effects

Acute liver congestion was observed in 93 suckling piglets suffering fatally from acute selenium poisoning after accidental oral intake of iron supplement contaminated with sodium selenite in Norway. A pooled liver sample from three piglets showed 26.5 µg selenium/g liver wet weight, compared to hepatic concentrations above 3 µg selenium/g liver as diagnostic for selenium toxicosis (Sivertsen *et al.*, 2003). Sheep ingesting lethal levels of sodium selenate at 5 mg/kg-day (Hopper *et al.*, 1985) or sodium selenite at 5 mg/kg-day (Smyth *et al.*, 1990) showed liver congestion, edema, and hemorrhage.

Heart effects

Negative acute effects on the cardiovascular system of selenite ingestion have been observed in experimental animals. A single 5 mg/kg dose of sodium selenite to lambs produced acute multifocal myocardial necrosis accompanied by pulmonary edema and hemorrhage, suggestive of left ventricular insufficiency (Smyth *et al.*, 1990). Ayaz *et al.* (2007) showed acute effects of 10^{-6} M to 10^{-3} M sodium selenite in perfused rat hearts. Contractility parameters were more affected in female hearts, whereas excitation was more affected in the males.

Subchronic toxicity

Table 9, adapted mainly from ATSDR (1996, 2003), summarizes lethal oral doses in subchronic animal studies of water-soluble selenium compounds, and two one-year exposure studies. Toxic effects appeared to be cumulative, in that lethality could occur after many days of treatment, and at much lower doses than in the acute studies. For example, sodium selenite given in drinking water at 0.84 mg selenium/kg-day for four to six weeks resulted in the death of four of six male Sprague-Dawley rats (Palmer and Olson, 1974). Sodium selenate given in the same manner and the same dose caused death of two of six rats.

Table 9. Summary of Lethal Doses in Subchronic or Longer Animal Studies

Species	Exposure parameters	Chemical species	Lethality Se (mg/kg-day)	Reference
rat	4 - 6 weeks, drinking water	sodium selenite	0.84, 4/6 died, male	Palmer and Olson, 1974
rat	6 weeks, feed	sodium selenite	0.48, 1/8 died, male	Halverson *et al.*, 1966
rat	13 weeks, drinking water	sodium selenite	1.67, 2/10 died, female	Abdo, 1994
rat	1 year, daily, drinking water	sodium selenite	0.28, 25/50 died at 58 days in males and at 160 days in females	Schroeder and Mitchener, 1971a
rat	14 days, drinking water	sodium selenate	1.1, 7/12 died, female	Wolfe and Kaiser, 1996
rat	4 - 6 weeks, drinking water	sodium selenate	0.84, 2/6 died, male	Palmer and Olson, 1974
rat	13 weeks, drinking water	sodium selenate	2.54, 20/20 died, male and female	Abdo, 1994
rat	1 year, daily, drinking water	potassium selenate	1.05, 1/3 died in males, 3/5 died in females at 1 year	Rosenfeld and Beath, 1954
rat	6 weeks, feed	organic selenium	0.4, 1/8 died, male	Halverson *et al.*, 1966
mouse	30 days, 6 days/week gavage	D,L-selenocystine	14.2, 15/15 died, male	Sayato *et al.*, 1993

Selenate and selenite showed similar toxicity in female rats, but males appeared to be more susceptible to selenite (Palmer and Olson, 1974; Schroeder and Mitchener, 1971a). Sodium selenite in drinking water at 0.28 mg/kg-day for one year did not kill female rats, whereas 50 percent of the males died after two months of treatment (Schroeder and Mitchener, 1971a).

Wistar rats survived 1.05 mg selenium/kg-day given in drinking water as potassium selenate for over eight months; however, three of five females and one of three males died by the end of one year (Rosenfeld and Beath, 1954). Sodium selenite given in drinking water at 0.28 mg selenium/kg-day resulted in the death of 25 of 50 male BLU:[LE] rats treated for 58 days, whereas 50 percent of the female BLU:[LE] rats died after receiving 0.28 mg/kg-day for 160 days (Schroeder and Mitchener, 1971a).

In a National Toxicology Program (NTP) study, all 10 male and 10 female Fischer 344/N (F344/N) rats died after consuming 2.54 mg selenium/kg-day of sodium selenate in drinking water for 13 weeks; two of 10 female rats died in the same study after dosing with sodium selenite at 1.67 mg/kg-day (Abdo, 1994). In a 30-day NTP study, seven of the 12 female Sprague-Dawley rats dosed with sodium selenate in drinking water at 1.1 mg/kg-day died after 14 days (Wolfe and Kaiser, 1996).

Jacobs and Forst (1981a) gave sodium selenite in the drinking water to male and female Sprague-Dawley rats for 35 days at 0, 1, 4, 8, 16, and 64 ppm selenium. Liver toxicity was observed at concentrations higher than 4 ppm. All rats survived in the 0, 1 and 4 ppm groups, survival decreased in the 8 and 16 ppm groups, and all rats died in the 64 ppm group. In a companion study in mice, sodium selenite was given in drinking water at 0, 1, 4, 8, 16, 32, and 64 ppm for 46 days to male and female Swiss mice (Jacobs and Forst, 1981b). Survival was significantly reduced in 18-week-old mice given 64 ppm selenium and to a lesser extent, 32 ppm. All seven-week-old and 18-week-old mice provided with 0 to 16 ppm selenium in the drinking water survived the 46-day treatment.

Mahan and Magee (1991) fed ninety pigs averaging 12.5 kg of body weight *ad libitum* on corn-soybean meal diets fortified with either sodium selenite or calcium selenite at 0.3, 5, or 15 ppm selenium for 35 days. Resulting doses were 0.014, 0.25, or 0.47 mg selenium/kg-day. This was a 2 × 3 factorial experiment conducted in three replicates of a randomized complete block design, to evaluate the effects of calcium selenite and sodium selenite at three different levels of selenium in the diets of growing swine on performance and tissue selenium concentrations. Growth and feed intake were similar in pigs fed 0.3 and 5 ppm of selenium from either selenium source. At the 15 ppm level body weight gain was 78 percent lower than controls, accompanied by decreased food intake. The pigs exhibited hoof cracking only at the two highest doses, 0.25 and 0.47 mg/kg-day. A NOAEL of 0.014 mg/kg-day was estimated based on the hoof effect. The results indicated that calcium selenite was as effective as sodium selenite using the criteria of growth, serum and tissue selenium concentrations, and GPx activities of growing swine, when fed at approved, marginally toxic, and toxic dietary selenium levels.

Several other studies in pigs were identified in the literature. These are summarized in Table 10, but are not described in detail here because the resulting NOAELs and LOAELs were higher.

Table 10, mainly adapted from ATSDR (1996, 2003), lists the NOAEL or LOAEL from subchronic oral animal studies with various selenium compounds.

Table 10. NOAEL and LOAEL for Subchronic Oral Exposures in Animals

Exposure duration and route	Chemical species	System endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Rat					
3 - 6 weeks, drinking water	sodium selenite	endocrine, body weight		0.64 female 0.64 female	Thorlacius-Ussing *et al.*, 1988
40 days, feed	sodium selenite	hematologic, endocrine, body weight	0.27 male 0.026 male 0.27 male	0.055 male	Eder *et al.*, 1995
2 months, feed	sodium selenite	hepatic, body weight	0.1 male 0.2 male	0.2 male	Bioulac-Sage *et al.*, 1992
8 weeks, feed	sodium selenite	respiratory, renal, cardiovascular, gastrointestinal, hepatic, body weight	0.45 male 0.45 male 0.45 male	0.45 male 0.25 male	Chen and Whanger, 1993; Chen *et al.*, 1993
10 weeks, drinking water	sodium selenite	immunological lymphoreticular		0.7 female	Koller and Exon, 1986; Koller *et al.*, 1986
3 months, once daily, feed	sodium selenite	hepatic		0.002 male	Kolodziejczyk *et al.*, 2000
110 days, feed	sodium selenite	endocrine, body weight	0.105 male	0.105 male	Behne *et al.*, 1992
12 - 14 weeks	sodium selenite	cardiovascular, hepatic, body weight		0.324 0.324 0.324	Turan *et al.*, 1999a
13 weeks, drinking water	sodium selenite	respiratory, cardiovascular, gastrointestinal, hematologic, musculoskeletal, hepatic, renal, endocrine, ocular body weight	1.67 female 1.67 female 1.67 female 0.86 female 1.67 female 1.67 female 0.28 female 1.67 female 0.98 male	1.67 female 0.5 female 1.59 male	Abdo, 1994
23 - 29 days, drinking water	sodium selenate	body weight	0.167 male 0.209 female	0.293 male 0.334 female	Wolfe and Kaiser, 1996
4 - 6 weeks, drinking water	sodium selenate	hepatic, body weight		0.84 male 0.42 male	Palmer and Olson, 1974
13 weeks, drinking water	sodium selenate	respiratory, cardiovascular, gastrointestinal, hematologic, musculoskeletal, hepatic,	1.57 male 1.57 male 1.57 male 0.92 male 1.57 male 0.92 male	1.57 male 1.57 male	Abdo, 1994

Exposure duration and route	Chemical species	System endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
		renal, endocrine,ocular, body weight	0.31 female 1.57 male 0.47 female	0.47 female 0.88 female	
6 weeks, feed	sodium selenate	body weight	0.125 male		Salbe and Levander, 1990
6 weeks, feed	sodium selenate	endocrine, body weight	 0.09 male	0.09 male	Hotz *et al.*, 1997
6 weeks, feed	L-seleno-methionine	body weight	0.125 male		Salbe and Levander, 1990
110 days, feed	L-seleno-methionine	endocrine, body weight		0.118 male 0.118 male	Behne *et al.*, 1992
6 weeks, feed	organic selenium	hematologic, hepatic, endocrine, body weight	0.24 male 0.32 male	0.32 male 0.44 male 0.44 male	Halverson *et al.*, 1966
Mouse					
14 days, drinking water	sodium selenite	hematologic, hepatic, renal, body weight immunological lymphoreticular	0.38 male 0.38 male 0.17 male 0.38 male 0.38 male	0.82 male 0.82 male 0.38 male 0.82 male 0.82 male	Johnson *et al.*, 2000
14 days, drinking water	sodium selenite	neurological	0.24 male	0.58 male	Tsunoda *et al.*, 2000
47 days, drinking water	sodium selenite	immunological lymphoreticular		0.173	Raisbeck *et al.*, 1998
12 weeks, feed	sodium selenite	hepatic, body weight	 0.2 male	0.2 male	Skowerski *et al.*, 1997b
13 weeks, drinking water	sodium selenite	respiratory, cardiovascular, gastrointestinal, hematologic, musculoskeletal, hepatic, renal, endocrine, ocular, body weight	3.83 female 3.83 female 3.83 female 3.83 female 3.83 female 3.83 female 0.91 male 3.83 female 1.61 male	 1.61 male 3.31 male	Abdo, 1994
12 weeks, feed	sodium selenate	hepatic, body weight	 0.2 male	0.2 male	Skowerski *et al.*, 1997a
13 weeks, drinking water	sodium selenate	respiratory, cardiovascular, gastrointestinal, hematologic,	7.17 female 7.17 female 7.17 female 7.17 female		Abdo, 1994

Exposure duration and route	Chemical species	System endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
		musculoskeletal, hepatic, renal, endocrine, ocular, body weight	7.17 female 7.17 female 1.07 male 7.17 female 1.87	1.87 male 2.95 male	
30 days, 6 days/week, gavage	D,L-seleno cystine	hepatic, renal, body weight	4.7 male 9.4 male	9.4 male 9.4 male	Sayato *et al.*, 1993
47 days, drinking water	D,L-seleno cystine	immunological lymphoreticular		0.173	Raisbeck *et al.*, 1998
90 days, gavage	D,L-seleno cystine	hepatic, body weight	2.4 male 2.4 male	4.7 male 4.7 male	Hasegawa *et al.*, 1994
14 days, drinking water	L-seleno-methionine	hematologic, hepatic, renal, body weight immunological lymphoreticular	1.36 male 1.36 male 1.36 male 1.36 male		Johnson *et al.*, 2000
47 days, drinking water	seleno-methionine	immunological lymphoreticular		0.173	Raisbeck *et al.*, 1998
14 days, drinking water	L-seleno-methionine	neurological	1.96 male		Tsunoda *et al.*, 2000
Rabbit					
3 months, feed	sodium selenite	cardiovascular, hematologic, body weight	0.4	0.4	Turan *et al.*, 1999b
Pig					
unspecified, feed	sodium selenite	dermal, body weight	0.4	0.4	Wahlstrom and Olson, 1959
10 days, capsule	sodium selenite	neurological		1.3	Wilson *et al.*, 1989
20 - 42 days, feed	sodium selenite	neurological	1	2.1	Wilson *et al.*, 1983
35 days, feed	sodium selenite	dermal, body weight	0.014 0.25	0.25 0.47	Mahan and Magee, 1991
8 weeks, feed	sodium selenite	hepatic, dermal, neurological	0.33 0.33	0.59 0.59	Mihailovic *et al.*, 1992a
8 weeks, feed	sodium selenate	hepatic, dermal, body weight		1.1 1.1	Baker *et al.*, 1989
16 ± 16 days	sodium selenate	respiratory, cardiovascular, hepatic, renal, dermal, body weight	1.25 1.25 1.25	1.25	Panter *et al.*, 1996
34 days,	unspecified	cardiovascular,		0.46	Stowe *et al.*, 1992

Exposure duration and route	Chemical species	System endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
feed	selenium	musculoskeletal		0.46	
31 ± 14 days	D,L-seleno-methionine	cardiovascular, hepatic, renal, dermal, body weight	1.25 1.25	 1.25	Panter *et al.*, 1996
7 weeks, feed	organic selenium	neurological		1.3	Baker *et al.*, 1989
Calf					
16 weeks, feed	sodium selenite	hepatic, dermal, hematologic, neurological		0.25 female	Kaur *et al.*, 2003
120 days, once a day, feed	sodium selenite	respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, ocular, dermal, neurological, immunological lymphoreticular	0.808 male 0.808 male 0.808 male 0.808 male 0.808 male 0.808 male 0.288 male 0.808 male 0.808 male	 0.808 male	O'Toole and Raisbeck, 1995
120 days, once a day, feed	seleno-methionine	respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, ocular, dermal, neurological, immunological lymphoreticular	0.808 male 0.808 male 0.808 male 0.808 male 0.808 male 0.808 male 0.158 male 0.808 male 0.808 male	 0.288 male	O'Toole and Raisbeck, 1995
Monkey					
30 days, once/day, nasogastric intubation, drinking water	L-seleno-methionine	dermal neurological	 0.08 female	0.01 female 0.12 female	Cukierski *et al.*, 1989

Growth retardation is a prominent symptom of alkali disease and to a lesser extent of blind staggers' disease (NRC, 1976). Body weight reduction, often accompanied by reduced food and water consumption, has been reported in rats, mice, rabbits, pigs, calves, and monkeys orally dosed with toxic levels of selenite, selenate, selenomethionine, or selenocystine, which may be related to adverse endocrine or neurological effects (see Table 10). Decreased body weight gain with a reduction in somatomedin C levels was exhibited in postweanling female Wistar rats exposed to

sodium selenite in drinking water for three to six weeks at 0.64 mg/kg-day (Thorlacius-Ussing *et al.*, 1988).

Thorlacius-Ussing *et al.* (1987) demonstrated that selenium retarded growth through a reduction in growth hormone secretion and circulating insulin-like growth factor-I levels. Less than three weeks after beginning ingestion of sodium selenite in drinking water at 10 mg/L, a significant growth retardation ($p < 0.0001$) was induced in 24 nursing rats (Thorlacius-Ussing, 1990). Sodium selenite in drinking water at 15 mg/L (5.0 mg selenium/L) induced growth retardation, accumulation of selenium in somatotrophs, lack of growth hormone response to growth hormone-releasing factor and an 80 percent reduction in serum somatomedin C in infant rats. In addition, it induced a slight reduction in serum albumin and occasionally slight central liver necrosis. Growth reduction was highly significant ($p < 0.0001$) after 14 days, and the difference increased until it became constant after 60 days (Thorlacius-Ussing *et al.*, 1988; Thorlacius-Ussing, 1990).

Ten male Wistar rats treated with sodium selenite in the drinking water at 10 mg/L (3.3 mg selenium/L) had significantly lower body weights compared with ten *ad libitum*-fed rats on day nine and ten pair-fed rats on day 14 ($p < 0.05$) (Gronbaek *et al.*, 1995). Selenium-treated rats had significantly shorter tibia lengths (3.46 ± 0.03 cm) compared with *ad libitum*-fed rats ($p < 0.001$) and pair-fed rats ($p < 0.05$) by the end of the study on day 35, whereas there was no difference between *ad libitum*-fed rats and pair-fed rats (3.77 ± 0.04 cm versus 3.60 ± 0.02 cm). Selenium treatment induced a significant reduction in circulating insulin-like growth factor-I on day 35 compared with *ad libitum*-fed and pair-fed rats ($p < 0.05$). Serum insulin-like growth factor-I and insulin-like growth factor-binding proteins (IGFBP) subjected to Western blots on days 0, 14 and 35 showed four distinct bands with apparent relative molecular weights of 38 to 47 kDa (doublet) (IGFBP-3), 30 kDa (IGFBP-1 and/or IGFBP-2), and 24 kDa (IGFBP-4).

Organic and inorganic selenium accumulated in somatotrophs of the rat anterior pituitary after administration of sodium selenite either by drinking water at 2.5 to 15 mg/L or by intraperitoneal injection at 5 to 20 mg/kg (Thorlacius-Ussing and Danscher, 1985; Thorlacius-Ussing and Jensen, 1988). Similar accumulation occured after the administration of L-selenomethionine by drinking water at 3.0 mg selenium/L for one, two or four weeks or by a single intraperitoneal injection at 3.7 mg selenium/kg (Gronbaek and Thorlacius-Ussing, 1990). During the first 48 hours after a single injection of sodium selenite or L-selenomethionine, a substantial increase in anterior pituitary staining was observed and precipitates could still be observed after two weeks. When rats were given selenium in drinking water for four weeks, an increasing amount of precipitate was observed during the first two weeks followed by a small decrease in staining intensity.

Secretion of growth hormone and somatomedin C in response to growth hormone-releasing factor in nursing male rat pups was reduced by exposure to 15 mg sodium selenite/L drinking (Thorlacius-Ussing *et al.*, 1987). Sodium selenite in drinking water at 2.5, 7.5, or 15 mg/L for 14 days caused selenium accumulation in the anterior pituitary (Thorlacius-Ussing, 1990). Selenium treatment also decreased growth rate of hypophysectomized rats treated with a minimal dose of growth hormone.

Both low and high selenium intakes altered thyroid hormone metabolism in rats. Male weanling Sprague-Dawley rats fed sodium selenite in food at 0.055 or 0.27 mg selenium/kg-day for 40 days showed approximately 50 percent decrease in serum levels of T_3 and a nonsignificant reduction of type I 5´-iodothyronine deiodinase activity compared to rats fed 0.009 or 0.026 mg selenium/kg-day (Eder *et al.*, 1995). Weanling male Sprague-Dawley rats fed sodium selenate in food at 0.09 mg selenium/kg-day for six weeks showed approximately 30 percent increase in thyroid stimulating hormones and a nonsignificant reduction of kidney type I 5´-iodothyronine deiodinase level compared to rats fed 0.009 mg selenium/kg-day (Hotz *et al.*, 1997). Male Wistar rats fed 0.105 mg/kg-day as sodium selenite or 0.118 mg selenium/kg-day as L-selenomethionine for three months showed a significant reduction in liver type I 5´-iodothyronine deiodinase activity compared with control rats fed 0.0015 mg selenium/kg-day as sodium selenite. There was also a 29 percent reduction in production rate of T_3 from T_4 and a 45 percent reduction in production rate of 3,3´-diiodothyronine from T_4 (Behne *et al.*, 1992).

In a study performed for NTP (Wolfe and Kaiser, 1996), Sprague-Dawley rats ingested sodium selenate in the drinking water. Males had an 11 percent reduction in body weight at 0.293 mg/kg-day for 29 days and a 20 percent reduction at 0.418 mg/kg-day for 25 days in males. Females had a 39 percent reduction in body weight at 0.418 mg/kg-day for 23 days. No body weight effects were observed over a 30-day period at 0.167 mg/kg-day in males or at 0.209 mg/kg-day in females. Body weights and feed and water consumption decreased with increasing dose compared with controls; final body weights at necropsy for the 0.8- and 1.1-mg/kg-day males were 12 percent and 20 percent lower than controls, respectively. Water consumption decreased in all treated groups and was dramatically reduced in the 1.1-mg/kg-day group, approximately 70 to 80 percent compared to the controls.

In the NTP 13-week study in F344/N rats, a NOAEL of 0.31 mg selenium/kg-day and a LOAEL of 0.47 mg selenium/kg-day were identified based on minimal renal papilla degeneration in the selenate-treated females (Abdo, 1994). A NOAEL of 0.28 mg selenium/kg-day and a LOAEL of 0.5 mg selenium/kg-day were identified based on mild renal papilla degeneration in the selenite-treated females.

Jacobs and Forst (1981a) treated male and female Sprague-Dawley rats for 35 days with 0, 1, 4, 8, 16, or 64 ppm sodium selenite in the drinking water. Effects on survival are discussed above. Liver toxicity was observed at concentrations higher than 4 ppm, with liver congestion, fatty degeneration of parenchymal cells, and necrosis. Body weight gain was normal in the 0, 1 and 4 ppm groups but substantially decreased in the 16 and 64 ppm groups. Serum alkaline phosphatase and glutamic-oxaloacetic transaminase (SGOT) increased with 16 and 64 ppm selenium.

Jacobs and Forst (1981b) administered sodium selenite in drinking water at 0, 1, 4, 8, 16, 32, and 64 ppm for 46 days to male and female Swiss mice, starting at 7 or 18 weeks of age. Effects on survival are discussed above. No effects on growth were observed up to 8 ppm. Selenium toxicity in the 18-week-old mice was less in females than in males. Only 64 ppm selenium caused a sharp reduction in body weight in young and adult mice of both sexes. Occasional liver and kidney congestion, liver necrosis, parenchymal cell degeneration, and bile duct proliferation observed were similar in control and treatment groups. Serum enzymes and pathology were normal in mice of both ages and sexes given

1, 4, and 8 ppm selenium. Serum alkaline phosphatase and glutamic-oxaloacetic transaminase increased with 32 and 64 ppm selenium.

Johnson *et al.* (2000) gave five adult male BALB/c mice per group drinking water containing sodium selenite at 0, 1, 3, or 9 ppm selenium, equivalent to 0.024, 0.17, 0.38, or 0.82 mg selenium/kg-day, for 14 days. At the highest dose there was about a 21 percent decrease in food consumption, a 43 percent decrease in water consumption, and 62 percent decrease in relative spleen weight. Water consumption was also reduced at 3 ppm. A marked decrease in body weight gain and relative organ weights was observed at 9 ppm and the thymus to body weight ratio was significantly reduced at 3 or 9 ppm. A 260 percent increase in the basal rate of proliferation in splenocytes resulted with exposure to 9 ppm. The production of proinflammatory cytokines in lipopolysaccharide-stimulated splenic macrophages was increased. Adult male BALB/c mice exposed to 1.36 mg selenium/kg-day in the form of L-selenomethionine in drinking water did not have the same effects as sodium selenite (Johnson *et al.*, 2000). Other studies by the same group indicated that 14-day oral administration of 3 or 9 ppm sodium selenite but not L-selenomethionine in drinking water altered dopamine turnover in the striatum of mice (Tsunoda *et al.*, 2000).

At an oral dose of 6 mg/kg-day selenite for consecutive 12 days, growth of mice was completely suppressed. At oral doses of 2 or 4 mg/kg-day for consecutive 15 days, selenite suppressed growth, depleted reduced glutathione, upregulated GPx, induced glutathione S-transferase, and inhibited liver catalase and superoxide dismutase activities. Alanine aminotransferase and aspartate aminotransferase increased in serum, indications of liver damage. Selenite increased the malondialdehyde content of liver. The results indicate that over a short-term, a high-dose of selenite caused pronounced oxidative stress and liver injury (Zhang *et al.*, 2005). Mice fed with sodium selenite at 0.05 mg/kg for 30 days showed significantly increased blood and liver selenium concentration and activity of blood GPx (Gao *et al.*, 2000). In selenium-deficient rats, selenite increased tissue selenium and GPx activity (Zhang *et al.*, 2001b) as in the mice, and stimulated the synthesis of GPx4 and TR.

Hawkes *et al.* (1992) administered L-selenomethionine at 0, 0.025, 0.15, or 0.3 mg selenium/kg-day for 30 days to 40 pregnant longtailed macaque monkeys. Erythrocyte and plasma selenium and GPx specific activities, hair and fecal selenium, and urinary selenium excretion increased with dose. Hair selenium was most sensitive to dose, with an 84-fold increase in the 0.3 mg/kg-day group relative to controls ($r = 0.917$). At 0.3 mg/kg-day, GPx1 and GPx3 specific activities increased 154 percent and 69 percent over controls, respectively. In a study by Cukierski *et al.* (1989), skin lesions appeared on the forearm of one of two female longtailed macaques administered 0.01 mg selenium/kg-day as L-selenomethionine in drinking water through nasogastric intubation once a day for 30 days. The limited number of animals precludes the identification of the dose as a LOAEL for dermal effects.

Tooth effects

After Hadjimarkos (1965) reported that urinary levels of selenium might be related to increased risks of dental caries in children in Oregon and Wyoming, Shearer and

Hadjimarkos (1973) and Shearer (1975) conducted exploratory studies in developing rats. They were interested in whether selenotrisulfides or selenoamino acids incorporated into developing proteins might be responsible for the increases in dental caries reported in experimental animals ingesting increased amounts of selenium. Initial studies were on the uptake of dietary inorganic and organic selenium by the molar teeth of mother rats and the developing molar teeth of their pups. Pregnant rats received drinking solutions containing 0.2 ppm sodium selenite plus 0.043 µCi [^{75}Se]sodium selenite/mL from day 10 of pregnancy until 13 days postpartum. The uptake of dietary [^{75}Se] into developing molar teeth was more extensive than the postdevelopmental uptake by mature teeth. Developing molar teeth incorporated more [^{75}Se] from dietary selenomethionine than from selenite, as did many hard and soft tissues studied. The major portion of [^{75}Se] in enamel and dentine was located in the protein fraction of these dental tissues. Dialysis experiments showed that the selenium in enamel and dentine existed in at least three forms: loosely bound selenium, proteinaceous selenotrisulfides, and proteinaceous stable selenium (Shearer and Hadjimarkos, 1973; Shearer, 1975). Selenium was taken up into the continuously-growing rat incisor in proportion to the amount of selenium in the diet (Johnson and Shearer, 1979).

Shearer and Ridlington (1976) found no evidence that 50 ppm sodium fluoride in drinking solutions interacted with any of four selenium compounds given at 1 or 3 ppm (sodium selenite, sodium selenate, D,L-selenomethionine, or D,L-selenocystine), in the hard and soft tissues of rats in terms of symptoms of selenium toxicity, soft tissue uptake of fluoride and selenium, histology of liver and kidney tissues, fluoride uptake into growing femur bones, and fluoride uptake onto calcified molar enamel.

In another study by the same group, moderate levels of dietary selenium at 0.8 ppm as sodium selenite or selenomethionine were fed to 10-day pregnant rats until the pups were weaned. This dose of selenium, present during tooth development, significantly reduced buccal caries in male pups fed a MIT--200 diet for seven weeks, compared to control rats or to rats receiving a higher level, 2.4 ppm of selenium (Britton *et al.*, 1980).

Meissner (1976) carried out two studies with Osborne-Mendel rats to evaluate whether high levels of selenium in drinking water had effects on caries or on the caries-protective effect of fluoride. Sodium selenate was either added to drinking water or mixed with a toothpaste containing sodium fluoride. In the first experiment, three groups of 20 rats each received sodium selenate at 1, 5 and 10 ppm in drinking water and the cariogenic diet Stephan 580; two other groups served as control, including one receiving the cariogenic diet and the other one getting the normal breeding diet. Nine of the 40 animals of the groups that had received the selenium doses of 5 and 10 ppm died. In the second experiment, all five groups of rats received the cariogenic diet Stephan 580; two of them got 4 ppm sodium selenate in drinking water. The molars of the lower jaws of one of these groups were brushed with a toothpaste containing sodium fluoride. The molars of the other group were treated with a paste, serving as a placebo. Two other groups were provided each with the paste containing fluoride and the paste serving as a placebo, but without selenium in their drinking water. The last group of 20 rats was used as a control group. Comparison of caries among the groups in both studies revealed no effect of sodium selenate on the protective effect of the fluoride.

Liver effects

Effects on liver size, liver appearance, and serum bilirubin were reported in early short-term studies with post-weanling rats fed seliniferous grain that provided dietary selenium concentrations of 13 to 100 ppm (Franke, 1934a; Franke and Potter, 1935; Munsell *et al.*, 1936; Smith *et al.*, 1937). A subchronic study by Harr *et al.* (1967) described three lesion patterns in the liver: acute toxic hepatitis with ascites and pleural effusion; chronic toxic hepatopathy with fibroplasia and bile duct hyperplasia; and nodular hyperplasia, with sodium selenite or sodium selenate at 0.25 mg selenium/kg-day or higher.

The toxic effects of seleniferous wheat on rat liver were confirmed by Halverson *et al.* (1966) who fed organic selenium as seleniferous wheat or sodium selenite to male post-weanling Sprague-Dawley rats for six weeks at 1.6, 3.2, 4.8, 6.4, 8, 9.6 or 11.2 ppm of selenium (equivalent to 0.13, 0.24, 0.32, 0.44, 0.52, 0.67, or 0.81 mg/kg-day, respectively). Reduced liver-to-body-weight ratios and increased bilirubin were reported at 6.4 ppm selenium as seleniferous wheat. At 8 ppm selenium, there was a decrease in liver weight, increase in spleen weight, and a decrease in hemoglobin. Mortality was observed in the groups fed 8, 9.6 and 11.2 ppm selenium as seleniferous wheat at incidences of 1/8, 5/8 and 8/8, respectively. A LOAEL of 0.44 mg selenium/kg-day was identified based on liver effects, reflected by a six-fold increase of serum bilirubin.

Liver nodular regenerative hyperplasia and increased relative liver weight were observed by Palmer and Olson (1974) in weaned male Sprague-Dawley rats fed a 4 ppm selenium diet as selenite after two months, compared with a control group fed a 0.4 ppm selenium diet. No significantly decreased body weights and normal liver function were observed, but portal pressure was 1.8 times higher ($p < 0.05$). Atrophic micronodular lobes and hypertrophic lobes were confirmed by light microscopy and electron microscopy. Bioulac-Sage *et al.* (1992) reported a NOAEL of 0.1 mg selenium/kg-day and a LOAEL of 0.2 mg selenium/kg-day in Sprague-Dawley rats based on liver effects. Two of six Sprague-Dawley rats administered 0.84 mg selenium/kg-day as sodium selenate in drinking water for four to six weeks died, and all six rats had liver cirrhosis.

Diffuse panlobular vacuolar changes were reported in weanling male Sprague-Dawley rats fed sodium selenite in the diet for eight weeks at 0.45 mg/kg-day (Chen *et al.*, 1993; Chen and Whanger, 1993, 1994). In male ICR mice orally administered 4.7, 9.5, 14.1, or 18.9 mg selenium/kg as D,L-selenocystine for 30 days at six days/week, body weight gain significantly decreased with the increase of dosage, and all mice died within the exposure period at the two high doses (Sayato *et al.*, 1993). Liver damage was reported as an increase in vacuolation of centrilobular and peripheral hepatocytes. A NOAEL of 4.7 mg selenium/kg-day and a LOAEL of 9.4 mg selenium/kg-day were identified, based on significant two- to three-fold increases in aspartate aminotransferase and alanine aminotransferase in plasma. Liver damage in the form of yellow atrophy was reported in rats orally administered 2.1 mg selenium/kg-day as sodium selenate for 30 days.

Wistar rats fed 0.324 mg selenium/kg-day as sodium selenite in food for 12 to 14 weeks showed degenerative changes in the liver (Turan *et al.*, 1999a).

In contrast to these studies, F344/N rats given sodium selenate in drinking water for 13 weeks in a NTP study had an increased level of serum bile acids, suggesting cholestasis,

at 1.57 mg selenium/kg-day, but no hepatic effects were noted at 0.92 mg/kg-day. In companion NTP 13-week drinking water studies, hepatic effects were not observed in $B6C3F_1$ mice treated with sodium selenate at doses up to 7.17 mg selenium/kg-day, sodium selenite at doses up to 3.83 mg selenium/kg-day, or in F344/N rats treated with sodium selenite at doses up to 1.67 mg selenium/kg-day (Abdo, 1994).

Ultrastructural examination of adult male Balby mice fed 0.2 mg selenium/kg-day as sodium selenite or sodium selenate in food for 12 weeks revealed hepatocytes with extremely large and irregularly shaped vacuoles in the cytoplasm, numerous damaged mitochondria, a large number of lipid droplets, and numerous lysosomes (Skowerski *et al.*, 1997a,b).

Kolodziejczyk *et al.* (2000) fed male Wistar rats 0.002 or 0.005 mg selenium/kg-day as sodium selenite in food for three months. Increased activity was noted in the enzymes of aerobic and anaerobic metabolic pathways as well as liver damage that increased with dose. A distinct swelling of Kupffer cells in dilated sinusoidal vessels mainly in the proximity of portal fields, and occasional necrotic areas comprising single groups of hepatocytes were observed in rat livers at the 0.002 mg/kg-day dose. Infiltrations of mononuclear cells into portal canals, sporadic areas of necrosis within individual lobules, and weak activation of Kupffer cells were observed in rats dosed at 0.005 mg/kg-day. No evaluation of statistical significance was provided in the published report.

Wycherly *et al.* (2004) studied whether high dietary intake of inorganic selenium may promote *in vivo* DNA oxidation as shown by increased concentration of 8-hydroxy-2'-deoxyguanosine in liver DNA. Weanling male Sprague-Dawley rats were fed a Torula yeast-based, selenium-deficient diet supplemented with 0, 0.15, or 2.0 ppm selenium as sodium selenite for 10 weeks, then were injected with 0, 0.1, or 10 mg/kg of the prooxidant carcinogen N-nitrosodiethylamine. Selenium-dependent GPx1 gene expression and enzyme activity were dramatically reduced by dietary selenium deficiency but were unaffected by carcinogen administration. There were no significant main or interactive effects of selenium or carcinogen on activity or gene expression of the DNA repair enzyme 8-oxoguanine glycosylase I (Wycherly *et al.*, 2004).

Hepatic lesions have also been observed in birds. For example, mallards fed more than 80 mg selenium/kg as selenomethionine may die within two weeks of exposure with massive panacinar acute multifocal hepatocellular necrosis. Congestion of the liver has also been noted in herbivores given lethal doses of sodium selenite or sodium selenate, but there is no convincing evidence for hepatic necrosis or cirrhotic lesions (ATSDR, 1996).

Heart effects

Cardiac damage was observed in weanling Wistar rats fed 0.324 mg selenium/kg-day as sodium selenite in food for 12 to 14 weeks. Severe diffuse degenerative changes including edema in the subendocardial connective tissue and interfibers of prevascular regions, and myofibril swelling with profuse intercellular edema were reported. Irregular myocyte borders, loss of striations, and degeneration of the sarcolemma and myofibril structure and order were observed (Turan *et al.*, 1999a). New Zealand white rabbits fed 0.317 mg selenium/kg-day as sodium selenite in food for three months showed distinct,

degenerative changes in heart indicating disintegration of the internal structure of the myocytes, compared to the hearts of control rabbits fed 0.007 mg selenium/kg-day (Turan *et al.*, 1999b).

Pigs ingesting feed containing excess sodium selenite, as high as 54.6 mg selenium/kg of feed, for up to eight weeks exhibited consistent focal degeneration and necrosis of the myocardium and skeletal muscles (Penrith, 1995). Myocardial lesions such as widespread hypertrophy, atrophy, and disorganization of fibers, occasional fibrosis, and marked meddial hypertrophy of the arterioles were reported in heart tissues from pigs that died after consuming the feed for one to seven days (Penrith and Robinson, 1996). Myocardial lesions including vacuolation and pyknosis of nuclei were noted in pigs fed an unspecified form of selenium at 0.46 mg selenium/kg-day for 34 days (Stowe *et al.*, 1992), but were less prominent than reported by Penrith and Robinson (1996). Harr *et al.* (1967) reported myocardial hyperemia, hemorrhage, and degeneration as well as pericardial edema in young rats given sodium selenite or sodium selenate in the feed at 0.5 mg selenium/kg-day. The exposure duration required to produce these effects was not specified.

Blood effects

The hematocrit was decreased in rats fed seleniferous wheat doses of 0.32 to 0.56 mg selenium/kg-day for six weeks (Halverson *et al.*, 1966). Hemoglobin levels were decreased in male Sprague-Dawley rats given sodium selenite at 5 to 15 ppm in the diet (Halverson *et al.*, 1970). The decrease was attributed to a hemolytic effect of selenite. Selenite is taken up into red blood cells through the anion exchanger on its plasma membrane in rats and then may be reduced to selenide by glutathione (Hsieh and Ganther, 1975). Spallholz (1994) has shown that sodium selenite, in the presence of a thiol donor such as glutathione, will cause hemolysis *in vitro*. The effects were attributed to selenotrisulfide intermediates and to the generation of superoxide radicals. Selenotrisulfide binds to human hemoglobin *in vitro* through its thiol groups with high affinity after formation of glutathione selenotrisulfide in red blood cells from selenite and glutathione (Haratake *et al.*, 2005). In contrast, this hemolytic effect was not seen with sodium selenate or with selenomethionine. Sodium selenite at 1.67 mg selenium/kg-day or sodium selenate at 1.57 mg selenium/kg-day given in drinking water for 13 weeks to rats increased the hematocrit. This effect was attributed to decreased water intake (Abdo, 1994).

Immune system effects

Immunotoxic effects of sodium selenite or sodium selenate have been observed in some experimental animals (Spallholz *et al.*, 1990). Decreased delayed-type hypersensitivity, increased thymus weight, reduced humoral IgG antibody production in response to an administered antigen, and reduced prostaglandin synthesis were observed in female Sprague-Dawley rats treated orally with sodium selenite in drinking water at 0.7 mg/kg-day for 10 weeks. At lower doses of 0.07 or 0.28 mg/kg-day, natural killer cell cytotoxicity was significantly increased (Koller and Exon, 1986; Koller *et al.*, 1986). Raisbeck *et al.* (1998) found that sodium selenite, selenocystine, or selenomethionine

administered to BALB/c mice at 7 ppm in drinking water for 47 days decreased the antibody response to hen egg albumin. BALB/c mice, five adult males per group, ingested drinking water containing 0, 1, 3, or 9 ppm selenium as sodium selenite (0.024, 0.17, 0.38, or 0.82 mg selenium/kg-day) for 14 days showed about 62 percent decreased relative spleen weight with a decrease of 21 percent in food consumption and 43 percent in water consumption (Johnson *et al.*, 2000).

Glenn *et al.* (1964a,b,c) found that two-year-old lambs or pregnant ewes fed sodium selenate in gelatin capsules with a time-weighted average dose of 0.65 or 0.9 mg selenium kg/day over a 171-day period showed atrophy of lymphoid centers in the spleen, in regional lymph nodes, and in Peyer's patches in the intestinal wall. Reduced leukocyte function, decreased forced antibody response, and diminished mitogenic response to concanavalin A and pokeweed mitogen were observed in pregnant cows fed diets containing 0.135 mg selenium/kg-day for three months compared to controls fed 0.005 mg selenium/kg-day (Yaeger *et al.*, 1998).

Chronic toxicity

Table 11, mainly adapted from ATSDR (1996, 2003), lists the NOAEL or LOAEL from animal studies with chronic oral exposure to sodium selenate, sodium selenite, or organic selenium compounds. The highest NOAEL value for various toxic effects and all reliable LOAEL values are listed.

In a National Cancer Institute chronic oral study to resolve the issue of selenium carcinogenicity, diets containing sodium selenite or sodium selenate at 0.5 to 16 ppm were fed to 1,437 Wistar rats for their lifetime (Harr *et al.*, 1967; Tinsley *et al.*, 1967). Soft bones were observed at 0.2 mg/kg-day but not at 0.1 mg/kg-day; liver hyperplastic lesions and nephritis were observed at 0.1 mg/kg-day but not at 0.025 mg/kg-day. Lifespan was reduced to 60 to 100 days in the rats fed selenate or selenite at 0.5 mg selenium/kg-day versus controls at about 500 days.

Nelson and Fitzhugh (1943) conducted chronic toxicity studies of selenium in rats. Female Osborne-Mendel rats fed 0.25 or 0.5 mg selenium/kg-day as seleniferous corn or wheat for two years showed no toxic effect on respiratory, gastrointestinal, musculoskeletal, and endocrine systems, as well as no dermal effects. However, cirrhosis of the liver was reported at 0.25 mg/kg-day and higher. Reduced body weight gain in young rats and weight loss in older rats was documented at 0.5 mg selenium/kg-day. Hepatic lesions and ascites were also reported in rats, rabbits, and cats fed sodium selenite and selenate (Munsell *et al.*, 1936; O'Toole and Raisbeck, 1998).

Schroeder and Mitchener (1972) exposed Swiss mice for their lifetime to sodium selenate or sodium selenite in drinking water at 0.57 mg selenium/kg-day. They observed amyloidosis of the lungs, heart, liver, kidney, adrenal gland, and pancreas in 58 percent of selenium treated mice versus 30 percent of control mice ($p < 0.001$), but data for individual organs were not provided. Poor quality of the hair coat was also reported in the treated mice. No effect on body weight was observed.

Table 11. NOAEL or LOAEL of Chronic Oral Exposure in Animals

Exposure duration and route	Chemical species	System endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Rat					
24 months, feed	organic selenium	respiratory, gastrointestinal, musculoskeletal, hepatic, endocrine, dermal body weight	0.5 female 0.5 female 0.5 female 0.5 female	0.25 female 0.5 female	Nelson and Fitzhugh, 1943
lifetime, feed	sodium selenite	musculoskeletal, hepatic, renal, lifespan	0.1 0.025	0.2 0.1 0.5	Harr *et al.*, 1967; Tinsley *et al.*, 1967
lifetime, feed	sodium selenate	musculoskeletal, hepatic, renal, lifespan	0.1 0.025	0.2 0.1 0.5	Harr *et al.*, 1967; Tinsley *et al.*, 1967
Mouse					
lifetime, drinking water	sodium selenite	respiratory, cardiovascular, hepatic, renal, endocrine, dermal, body weight	0.57	0.57 0.57 0.57 0.57	Schroeder and Mitchener, 1972
lifetime, drinking water	sodium selenate	respiratory, cardiovascular, hepatic, renal, endocrine, dermal, body weight	0.57	0.57 0.57 0.57 0.57	Schroeder and Mitchener, 1972

Jacobs and Forst (1981a) gave sodium selenite in drinking water at 0, 1, 4, 8, 16, and 64 ppm selenium to male and female Sprague-Dawley rats for one or two years. Toxic effects were observed at above 4 ppm. Microscopic evaluation showed liver congestion, fatty degeneration of parenchymal cells, and necrosis. All rats survived in the 0, 1 and 4 ppm groups, survival decreased at 8 and 16 ppm, and all rats died at 64 ppm. One-year survival in the 0, 1, or 4 ppm groups was above 90 percent and 2-year survival was above 50 percent. Weight gains were equivalent for the 0, 1 and 4 ppm groups. With increased age there was a slight reduction in hemoglobin and white blood cells, which was greater in selenium-treated than in control rats. Serum components including alkaline and acid phosphatase, serum glutamic-oxaloacetic transaminase, protein, glucose, and sialic acid were equivalent from 0 through 4 ppm. Selenium levels in the treated rats were twice those in the controls and liver GPx was half. Data presented were for male rats with reference to similar data on females. The parameters measured were dependent on the age of the rat when the treatmens was initiated.

Jacobs and Forst (1981b) reported reduced white blood cell count and increased alkaline phosphatase and glutamic-oxaloacetic transaminase in female Swiss mice given 8 ppm

selenium for 50 weeks, compared to 0, 1, and 4 ppm dose groups. The survival of selenium-treated groups was more than 90 percent and that of controls was only 72 percent after 50 weeks. All mice gained weight, but the group treated with 8 ppm selenium gained half as much as other groups. Both liver selenium and GPx activity increased in selenium-treated mice compared to controls at 25 and 50 weeks.

The chronic manifestations of selenium toxicity in laboratory animals, ecological contamination incidents, and in use of selenium supplements in animal husbandry are complex and depend on the species, the chemical form of selenium, and the dose, route, and frequency of administration. Some of the tissue targets of selenium toxicity after chronic administration in mammals and birds are shown in Table 12, adapted from O'Toole *et al.* (1996) and O'Toole and Raisbeck (1995, 1998).

Table 12. Chronic Lesions of Selenosis in Mammalian and Avian Species

Species	Hoof or nail	Horn	Hair or feather follicles	Liver	Heart	Emaciation	Spinal cord	Anemia	Terata
man	+	NA	+	-	-	-	-	-	-
cattle	+	+	+	±	-	+	-	±	-
sheep	-	-	-	+	+	+	-	-	-
pig	+	NA	+	-	-	-	+	±	-
dog	-	NA	-	+	-	+	ND	+	-
cat	-	NA	-	+	-	+	ND	ND	-
rat	-	NA	-	+	+	-	-	+	-
monkey	+	NA	-	-	-	-	-	-	-
mallard	+	NA	+	-	-	+	-	-	+

+, clearly present; ±, not strongly evident; -, not evident;
NA, not applicable; ND, not done

For ecological contamination, reports of poisonings in cattle, sheep, horses and other domestic animals first appeared in the early part of the 20th century in scattered areas of South Dakota, North Dakota, Nebraska, Wyoming, Utah, and other western states where new lands were being opened up for farming (Edmondson *et al.*, 1993; O'Toole and Raisbeck, 1995, 1998; O'Toole *et al.*, 1996). These incidents led to the discovery of the toxic properties of plants that accumulate selenium (Rosenfeld and Beath, 1964).

The term "alkali disease" was applied to animals with general dullness, lack of vitality, emaciation, stiffness, and lameness. Horses and cattle lost hair, and hoofs became loose and often sloughed off. Different degrees of severity were observed, with more serious cases involving blindness, loss of appetite, emaciation, and an unwillingness of animals to move about because of lameness and hoof damage. This condition is now recognized to result from excess selenium in the animals' diets (O'Toole *et al.*, 1996). In China, Yang *et al.* (1981) reported ox alkali disease with deformed hoof in Enshi in 1966.

The term "blind staggers' disease" was also used to describe a neurological condition in cattle in these regions (Rosenfeld and Beath, 1964). Blind staggers' disease is a condition of motor incoordination, impaired vision, aimless wandering behavior, lassitude and ultimately paralysis and death. It has been difficult to reproduce this condition in cattle with sodium selenite or sodium selenate and it has been suggested that the term "blind staggers" is not applicable to toxicity caused by an excess of inorganic selenium salts, but may instead have been a result of other factors in seleniferous plants or in a deficient diet (O'Toole *et al.*, 1996). In China, Yang *et al.* (1981) reported blind staggers' disease in baby swine in Enshi in 1966. The newborn pigs ceaselessly walked in circles and died young. Yang *et al.* (1981) considered baby swine survival and chicken egg hatchatability as the most sensitive indicators of endemic selenosis in Enshi.

A common theme of chronic selenium toxicity in animals is involvement of keratin-forming cells or keratinocytes (O'Toole *et al.*, 1996). These cells produce the hard sulfur-rich keratins in hooves, horns, hair, feathers, beak, and digital nails. Pathological changes in the keratinocytes of the stratum spinosum result in poor quality keratin in the cornified layer of the skin and, in some instances, progress to necrosis of the epithelium. In avian species, these effects are manifested as disheveled plumage, loss of feathers, loss of nails, and altered growth. In herbivores such as cattle, horses, and goats, the changes are in the hooves and hair of the mane and tail. Loss of the mane and tail may be the earliest clinical sign in herbivores, followed by cracks in the hoof wall. The lesions may develop within five days of poisoning, although longer periods are more common. The damage to the hoof in severe cases is painful and the animals are reluctant to walk for food, hence there is weight loss. Histological changes are found in the cells that produce keratin in the hoof. Swine also develop lesions of the hair and hooves. Loss of hair and nail lesions also develop in primates, but have not been described in common laboratory species such as the mouse, rat, dog, or cat.

Poor quality of the hair coat was reported in treated Swiss mice following lifetime exposure to sodium selenate or sodium selenite in drinking water at 0.57 mg selenium/kg-day (Schroeder and Mitchener, 1972). Sodium selenite caused cell death and an inhibition of cell growth in three types of human skin cells tested *in vitro*, including normal keratinocytes, normal skin melanocytes, and neoplastic melanoma cell line HTB140 capable of forming metastases, at a concentration approximately 50 times lower than sodium selenate causing the same effects (Bandura *et al.*, 2005).

Developmental and reproductive effects

Barlow and Sullivan (1982) reviewed the evidence from animal studies and concluded that the reproductive organs and the developing fetus are sensitive to selenium exposure. Chronic exposure to low and otherwise nontoxic doses of selenite or selenate has been reported to affect fertility in rodents, and reduce the viability of the young animals managing to reproduce (Willhite, 1993).

Table 13, mainly adapted from ATSDR (1996, 2003), lists the NOAEL or the LOAEL for developmental or reproductive effects from animal studies with acute, subchronic and chronic oral exposure to various selenium compounds. The highest NOAEL value after

oral exposure to selenite, selenate and L-selenomethionine is listed. All reliable LOAEL values after oral exposure to selenite, selenate and L-selenomethionine are also listed.

Table 13. NOAEL or LOAEL of Animal Developmental and Reproductive Effects

Exposure duration and route	Chemical species	System endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Hamster					
once, gestation day 8, gavage, water	selenite	developmental	7.1	7.9	Ferm *et al.*, 1990
once, gestation day 8, gavage, water	selenate	developmental		7.1	Ferm *et al.*, 1990
once, gestation day 8, gavage, water	L-seleno-methionine	developmental		5.9	Ferm *et al.*, 1990
Rat					
8 weeks, drinking water	selenite	developmental		0.64	Thorlacius-Ussing, 1990
5 weeks, feed	sodium selenite	reproductive		0.1 male	Kaur and Parshad, 1994
12 -14 weeks, drinking water	sodium selenite	reproductive		0.234 male	Turan *et al.*, 1999a
13 weeks, drinking water	sodium selenite	reproductive	0.5 female	0.17 male 0.86 female	Abdo, 1994
13 weeks, drinking water	sodium selenate	reproductive		0.29 male 0.31 female	Abdo, 1994
1 year, daily, drinking water	selenate	reproductive	0.21	0.35	Rosenfeld and Beath, 1954
Mouse					
pregestation 30 days and gestation day 0 - 18, drinking water	sodium selenite	developmental, reproductive	0.17 0.17 female	0.34 0.34 female	Nobunaga *et al.*, 1979
13 weeks, drinking water	sodium selenite	reproductive	3.83 female 3.31 male		Abdo, 1994
13 weeks, drinking water	sodium selenate	reproductive	7.17 female 5.45 male		Abdo, 1994
3 generation, drinking water	sodium selenate	developmental, reproductive		0.57 0.57	Schroeder and Mitchener, 1971b

Exposure duration and route	Chemical species	System endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Rabbit					
6 weeks, once a week, gavage, drinking water	sodium selenite	reproductive		0.001 male	El-Zarkouny *et al.*, 1999
Pig					
not specified, feed	sodium selenite	developmental, reproductive		0.4 0.4	Wahlstrom and Olson, 1959
Cattle					
3 months, feed	sodium selenite	developmental	0.265		Yaeger *et al.*, 1998
Monkey					
30 days, once/day, nasogastric intubation, water	L-seleno-methionine	reproductive	0.06 female	0.08 female	Cukierski *et al.*, 1989

Reproductive effects

In experimental animals, high selenium levels produce tissue damage and lead to testicular degeneration (Hansen and Deguchi, 1996). Male and female Wistar rats fed wheat containing 24.6 ppm of naturally occurring selenium (Franke, 1934a,b; Franke and Potter, 1934) or control wheat with sodium selenite added at 22.3, 33.5 or 52.1 ppm (Franke and Potter, 1935, 1936) had excess mortality. Those that were able to survive the toxic diet showed subnormal growth, underdeveloped and degenerated reproductive organs, and reproduction loss.

Testes, mainly spermatozoa, contain the highest concentration of selenium of any tissue in the mammalian reproductive system after a single oral administration (Behne *et al.*, 1986). Administration of [^{75}Se] in sodium selenite to mice (Gunn *et al.*, 1967) or rats (Brown and Burk, 1973; Calvin and Cooper, 1979; Calvin *et al.*, 1981, 1987; Gunn and Gould, 1970; Hawkes *et al.*, 1985) resulted in accumulation of selenium in the testis and incorporation into developing spermatozoa. The retention of selenium in the testis was especially significant in rats fed with a selenium-deficient diet, resulting in morphological defects in the midpiece of sperm (Behne *et al.*, 1982; Behne and Hofer-Bosse, 1984; Brown and Burk, 1973; Wallace *et al.*, 1983a,b; Wu *et al.*, 1969, 1979). In rats, dietary intake of selenium at 6 and 8 ppm for six and nine weeks affects the oxidoreductase activity of glutathione and resulting in oxidative damage to testis (Kaur *et al.*, 1999).

Male Wistar rats fed with sodium selenite in drinking water at 4, 8 or 16 ppm (0.55, 1.1 or 2.2 mg selenium/kg-day) for 240 days exhibited decreased body weight, increased relative testis weight, damaged tubules, intertubular edema, oligospermia, vacuolization of spermatids, as well as changes in specific testicular enzymes mainly at the 16 ppm

dose. A NOAEL was not identified (Nebbia *et al.*, 1987). Testicular hypertrophy was reported in male weanling Wistar rats fed 0.234 mg selenium/kg-day as sodium selenite in water for 12 to 14 weeks (Turan *et al.*, 1999a). Male New Zealand white rabbits gavaged with 0.001 mg selenium/kg-day as sodium selenite in drinking water once a week for six weeks had a significant 49 percent reduction in serum testosterone levels (El-Zarkouny *et al.*, 1999).

Kaur and Bansal (2004a,b,c) found that selenium deficiency in mice affected testicular steroidogenesis, caused DNA fragmentation, and disrupted the spermatogenic process with a reduction in mature sperm, which they attributed to excessive oxidative stress. Three groups of male Balb/c mice weighing 25 g were fed diets varying in selenium level for eight weeks. The first group was fed a yeast-based selenium-deficient diet at 0.02 ppm, while the second and third groups were fed the same diet supplemented with 0.2 ppm and 1 ppm selenium as sodium selenite, respectively. The first group of mice had significant decreases in serum and testis selenium level, liver and testis GPx activity, serum luteinizing hormone, follicle-stimulating hormone, testosterone, testicular lumen size,testicular lactate dehydrogenase-X level, germ cell population (with spermatids and mature sperm affected the most), and in sperm number and motility. The first group also had a significant increase in the testis glutathione-S-transferase activity. DNA fragmentation was observed in both the first and third groups; however, the damage was more prevalent in the first group. An increase in testis selenium level was observed in the third group, compared to the second group. Displacement of germ cell population was only observed in the third group.

Kaur and Kaur (2000) fed adult male albino rats 6 and 8 ppm selenium in diet for six and nine weeks. The rats showed dose-time-dependent reduction in body weight and reproductive organ weights, increase in number of morphologically abnormal spermatozoa, and reduction in tubular diameter, epithelial height, number of spermatogenic cells and disintegration of cellular associations in the seminiferous tubules of testes. There also was a reduction in the diameter of cauda epididymal tubules and pseudostratification of their epithelial lining.

Kaur and Parshad (1994) observed a dose-related increase in abnormal sperm in cauda epididymis, a decrease in live sperm, and a decrease in testicular and cauda epididymis weights in wild-caught house rats exposed to sodium selenite in the diet at 2 or 4 ppm of selenium (0.1 and 0.2 mg selenium/kg-day) for five weeks compared to controls. The percentage of abnormal sperm was 3.9 percent at 0.1 mg/kg-day and 24.6 percent at 0.2 mg/kg-day, and the abnormalities were mainly in the midpiece region of the flagellum.

Abdo (1994) observed decreased sperm counts in F344/N rats provided with 0.29 mg selenium/kg-day of sodium selenate or 0.17 mg selenium/kg-day as sodium selenite in drinking water for 13 weeks in a study performed by the NTP. Sodium selenate and sodium selenite also caused increases in estrous cycle length in female F344/N rats. Sodium selenite also caused an increase in estrous cycle length in female mice. Vaginal cytology of female rats indicated that the rats spent more time in diestrus and less time in proestrus and estrus than the controls. This effect occurred at 0.31 mg selenium/kg-day following selenate treatment and at 0.86 mg selenium/kg-day following selenite treatment. The animals in these studies were not mated, so it is not known if the effects on sperm or the estrous cycle had any effect on fertility (Abdo, 1994).

In another study performed by NTP (Wolfe and Kaiser, 1996), designed to extend the previous NTP studies (Abdo, 1994), a truncated test was used to screen for both the short-term reproductive and developmental toxicity of sodium selenate. Sodium selenate was administered in the drinking water for 30 days at 0, 7.5, 15 or 30 ppm (0.418, 0.8 or 1.1 mg/kg-day) to Sprague-Dawley rats. One group of male (10 per group) and three groups of female rats designated as Group A (periconception exposure, 10 per group), Group B (gestational exposure, 13 per group), and Group C (vaginal cytology, 10 per group) were used for each dose level. Sodium selenate produced minor effects on male reproductive function in rats. Sperm endpoints were generally unchanged and microscopic lesions were not noted in the kidney, liver, testis, or epididymis.

Sodium selenate produced reproductive toxicity in both Group A and B female Sprague-Dawley rats, mainly at 30 ppm. There was an increased gestation length and decreased number of live pups, live pup weight, proportion of pups born alive, number of implants and corpora lutea per litter, and pup survival. However, the toxicity could not be separated from the effects due to reduced water consumption. Mortality was observed only in the 30 ppm group; seven of the 12 females died after 14 days of treatment. In the 30 ppm females in both Group A and B, significantly fewer implants per litter and corpora lutea, as well as a decreased number of live fetuses per litter were observed, compared to controls. The 30 ppm Group C females had a 22 percent increase in estrous cycle length; however, a NOAEL was not identified. Significant body weight reduction was observed in all dosed groups, for example, 36 percent weight reduction was reported in the female rats receiving 7.5 ppm or 0.418 mg/kg-day.

Selenium administered in the diet or in drinking water over one month affected the fertility of female animals when the intake was sufficiently high to cause general toxicity (Barlow and Sullivan, 1982; Nobunaga *et al.*, 1979). Nobunaga *et al.* (1979) found a small increase in the number of abnormal length estrous cycles in female IVCS mice exposed to sodium selenite at 0.34 mg selenium/kg-day in drinking water for a total of 48 days beginning 30 days before mating and ending 18 days after pregnancy. A NOAEL of 0.17 mg selenium/kg-day and a LOAEL of 0.34 mg selenium/kg-day were identified based on proportion of mice with longer estrus cycles increasing by 11.8 percent.

Similarly, an altered menstrual cycle was reported in female longtailed *Macaque* monkeys administered 0.08 or 0.12 mg selenium/kg-day as L-selenomethionine in drinking water through nasogastric intubation once a day for 30 days. A NOAEL of 0.06 mg selenium/kg-day and a LOAEL of 0.08 mg selenium/kg-day were identified based on altered menstrual cycles (Cukierski *et al.*, 1989).

In a study of the effect of selenium on fertility in Duroc pigs, females fed sodium selenite at 0.4 mg selenium/kg-day from eight weeks of age exhibited reduced rates of conception and also produced offspring with significantly reduced birth weight and weaning weights in the first and second litters. A LOAEL of 0.4 mg selenium/kg-day was identified based on decreased fertility and maternal toxicity (Wahlstrom and Olson, 1959).

Pregnant (Smith, 1983; Yonemoto *et al.*, 1983) or nonpregnant (Plasterer *et al.*, 1985) female CD1 mice given an oral dose of 7 mg/kg-day of sodium selenite for eight consecutive days was lethal to 10 percent of the tested mice, but showed no significant effects on reproductive indices.

In a three-generation reproduction study, sodium selenide at 3 ppm selenium (0.57 mg selenium/kg-day) in the drinking water of Charles Rivers CD breeding mice resulted in excess death before weaning, runts, and failures of about half of the pairs to breed (Schroeder and Mitchener, 1971b). Similar chronic exposure of rats to otherwise nontoxic doses of selenium was shown to reduce the viability of the offspring of pairs that are able to conceive (Wahlstrom and Olson, 1959).

In a two-generation study in Wistar rats, potassium selenate was administered in drinking water at 1.5, 2.5 or 7.5 ppm of selenium, equivalent to 0.21, 0.35 or 1.05 mg selenium/kg-day (Rosenfeld and Beath, 1954). The dose of 0.21 mg selenium/kg-day for one year had no effect on reproduction. At 0.35 mg selenium/kg-day for one year, the number of young successfully reared by the females was reduced by 50 percent and the body weight of the females was approximately 20 percent less than that of the control females. Decreased fertility and pup survival were noted at 1.05 mg selenium/kg-day. The value of this study is limited, however, because the report did not indicate which factors contributed to the 50 percent reduction in successfully reared young.

High selenium levels in the body during the oestrous cycle preceding mating affected the number of ovulations, implantations and live embryos (Parshad, 1999). Intraperitoneal injections of sodium selenite at 2 or 4 mg selenium/kg to normally four day cycling female Wistar rats grouped based on stage of the reproductive cycle daily for 30 days had no effect on the duration of the first two oestrous cycles, but afterwards the rats remained at the dioestrous stage. Their ovaries developed cystic follicles. A single injection of selenite during the oestrous cycle preceding mating affected the implantation and pregnancy outcome in a dose-related manner. A single dose containing 2 mg/kg administered either at the proestrous or oestrous stage had no effect on oestrous cyclicity, ovarian follicles, ovulation, implantation and pregnancy outcome on day 14 of gestation. However, a daily dose at this level during the four-day oestrous cycle reduced the number of corpora lutea and implantations, compared to saline injected controls. Contrarily, a single dose of selenite at 4 mg/kg at the proestrous stage reduced the conception rate, number of live embryos, and implantation sites on day 14 of gestation. Selenite at 4 mg/kg during oestrous or throughout the cycle decreased the number of implantations and increased the resorption rate per litter on day 14 of gestation.

Developmental effects

The ability of selenium to pass across the placenta to the fetus has been documented (Westfall *et al.*, 1938; Willhite *et al.*, 1990). Selenium also is delivered in the milk to suckling young (Barlow and Sullivan, 1982; Hidiroglou, 1982). Thus signs of selenosis may appear in newborn animals, as well as developmental anomalies (Grabek *et al.*, 1991). Encephaloceles were seen in the dams of hamsters given sodium selenate and selenite orally, while teratogenic response following selenomethionine administration was accompanied by maternal toxicity. Fetotoxicity was found in the dams of mice fed sodium selenate but teratogenicity studies in monkeys were negative (Fan, 1992).

Danielsson *et al.* (1990) reported on the placental transfer and distribution pattern of selenium in embryonic and fetal tissues in mice after intravenous injection of [^{75}Se]sodium selenite or [^{75}Se]sodium selenate at 45 µg selenium/kg in early, mid, and

late pregnancy. The distribution patterns of selenite or selenate were similar in maternal tissues with higher uptake of selenite in the liver. Placental transfer increased with time and with progression from embryonic through fetal stages. Fetal uptake increased with increasing age but was lower than in maternal tissues, except for the fetal eye. The distribution patterns of selenite or selenate were similar in embryonic and fetal tissues with high uptake in the neuroepithelium, liver, renal cortex, skeleton, and eye. Selenite was more toxic than selenate *in vitro* with either mouse blastocysts or chick embryo fibroblasts differentiating into chondrocytes to compare the direct toxicity in early embryonic stages.

Nishimura *et al.* (1991) injected [^{75}Se] intravenously to pregnant rats between the 7th and 20th days of gestation. The relative concentrations in the fetal membrane, placenta, and maternal blood were higher than those in the fetus, and decreased with time after administration. Transplacental passage of [^{75}Se] in the seven-, 14- and 20-day pregnant rats amounted to 0.8, two and three percent of the initial dose, respectively. In the same study, one to three percent of a dose of [^{75}Se] was transferred to sucklings via the milk.

Sodium selenite fed in drinking water to Swiss mice at 1 or 5 ppm from day one of lactation, continued daily for 14 or 21 days, was shown to pass from the nursing mother by way of milk to the progeny (Chhabra and Rao, 1994). At 5 ppm, selenite decreased the hepatic cytochrome P-450 b_5 content, glutathione S-transferase, glutathione reductase, and GPx both in the dams and in the pups translactationally exposed for 14 days.

Juszkiewicz *et al.* (1993) injected sodium selenite subcutaneously to female rats on gestation day nine in single doses of 0.5, 1, and 2 mg selenium/kg, or on gestation days six to 15 at 0.5 mg/kg-day. No evidence of maternal toxicity or teratogenic malformation were observed in the 21-day dams and fetuses. The reproductive and fetal development parameters were found to be better in groups treated with single doses of selenium at 0.5 and 1 mg/kg than in controls. Some increase in resorptions was recorded in rats receiving selenium in a dose of 0.5 mg/kg-day from the sixth to 15th days of gestation.

Danielson *et al.* (1988) reported adverse eye effects in 22 mated three-month-old female Sprague-Dawley rats given sodium selenite solution by gavage at 15 mg selenium/kg once daily during days seven to nine of gestation. Only three produced surviving litters. In one litter, six out of 13 fetuses showed anophthalmia in addition to skeletal defects. Three of the six fetuses with anophthalmia also showed exencephalus. The other two surviving litters showed skeletal defects.

Shearer *et al.* (1983) demonstrated that oral administration of selenium caused cataract formation in young rats. On days six through 10 postpartum, the suckling pups of four Sprague-Dawley derived COB female rats were given orally 0.01 mL of 0.5 M glucose solution containing 0, 0.11, 0.23, or 0.46 µmole selenium as sodium selenite. Bilateral nuclear cataract was produced in all pups swallowing 0.23 or 0.46 µmole selenium. The dose required for the eye effect when given orally for five days (0.23 µmole) was similar to that needed by repeated injections for five days (0.15 µmole) as described below. The cataracts formed were similar in appearance and severity to those described below for pups receiving selenium by injection.

Either selenate or selenite has been reported to induce lens cataract in newborn rats caused by embryonic concentration of selenium at doses given by injection to the mother

higher than 3 mg selenium/kg (Danielson *et al.*, 1988; Ostadalova *et al.*, 1978; Ostadalova and Babicky, 1980). In contrast to other tissues, the fetal eye uptake of either selenate or selenite was higher than the maternal eye. An accumulation in the fetal eye was observed seven days after a single subcutaneous injection to the mother on day 12 of gestation (Danielsson *et al.*, 1990). Selenomethionine was reported to be cataractogenic in rats after subcutaneous injections, probably due to interference with glutathione metabolism (Rossoff, 2002).

Huang *et al.* (1992) reported that nuclear cataracts occurred in both young Wistar (up to 13 days of age) and Sprague-Dawley (up to 12 days of age) rats after five subcutaneous injections of selenite over eight days, with a cumulative dose of 6.9 to 8.6 mg/kg selenite. Glutathione content decreased within the first 24 hours to 40 to 50 percent of controls at the time of nuclear opacification and remained at 60 percent of the controls. Before and during nuclear cataract formation, changes in DNA metabolism including damage, repair, and replication evaluated *in vitro* by measuring incorporation of ^{3}H-thymidine in rat lens following a single cataractogenic dose of sodium selenite at 30 nmole/g was observed within hours of exposure (Huang *et al.*, 1990).

Ostadalova *et al.* (1979, 1982, 1988) and Ostadalova and Babicky (1983, 1984) also reported nuclear cataract formation in Wistar rats injected with selenite. The cataract formation was accompanied by a two- to three-fold increase of calcium concentration and a decrease of protein content in the blood. A minimum initial dose of 6 nmole selenite/g and a final dose given at day 21 were required for cataract development. This multiple low dose protocol resulted in nuclear cataracts similar in appearance to those observed with a single, acute high dose of selenite; however, the enhanced perinatal sensitivity only occurred at doses close to lethal doses as shown in the acute studies (Ostadalova *et al.*, 1978, 1979, 1982, 1988; Ostadalova and Babicky, 1980, 1983, 1984).

Li *et al.* (1999), using the whole embryo rotated culture technique, showed synergistic developmental toxicity of a combination of selenium, fluoride and arsenic on rat embryos at day 9.5 of gestation. Mixtures with different levels of these three chemicals in combination resulted in different toxicity. The low level combinations (selenium 0.5 µg + fluoride 2.5 µg + arsenic 0.5 µg/mL culture media) mainly caused a teratogenic effect. High level combinations (selenium 2.0 µg + fluoride 10 µg + arsenic 1.0 µg/mL culture media) caused a lethal effect.

In studies of laboratory mammals, the administration of inorganic selenium compounds at levels that are not maternally toxic has not produced terata (Bergman *et al.*, 1990; Ferm *et al.*, 1990; Poulsen *et al.*, 1989; Rosenfeld and Beath, 1954; Schroeder and Mitchener, 1971b; Tang *et al.*, 1991; Thorlacius-Ussing, 1990). Bergman *et al.* (1990) found no negative effects on pups born to mother rats given up to 5 ppm dietary sodium selenite during gestation. Nobunaga *et al.* (1979) found that administration of sodium selenite in drinking water at 0.34 mg/kg-day for 30 days before mating and for 18 days during pregnancy slightly, but significantly, reduced fetal growth in mice, with no effect on fetal growth at 0.17 mg/kg-day. Selenium administered as potassium selenate in drinking water to male and female rats at a dose of 1.05 mg/kg-day for one to eight months for two successive generations did not cause congenital malformations (Rosenfeld and Beath, 1954). Similarly, administration of 0.57 mg/kg-day as sodium selenate in the drinking water of breeding mice for three generations did not have teratogenic effects, although

there was an increased incidence of fetal deaths, and a high proportion of the surviving offspring were runts (Schroeder and Mitchener, 1971b). Decreased weight gain of pups during lactation of female Wistar rats exposed to sodium selenite at 0.64 or 0.96 mg/kg-day in water for eight weeks was observed, which was suggested as associated with a reduction in somatomedin C levels (Thorlacius-Ussing, 1990).

Developmental toxicity studies in Syrian LKV golden hamsters failed to demonstrate an adverse response of selenium except at maternally toxic doses (Holmberg and Ferm, 1969). A following study of selenite, selenate, or L-selenomethionine in pregnant hamsters again failed to elicit a teratogenic response except at doses that produced maternal intoxication (Ferm *et al.*, 1990). Ferm *et al.* (1990) administered a single dose of selenium at 5.9 to 8.7 mg/kg to pregnant hamsters during the critical stages of embryogenesis through oral, intravenous, and osmotic minipump infusion. When the offspring were examined on day 13, malformations, mainly encephalocele and excencephaly, were observed in the higher dose groups with oral or intravenous selenite and selenate. However, this was accompanied by maternal toxicity manifested by inanition, lethargy, and weight loss. Fetal body weights and lengths were reduced in a dose-dependent manner. Single oral doses of L-selenomethionine above 77 µmole/kg induced similar malformations but not when the dose was delivered orally over four days or by minipump over several days. For selenite, a NOAEL of 7.1 mg selenium/kg-day was identified, and a LOAEL of 7.9 mg selenium/kg-day was identified, based on encephalocele and decreased crown-rump length. For selenate, a LOAEL of 7.1 mg selenium/kg-day was identified based on encephalocele. For selenomethionine, a LOAEL of 5.9 mg selenium/kg-day was identified based on decreased fetal crown-rump length.

Day eight pregnant Syrian Lak:LVG(SYR) golden hamsters were used to evaluate the pharmacokinetics of selenate or L-selenomethionine, the selenium forms that predominated in water and plants in the Kesterson area, after a single oral or intravenous dose of 60 µmole/kg (Willhite *et al.*, 1990). No teratogenic response was found at doses less than those associated with maternal intoxication, which the authors concluded cannot be attributed to lack of selenium accumulation in early embryonic and placental tissue. However, as noted by there authors, there are some difficulties in interpreting the results due to the limited time points studied and limited clinical and experimental data on transplacental transfer of selenium.

In a teratology study in Cynomolgus macaque (*Macaca fascicularis*) monkeys, no gross abnormalities or growth retardations were observed in fetuses from mothers administered L-selenomethionine even at doses causing maternal toxicity such as anorexia, vomiting, and body weight reduction (Tarantal *et al.*, 1989, 1991). L-selenomethionine, the most potent avian teratogen among selenium compounds, was given by nasogastric intubation once a day at 0.003, 0.025, 0.15, or 0.30 mg selenium/kg-day during gestational days 20 to 50 with 10 animals per group; the highest two doses were maternally toxic. Weight loss and vomiting was observed at the 0.15 mg/kg-day group. In a separate study, forty pregnant Cynomolgus monkeys treated daily from gestational day 20 to 50 by nasogastric intubation of 0, 0.025, 0.15, or 0.3 mg/kg-day as L-selenomethionine again showed no teratogenic effects (Hawkes *et al.*, 1994).

Selenium caused morphological abnormalities in specific sites in cultured rat embryos (Usami and Ohno, 1996). Wistar rat postimplantation embryos at day 9.5 of gestation were cultured *in vitro* for 48 hours in the presence of one of four selenium compounds. Sodium selenite, sodium selenate, D,L-selenomethionine, and D,L-selenocystine were lethal to the embryos at 20, 300, 1,000, and 10,000 µM selenium, respectively. In the surviving embryos, abnormalities were observed mainly in the head region, such as deformed optic vesicle and swollen rhombencephalon. Each of the four selenium compounds reduced the embryonic viability and growth.

Tabata *et al.* (1993) examined the effects of depletion of glutathione, the major intracellular reducing agent, on selenium teratogenicity in cultured rat embryos at day 9.5 of gestation. The embryos were cultured for 48 hours in the presence of selenium as sodium selenite at 10 and 20 µM or sodium selenate at 30 and 100 µM, with or without 0.1 mM L-buthionine-(*S, R*)-sulfoximine. With L-buthionine-(*S, R*)-sulfoximine, the reduced glutathione content in the embryos was decreased to about 50 percent. Selenite or selenate alone increased the incidence of abnormalities of the embryos in a concentration-dependent manner. The incidence of selenite-induced abnormalities of the embryos was significantly decreased with L-buthionine-(*S, R*)-sulfoximine. By contrast, the incidence of selenate-induced abnormalities was significantly increased with L-buthionine-(*S, R*)-sulfoximine. Thus embryonic glutathione and the selenium redox state appear to be involved in the mechanisms of selenium teratogenicity.

Livestock and Ecological studies

Adverse effects of excess selenium have also been shown on reproduction and development in livestock and avian species (Franke and Tully, 1935; Franke *et al.*, 1936; Rosenfeld and Beath, 1947, 1964; Gruenwald, 1958; Wahlstrom and Olson, 1959; Dinkel *et al.*, 1963; Harr and Muth, 1972; Palmer *et al.*, 1973; Puzanova *et al.*, 1976; Fitzsimmons and Phalaraksh, 1978; Olson, 1978; Smith *et al.*, 1979; Khan and Gilani, 1980; Lo and Sandi, 1980; Pond *et al.*, 1983; Heimann *et al.*, 1984; Ohlendorf *et al.*, 1986, 1989; Puzanova, 1986; Penava *et al.*, 1987; Hoffman and Heinz, 1988; Poulsen *et al.*, 1989; James, 1990; Willhite *et al.*, 1990; Grabek *et al.*, 1991; Mohammed *et al.*, 1991; Heinz and Fitzgerald, 1993; Willhite, 1993; Green and Albers, 1997; Heinz and Hoffman, 1998; Yaeger *et al.*, 1998; Fairbrother *et al.*, 1999; Panter *et al.*, 1999; Kolev *et al.*, 1999; Kim and Mahan, 2001a,b,c; Lemly, 2002; Spallholz and Hoffman, 2002; Rossoff, 2002).

Selenium bioaccumulation has been held responsible for reproductive failure among waterfowl at the Kesterson Reservoir and the Tulare Lake Basin in California, the Ouray and Stewart Lake Wildlife Refuges in Utah, the Carson Sink in Nevada (Hoffman, 2002; Hoffman *et al.*, 2002; Willhite *et al.*, 1990; Benson *et al.*, 1990), and the Belews Lake in North Carolina (Adams *et al.*, 1998)

Selenium is readily accumulated by aquatic organisms and toxicity can be acute via water column exposure and chronic via food chain exposure (Chapman, 1999; Hamilton, 2004; Lemly, 2004; Chapman *et al*., 2009). The increased flux of selenium into aquatic ecosystems due to anthropogenic activities, such as selenium in overflow water from coal burning power plant settling basins or selenium leaching from the soil into water systems

used for irrigation in highly seleniferous areas, has resulted in death, teratogenesis, reproductive impairment and decreased populations in fish (Crane *et al.*, 1992; Hamilton *et al.*, 2000; Hermanutz *et al.*, 1992; Knight, 1989; Saiki and Ogle, 1995; Schultz and Hermanutz, 1990). A link between selenium-induced anemia signs in fish in natural habitats and the sensitivity of fish hapatic and gill δ-aminolevulinate dehydratase inhibition by selenium has been suggested (Antunes Soares *et al.*, 2005). Fish reproduction can be adversely affected when dietary selenium levels exceed about 4 µg/g on the dry weight basis. Conversely, dietary levels of selenium below about 0.1 to 0.5 µg/g cause nutritional deficiencies in fish, as in domestic animals and wildlife (Ohlendorf, 1999). Invasive species such as zebra mussels bioconcentrate selenium to toxic levels which can cause death or reproductive problems in waterfowl that eat them (Pelley, 2003). There is a parallel between selenium concentrations in fish eggs, incidence of teratogenic deformities in larvae, and magnitude of reproductive failure (Lemly, 1997). Teratogenic defects identified included lordosis, kyphosis, scoliosis, and head, mouth, and fin deformities. Other abnormalities observed were edema, exophthalmus, and cataracts (Lemly, 1993b). Biological effect thresholds for the health and reproductive success of freshwater and anadromous fish were suggested as: whole body 4 µg/g, skeletal muscle 8 µg/g, liver 12 µg/g, ovaries and eggs 10 µg/g (Lemly, 1993a). Ecological effects of selenium have been recently reviewed and summarized in a Pellston Workshop (Chapman *et al.*, 2009).

Genetic toxicity

The selenium genotoxicity literature has been reviewed by Shamberger (1985), Abdo (1994) and ATSDR (2003). Both mutagenic and antimutagenic activities have been observed with inorganic selenium compounds; the effects are determined by the chemical form and dose of selenium. At submicromolar concentrations, selenium compounds reduced the actions of known mutagens (i.e., antimutagenic effects). At a higher, narrow range of concentration, usually 0.1 to 1 micromolar (µM), mutations or clastogenic effects are observed in some systems. Still higher concentrations are frequently lethal to test organisms (Arciszewska *et al.*, 1982; Kramer and Ames, 1988; Shamberger, 1985).

Sodium selenite and sodium selenate produced mixed results in prokaryotic bacterial mutagenicity test systems *in vitro*. Lofroth and Ames (1978) reported that selenite showed mutagenic activity in the standard *Salmonella* plate incorporation test, and selenate gave rise to base-pair substitutions at about 0.03 revertants/nmole without S9 activation. Noda *et al.* (1979), testing without activation, found that sodium selenite and sodium selenate were both mutagenic in *Salmonella* strain TA100, but only in the narrow concentration range of 1 to 8 µmoles per plate. Higher concentrations, up to 12 to 14 µmoles per plate, were inactive. Minimal effects were found in strains TA98 and TAl537. Sodium selenate was also reported to induce gene mutations in strain TA1535 but not in TA100 in a standard plate incorporation assay without activation at doses of 6 to 20 mg/plate (Arlauskas *et al.*, 1985). Using TA104, a strain that is designed to be sensitive to oxidizing agents, Kramer and Ames (1988) found that sodium selenite was mutagenic at 4,000 µg/plate. The mutagenic activity appeared to be dependent on formation of reactive oxygen species (ROS).

Chortyk *et al.* (1988) showed that sodium selenite was not mutagenic in TA1538 or TA1978 at 80 µg/plate, but when added with tobacco smoke condensate, it was highly effective in reducing the mutagenic activity of the smoke condensates. Arciszewska *et al.* (1982) and Prasanna *et al.* (1987) found that sodium selenate or sodium selenite can decrease the number of revertants induced in TA98 and TAl00 by mutagenic polynuclear aromatic hydrocarbon such as 3-methylcholanthrene and dimethylbenzanthracene. The antimutagenic actions may be due to interference with enzymatic activities of the liver homogenate S9 required for the activation of the polynuclear aromatic hydrocarbons (Prasanna *et al.*, 1987).

Sodium selenite was mutagenic in *Bacillus subtilis* transformation assay without activation (Nakamuro *et al.*, 1976), but was not mutagenic in *B. subtilis* rec assay without activation (Noda *et al.*, 1979). Sodium selenate was mutagenic in *B. subtilis* rec assay without activation (Kanematsu *et al.*, 1980), but was inactive at concentrations up to 1.6 mM when tested in a recombinant transformation assay based on a differential growth inhibition in wild type versus repair-deficient strains of *B. subtilis* (Nakamuro *et al.*, 1976). Anjaria and Madhvanath (1988) reported that concentrations of sodium selenite at 10^{-2} to 10^{-3} M were mutagenic to diploid yeast *Saccharomyces cerevisiae* BZ34 and D_7.

Sodium selenite produced more consistently positive responses in a number of genetic toxicity tests based on mammalian cell cultures than did sodium selenate, but these inorganic salts produced mixed results in eukaryotic cell test systems *in vitro*. The positive responses did not require the presence of a liver S9 activation system and were potentiated by reduced glutathione. Sodium selenite and sodium selenate both induced chromosomal aberrations in Chinese hamster ovary cells without activation (Whiting *et al.*, 1980). Nakamuro *et al.* (1976) observed chromosomal aberrations in human leukocytes exposed to sodium selenite at 26×10^{-5} M without activation. No increases were observed with similar concentrations of sodium selenate without activation. However, Biswas *et al.* (1997) observed chromosomal aberrations in human lymphocytes induced by sodium selenate without activation.

Sodium selenite was more clastogenic than sodium selenate to human peripheral blood lymphocytes *in vitro* (Biswas *et al.*, 2000). Sodium selenite at 2.9×10^{-5} M or sodium selenate at 2.65×10^{-5} M was lethal without the formation of blast cells. Sodium selenite at 5.8×10^{-6} M, or sodium selenate at 1.06×10^{-5} M, were mitostatic. Sodium selenite at 0.2, 1.16, or 2.9×10^{-6} M, or sodium selenate at 1.06, 5.3 or 2.65×10^{-6} M, induced dose related chromosomal aberrations and reduced cell division.

Sodium selenite and selenomethionine were clastogenic *in vitro* for sister chromatid exchanges in cultured human lymphocytes (Khalil, 1989). The yield of abnormal metaphases in human lymphocyte cultures, 24 hours after stimulation with phytohemagglutinin and scored for chromosomal aberrations at 48 hours, was dose dependent at concentrations of 8.0×10^{-8} M to 8.0×10^{-5} M of sodium selenite or selenomethionine without activation. At 8.0×10^{-5} M, the proportion of aberrant cells reached 53.5 percent and 43.0 percent for selenite and selenomethionine, respectively. The selenium-induced chromosomal aberrations were primarily of the chromatid type and included breaks and fragments. Chromosomal exchanges were less frequent and included triradials and quadriradials. Selenocystine tested positive for sister chromatid exchanges in cultured human lymphocytes (Khalil, 1994).

Sodium selenite induced DNA fragmentation, DNA-repair synthesis, chromosome aberrations and mitotic inhibition in cultured human fibroblasts at 8×10^{-5} to 3×10^{-3} M (Lo *et al.*, 1978). Incubation with mouse liver S-9 microsomal fraction increased the capacity of selenite to induce chromosome aberrations, DNA-repair synthesis and lethality. Sodium selenate at 8×10^{-5} to 3×10^{-3} M induced a small but significant DNA-repair synthesis, and could not be activated by incubating with a S-9 preparation.

Ueda *et al.* (1997) compared the effect of sodium selenite or its major metabolism excretory product trimethylselenonium ion on the cytotoxicity of dimethylarsinic acid and on induction of tetraploidy by dimethylarsinic acid in Chinese hamster V79 cells. (Tetraploidy is a form of aneuploidy that is known to induce carcinogenesis.) The cytotoxicity of sodium selenite was 1,000-fold greater than that of trimethylselenonium which was about the same as dimethylarsinic acid. The mitotic index for dimethylarsinic acid was increased by both selenium compounds at low concentrations and decreased by them at high concentrations. The tetraploid index for dimethylarsinic acid decreased with increasing concentrations of both selenium compounds.

Whiting *et al.* (1980) reported that baseline effects of sodium selenate and sodium selenite on *in vitro* unscheduled DNA synthesis were up to 13 grains per nucleus at concentrations of 10^{-5} to 10^{-3} M, but were significantly enhanced to 74 to 114 grains per nucleus by the addition of glutathione at 10^{-3} M. The effects of sodium selenate were only apparent at 10^{-3} M, but effects with sodium selenite were observed at 10^{-5} M in the presence of glutathione. No unscheduled DNA synthesis was detected in cells treated with selenocystamine or selenomethionine, with or without added glutathione. However, selenocystine alone at 10^{-4} to 10^{-3} M induced a low level of unscheduled DNA synthesis; glutathione enhanced the DNA-damaging effect of selenocystine. The maximum amount of unscheduled DNA synthesis at 22 grains per nucleus occurred in the presence of 10^{-2} M glutathione. This was about one-fifth of that detected in cells treated with inorganic selenium compounds and 10-fold lower concentrations of glutathione at 10^{-3} M. In the absence of glutathione, these inorganic selenium compounds induced moderate frequencies of chromosome aberrations, up to 11 percent. Glutathione at 10^{-3} M also enhanced the clastogenic and cytotoxic effects of selenite and selenate in Chinese hamster ovary cells. Glutathione at 10^{-4} M or 10^{-2} M caused less enhancement of DNA damage and toxicity on unscheduled DNA synthesis in cultured human fibroblasts and on chromosome aberration in Chinese hamster ovary cells. Whiting *et al.* (1980) suggested that the thiol groups provided by glutathione might aid in the formation of toxic selenotrisulfide intermediates that interact with DNA.

Snyder (1987) confirmed the observation by Whiting *et al.* (1980) that sodium selenite induces substantial DNA damage in human cells. Snyder (1987) showed a dose-dependent decrease in cloning efficiency of fibroblasts treated with 0.1 to 1×10^{-3} M of sodium selenite. Snyder (1987) concluded that DNA strand breakage was not due to free oxygen radical production. They also observed an enhanced sensitivity of cells to damage in the presence of reduced glutathione and, to a lesser degree, serum. Sodium selenate and sodium selenite at 10^{-4} to 10^{-3} M induced unscheduled DNA synthesis in human fibroblasts (Lo *et al.*, 1978).

In vitro exposure of rat lymphocytes to sodium selenite at concentrations of 7.5×10^{-6} M, 1×10^{-5} M, or 2.5×10^{-5} M showed that selenite was clastogenic. However, *in vivo*

intravenous injection of sodium selenite up to 6 mg/kg to rats caused no significant increase in abnormal metaphases in lymphocytes. Nevertheless, intravenous injection twice at 5 and 6 mg/kg caused a significant increase in abnormal metaphases in rat bone marrow with induced chromosomal aberrations (Newton and Lilly, 1986).

Sister chromatid exchanges were induced *in vitro* by sodium selenite in Chinese hamster V79 cells (Sirianni and Huang, 1983) and in human lymphocytes cultured in whole blood (Ray and Altenburg, 1980). Selenite induction of sister chromatid exchanges in human lymphocytes required the presence of reduced glutathione (Ray, 1984). Sodium selenate was not active in this *in vitro* system at concentrations of 8×10^{-5} M (Ray and Altenburg, 1980). Newton and Lilly (1986) examined the clastogenic effects of sodium selenite on the chromosomes of rat lymphocytes and bone marrow. *In vitro* exposure of rat lymphocytes to sodium selenite showed clastogenicity at 7.5 to 25×10^{-6} M.

Lu *et al.* (1994a) examined effects of sodium selenite on DNA integrity, cell viability, and long-term proliferative potential of mouse leukemic L1210 cells. Selenite treatment at 1 to 10 µM resulted in concentration-dependent increases in DNA single strand and double strand breaks. Analysis of the pathways of enzymatic activity suggested that activation of endonucleases by selenite may have facilitated DNA double strand breaks and apoptotic cell death. It was suggested that this action might contribute to the anticancer actions of selenite against neoplastic cells.

A few reports are available on the effects of sodium selenite and sodium selenate on *in vivo* genetic toxicity assays. Norppa *et al.* (1980a) found increased chromosomal aberrations and sister chromatid exchanges in bone marrow cells of Chinese hamsters injected intraperitoneally with sodium selenite at 3 to 4 mg/kg.

Biswas *et al.* (1997) administered sodium selenite and sodium selenate by gavage to male Swiss mice at 7, 14, 21 and 28 mg/kg. These four doses were equivalent to one-eighth to half of the LD_{50} for sodium selenite and one-tenth to two-fifth of the LD_{50} for sodium selenate. Animals were sacrificed 6, 12, 18 and 24 hours later, and chromosome preparations were made from the bone marrow. Both salts induced chromosome breaks and spindle disturbances in bone marrow cells in a dose-related fashion. Sodium selenite was more cytotoxic than sodium selenate, and sodium selenite induced a slightly higher frequency of chromosomal aberrations than sodium selenate. Biswas *et al.* (1999b) also gavaged mice with sodium selenite and sodium selenate in aqueous solutions at 7, 14, 21, and 28 mg/kg to mice, and found similar effects on chromosome structure and spindle formation. The frequencies of aberrations induced were directly proportional to the doses and increased with exposure duration.

Gavage administration of sodium selenite one hour before sodium arsenite to Swiss mice at one-tenth of the LD_{50} significantly reduced the *in vivo* clastogenic effects of arsenite, including chromosomal aberrations and damaged cells from chromosome spreads in bone marrow cells recorded 24 hours after arsenite exposure (Biswas *et al.*, 1999a,b). The protective effect of selenite was less when the salts were given together, and there was no protection when arsenite was given before selenite (Biswas *et al.*, 1999a).

Results of other *in vivo* chromosomal aberration tests in mice (Norppa *et al.*, 1980b) and rats (Newton and Lilly 1986) were negative for sodium selenate. Itoh and Shimada (1996) examined the clastogenic effects in mouse bone marrow cells *in vivo* and found

that sodium selenate was not active for micronuclei induction at the tested doses of 3.75, 7.5 and 15 mg/kg intraperitoneally.

Biswas *et al.* (2000) found that *in vitro* exposure of human peripheral blood lymphocytes to sodium selenite was more clastogenic than exposure to sodium selenate.

Choy *et al.* (1989) found increased micronuclei in the bone marrow of one adult *Macaca fascicularus* monkey treated with L-selenomethionine at 600 µg/kg-day, a lethal dose for this species, for 15 days. However, nasogastric intubation of lower doses to pregnant macaques at 150 and 300 µg/kg-day at gestation days 20 to 50 did not affect the number of micronuclei in whole blood and bone marrow erythrocytes in the mother or the fetus.

Overall, the results from various genotoxicity assays seem to indicate that sodium selenite at relatively high concentrations *in vitro* at 10^{-6} to 10^{-3} M may exert a mutagenic and clastogenic effect. Near-lethal doses of sodium selenite have to be administered to animals to show this effect *in vivo* (Biswas *et al.*, 1999a,b). Sodium selenite is more potent than sodium selenate, and its actions may be potentiated in the presence of thiol donors such as glutathione. There are no published reports of genotoxic effects of sodium selenite, sodium selenate, or selenomethionine in humans.

Carcinogenic effects

The early literature on tumor-related toxicity of selenium in mammals was reviewed by Moxon and Rhian (1943). This review noted that rats fed seleniferous diets showed atrophied, necrotic, cirrhotic and hemorrhagic changes in the liver. Nelson and Fitzhugh (1943) were the first to suggest that selenium may produce liver carcinoma in rats. They fed Osborne-Mendel rats for two years on seleniferous wheat or corn or ammonium potassium selenide at 5, 7 or 10 ppm selenium. Histological examination of the liver suggested that adenoma or low-grade carcinomas developed in cirrhotic livers of 11/53 animals receiving selenium after 18 to 24 months on the diet. No tumors were reported in 73 rats living less than 18 months and the incidence of spontaneous hepatic tumors at 18 to 24 months was estimated as less than one percent. Incidence of lymphosarcoma, leukemia and spontaneous tumors of viscera other than liver were not different between the selenium-treated animals and other rats kept in the laboratory. The authors commented on the uncertainties of differentiating adenoma and carcinoma and on the borderline interpretation of nonmalignant and malignant tumors.

The National Cancer Institute sponsored an extensive study on selenium carcinogenicity in rats. Diets containing 0.5 to 16 ppm sodium selenite or sodium selenate were fed to 1,437 Wistar rats for their lifetime (Harr *et al.*, 1967; Tinsley *et al.*, 1967). No excess tumors were reported in rats fed these diets. Nonneoplastic liver effects such as hyperemia, cellular degeneration, binucleation of cells and mild hepatocyte proliferation were observed at concentrations of 4 ppm and higher.

Schroeder and Mitchener (1971a) administered sodium selenate or sodium selenite in drinking water to Long-Evans rats (approximately 50 male and 50 female rats per group at study initiation). Animals received 2 ppm (0.28 mg selenium/kg-day) for one year, then 3 ppm (0.42 mg selenium/kg-day) for the remainder of the study. The animals were observed for the duration of their natural lifespan of approximately 36 months. There

was suggestive evidence that selenate may have prolonged the lifespan of female rats and increased body weight gain, but the data were complicated by an epidemic of viral pneumonia that struck the colony when the rats were 21 months old and caused significant mortality. The authors claimed that the incidence of all tumors and of malignant tumors was statistically significantly increased in the selenate-treated rats compared with the controls. However, the statistical analysis failed to account for the fact that the selenium-treated rats lived longer than the controls. Incidence of all tumors in controls, selenate- and selenite-treated rats was 20/65 (30.8 percent), 30/48 (62.5 percent) and 4/32 (12.5 percent), respectively. Incidence of malignant tumors in the same groups was 11/65 (16.9 percent), 20/48 (41.7 percent) and 4/32 (12.5 percent), respectively. The earliest tumor occurred on day 833 in the control males, on day 633 in the control females, on day 344 in selenate-treated males and on day 633 in selenate-treated females. This study was not considered adequate for assessment because only the heart, lung, liver, kidney, and spleen tissues from necropsied animals were examined histologically, and an increase in longevity was observed in selenate-treated female rats. In addition, not all autopsied animals were histologically examined, and high mortality during the study as a result of a virulent pneumonia epidemic complicates the analysis.

Subsequently, Schroeder and Mitchener (1972) administered 3 ppm sodium selenate or sodium selenite in drinking water to Swiss mice (50 per sex per group). In the control group 23 of 119 animals (19 percent) had tumors, with 10 of the dosed animals developing malignant tumors (10/119, eight percent). Selenium-fed mice showed 13/88 (15 percent) tumors, all malignant. The authors concluded that selenium administered under these conditions did not affect the spontaneous incidence of tumors in mice.

Other studies indicate that selenium treatment may enhance neoplastic processes in the presence of a cancer initiator. Ankerst and Siogren (1982) found selenium treatment of rats increased the incidence of 1,2-dimethylhydrazine-induced tumors of the small intestine, while decreasing the incidence of tumors of the large intestine. These authors also found selenium treatment increased the incidence of virally-induced rat mammary tumors. Dorado *et al.* (1985) found that dietary exposure to either 4 or 6 ppm sodium selenite of rats treated with diethylnitrosamine after partial hepatectomy, then promoted with phenobarbital, showed modest reductions in the incidence of hepatic tumors, but nearly twice the incidence of renal tumors. Perchellet *et al.* (1987) noted that sodium selenite applied to the skin of mice may enhance the incidence of papillomas induced by topically applied 7,12-dimethylbenzanthracene. Birt (1989) and Birt *et al.* (1989) summarized studies in Syrian hamsters and SENCAR mice that showed selenium supplementation as being effective in inhibiting colon and mammary carcinogenesis, but possibly enhancing nitrosamine-induced carcinogenesis in skin, liver and pancreas. Woutersen *et al.* (1999) reported that cell proliferation in azaserine-induced preneoplastic pancreatic acinar lesions in male weanling SPF Wistar rats was higher in rats given selenium at 2.5 mg/kg, with or without β-carotene at 60 mg/kg via the diet, than in controls. Chen *et al.* (2000) noted that sodium selenate at 1.7 mg/kg in the diet for 40 weeks may have increased esophageal tumor incidence in a rat experimental model.

Toxicological Effects in Humans

Acute toxicity

A limited number of cases of acute selenium poisoning in humans has been reported (ATSDR, 2003; SCF, 2000; WHO, 1987). Most cases have involved occupational exposures such as workers in copper smelters or selenium rectifier plants due to inhalation of selenium-containing aerosols that are not soluble in water. Some have involved accidental consumption of various inorganic selenium compounds or organic selenium products (Combs, 2001), while other cases have involved selenium poisoning due to malicious or homicidal intent (Spiller and Pfiefer, 2007). These cases have demonstrated that acute selenosis can produce a garlic-like breath odor indicative of exhaled dimethyl selenide (NRC, 2000). Other symptoms of acute selenosis include nausea, vomiting, diarrhea, hypotension, respiratory distress, irritability, profound weakness and fatigue (NRC, 2000). Typical chronic selenosis symptoms such as hair dryness, hair loss, nail damage, and tenderness and swelling of fingertips may occur occasionally in acute selenosis. Approximate intake of up to 250 mg selenium as a single dose or 27 mg to 31 mg of multiple doses resulted in acute toxicity (SCF, 2000). Selenite, selenate, and selenomethionine are among the most acutely toxic selenium compounds (Combs and Combs, 1986).

A fatal case of acute poisoning in a 75-year-old Caucasian man who ingested about 10 g sodium selenite in water was reported in Australia (See *et al.*, 2006; Williams and Ansford, 2007). The man began to feel unwell about 60 to 90 minutes after the ingestion and complained of diarrhea upon arrival at the hospital. Symptoms at about 3.5 hours after ingestion included vomiting, diarrhea, abdominal pain, poor perfusion, hypotension, and hypokalaemia. He suffered a cardiac arrest about some six hours after ingestion. Post-mortem examination revealed congested lungs with intra-alveolar hemorrhage, severe coronary artery atherosclerosis, and adenocarcinoma of the prostate. Post-mortem arterial selenium level was 3 mg/L, which is consistent with reported blood selenium concentrations of 0.4 to 6.7 mg/L or 0.5 to 18 mg/L in other fatal cases (Bedwal *et al.*, 1993). In comparison, a daily dose of about 400 µg selenium corresponding to a blood selenium level of 0.56 mg/L is suggested as the maximum safe daily intake (Yang *et al.*, 1989a,b), and symptoms of selenosis were found at a daily intake of about 910 µg selenium corresponding to a blood level of 1.05 mg/L (Whanger *et al.*, 1996).

A fatal case of acute selenium poisoning in a 32-year-old man was reported in Hungary (Farago and Horvath, 1988). The unconscious male was admitted to a hospital about 40 minutes after suicidal ingestion of an unknown amount of sodium selenite solution, and died about 45 minutes later. Symptoms included sweating, garlic breath, nonpalpable blood pressure, hurried inspiration, muscle fascicular convulsion, peripheral cyanosis, and pulmonary edema. The selenium level was 0.5 µg/g in blood, 1.4 µg/g in lung, 1.4 µg/g in liver, and 520 µg/g in stomach, compared to normal levels of about 0.18 µg/g in blood, 0.21 µg/g in lung, 0.34 µg/g in liver, and 0.17 µg/g in stomach.

A fatal case of acute poisoning in a 22-year-old woman ingesting about 20 mL of sodium tetraoxoselenate (VI) solution with suicidal intent was reported in Poland (Lech, 2002). Initial symptoms were gastrointestinal disturbances including vomiting, abdominal pain,

and diarrhea. Later a strong smell of garlic or rotten onion in her breath appeared, and she died of cardiovascular failure.

Gasmi *et al.* (1997) identified eighteen cases of human poisoning, half of which were not fully documented. Of the 17 patients that ingested water-soluble selenium compounds, sodium selenite accounted for six cases, and sodium selenate for one case. Selenic acid, selenious acid, or selenium dioxide, which has not been found in drinking water, accounted for 10 cases. Oral ingestion of sodium selenate, selenious acid, and selenium dioxide at quantities resulting in toxic effects has been reviewed (Lockitch, 1989). Cases of accidental overconsumption of selenium supplement tablets in children have been reported to cause acute toxicity in children (SCF, 2000).

Ingestion of 1.7 g sodium selenite by an adult resulted in abdominal pain, vomiting, and diarrhea that resolved after one week (Gasmi *et al.*, 1997). The single case of selenate ingestion occurred in New Zealand and the patient survived, probably because she was forced to vomit soon after exposure (Civil and McDonald, 1978). A 15-year-old girl swallowed 400 mL of a sheep drench containing 5 mg/mL sodium selenate, for a dose of 22.3 mg selenium/kg or a total of 1.16 g of selenium. Her initial serum selenium concentration was 3.1 µg/mL. She had a strong garlic breath odor and frequent, loose, gray bowel movements. The patient was treated with gastric lavage, forced diuresis with dextrose, and administration of vitamin C and dimercaprol. She had transient electrocardiographic abnormalities and elevation of serum bilirubin, but normal serum glutamic-oxaloacetic transaminase and alkaline phosphatase activity. The patient was discharged and remained well six months after the incident.

Sioris *et al.* (1980) reported in an abstract on five patients who became ill after ingestion of "large amounts of sodium selenite intended as a turkey feed supplement." All five developed nausea, vomiting, diarrhea, abdominal pain, chills, and tremors shortly after ingestion. All symptoms resolved within 24 hours and the patients were well at an eight-month follow-up examination (Gasmi *et al.*, 1997).

As discussed earlier in this report, selenium dioxide dissolves in water and forms soluble selenious acid but neither selenium dioxide nor selenious acid has been detected in drinking water. Selenious acid is one of the most acutely toxic inorganic selenium compounds (Ellenhorn and Barceloux, 1988) and ingestion of commercial gun-blueing products containing one to four percent selenious acid or selenium dioxide has usually proved fatal (Mack, 1990). Coma, asystole, and apnea were reported in a 17-year-old male following ingestion of approximately 10 g of selenium dioxide (Koppel *et al.*, 1986). Death occurred about 45 minutes after admission, with a postmortem blood selenium concentration of 38 mg/L (Gasmi *et al.*, 1997; Koppel *et al.*, 1986).

Kise *et al.* (2004) reported a case of a 48-year-old woman in Japan who consumed 2 g of selenium dioxide in a bottle of glass blue used for stained glass manufacture, which corresponds to about 10 times the lethal dose in animals. About two hours after ingestion, she showed mildly altered consciousness and hematemesis, and vomited garlicky-smelling blood. Her serum and urinary selenium levels were about 24 mg/L and 13 mg/L, respectively. She was discharged from the hospital uneventfully on the 16th day after taking the selenium, although endoscopic follow-up was continued for six years due

to mucosal corrosive damage throughout the oral cavity, esophagus, and stomach caused by the selenium.

Selenium poisoning in some areas of South America has been associated with consumption of nuts of *Lecythidaceae* species, a large family of trees (ATSDR, 2003). The nuts, which include the Brazil nut, *Betholletia excelsa*, and the sapucaia nut, *Lecythis elliptica*, can be an important part of the diet in the region. Their flesh is rich in oils and protein. Some of these trees are selenium accumulators and can highly concentrate the element in their nuts. Brazil nuts, for instance, have been found to contain over 50 ppm of selenium (Reilly, 2000). Kerdel-Vegas (1966) described acute intoxication among residents of a seleniferous area of Venezuela who consumed the nuts of *Lecythis ollaria*, known as "Coco de Mono." Signs of poisoning included nausea, vomiting, diarrhea, dizziness, hair loss, and nail damage. The breath of those who were affected by eating the nuts was reported to be foul. One two-year-old boy died, apparently due to poisoning from selenium in the nuts he had eaten. The principal selenium compound in the nuts was identified as selenocystathione (Olivares *et al.*, 1967). Other cases were reported in the early 1970s of children living in a seleniferous area in Venezuela with typical signs of selenium toxicity such as dermatitis, loose hair and damaged nails, with elevated serum and urine selenium levels.

Subchronic toxicity

ATSDR (2003) reported no cases of death due to intermediate-term oral ingestion of water-soluble selenium compounds in humans. In an incident in the U.S. in 1983 to 1984, 13 persons suffered toxic effects from ingesting supplement tablets that contained high levels of selenium, the result of a manufacturing error. Jensen *et al.* (1984) described in detail one New York case due to this event; it was also mentioned in a U.S. FDA Drug Bulletin (U.S. FDA, 1984) and in an abstract by Helzlsouer *et al.* (1984). The selenium level was about 27 mg per tablet, 182 times higher than labeled, comprised of at least 25 mg of sodium selenite and 4 to 5 mg of elemental or organic selenium. Those exposed had total intakes of 27 to 2,387 mg of selenium. Symptoms of poisoning included nausea and vomiting, a sour milk breath odor, nail changes, hair loss, fatigue, and irritability.

The report of Jensen *et al.* (1984) on this incident concerned a 57-year-old woman who consumed 77 tablets at one tablet per day. Her tablets were found to contain 31 mg of total selenium per tablet instead of 150 µg per tablet as labeled. The woman noticed pronounced hair loss about 11 days after starting the selenium supplement. This progressed to almost total alopecia over a two-month period. Later, horizontal white streaking on one fingernail, along with tenderness and swelling of the fingertip and a purulent discharge in the fingernail bed, was noted. These changes subsequently progressed to involve all fingernails. The fingernail from the originally affected finger was eventually lost. She also had periodic episodes of nausea and vomiting, a sour milk breath odor, and progressive fatigue. In March 1984, her serum selenium concentration was 528 µg/L, two to four times the normal level for the U.S. population. Her estimated cumulative selenium dose was 2,387 mg. This woman was also taking other vitamin

supplements, and selenium toxicity was considered to have been mitigated by concurrent ingestion of large doses of vitamin C at a minimum of 1 g/day.

Other persons who had consumed the mislabeled selenium pills had similar manifestations with varying degrees of severity (Helzlsouer *et al.*, 1984). All 13 persons experienced nausea following tablet ingestion, and seven reported abdominal pain and watery diarrhea. Other symptoms included garlic breath odor, nail and hair changes, dryness of hair, peripheral neuropathy, fatigue, and irritability. About half of the patients experienced hair loss, and about one third lost nails. Selenium levels in red blood cells were elevated for as long as five months following exposure. Selenium level in toenail samples was 8.1 ppm, the highest ever reported in the U.S. Laboratory evaluation of eight of the 13 cases, including the woman described by Jensen *et al.* (1984) who had taken the largest dose, showed no abnormality of liver and renal function.

Clark *et al.* (1996a) reported a case of selenium poisoning from a nutritional supplement. A 36-year-old man was referred to San Diego Regional Poison Center for evaluation of alopecia, nail changes, and paresthesias four weeks after initiating vitamins as part of holistic therapy for fatigue. The vitamin label indicated 5 µg selenium per six tablets, but the tablets contained 3,000 to 6,000 times the labeled amount, according to a U.S. FDA analysis. The man developed loose stools after taking two tablets every hour and reduced consumption to 10 tablets a day. Over the first week he developed hair loss, a tingling sensation in the extremities and scalp, and his fatigue became worse. He continued taking 10 tablets a day for the second week during which he became completely bald. He stopped the vitamins by that time and noted color changes in nails of his finger and toes several days later. He was examined two weeks after discontinuation of therapy with early regrowth of hair, yellow-white and red transverse lines on nails, and a serum selenium level of 8.26 µmol/L, compared to a laboratory normal range of 0.7 to 1.65 µmol/L. His serum selenium level returned to normal two months later.

Subchronic selenosis was reported in a 17-year-old male and a 11-month-old female, both cystic fibrosis patients, after consumption of yeast selenium containing primarily selenomethionine (Rossoff, 2002). Toxicity was exhibited in the teenage boy after consuming the selenium tablets at 400 µg/day for two weeks. Symptoms were observed in the baby girl after consuming a yeast selenium complex at 25 µg/day for two months.

Duffield *et al.* (1999) studied the effects of ingestion of a placebo or 10, 20, 30, or 40 µg selenium/day as L-selenomethionine daily for 20 weeks in a New Zealand study. The 52 adults including 17 men and 35 women aged 19 to 59 years had an initial low blood selenium without signs of deficiency at an unsupplemented intake of 28 to 29 µg/day. Decreased thyroxine T_4 concentrations were reported in the supplemented groups.

Hagmar *et al.* (1998) studied 68 Latvian adult male fish consumers from Russia, age 24 to 79 years, without measurements of dietary selenium intake in a cross sectional study conducted in Sweden. The mean plasma selenium level in those with a high fish intake, at 21 to 50 fish meals per month, was 81 percent higher than in those with lowest fish intake. A significant inverse correlation was reported between serum levels of selenium and SelP versus thyroid stimulating hormone.

Hawkes and Turek (2001) fed 11 healthy North American men aged 20 to 45 years a controlled diet of foods naturally high or low in selenium for 120 days while confined in

a metabolic research unit, in a randomized blinded study. Results suggested that dietary selenium in food modulated thyroid hormone metabolism and serum triiodothyronine T_3 concentrations, which led to changes in energy metabolism and subsequent changes in body weight and composition. Dietary selenium was 47 µg/day or 0.0006 mg/kg-day for the first 21 days, then changed to 14 µg/day or 0.0002 mg/kg-day in six subjects, or to 297 µg/day or 0.004 mg/kg-day in five subjects for the remaining 99 days. Serum T_3 decreased and thyroid stimulating hormone increased in the high selenium group, suggesting that a subclinical hypothyroid response was induced by the high selenium diet, about 0.085 mg/kg-day. T_3 and triacylglycerols increased, accompanied by losses of body fat in the low selenium group, about 0.004 mg/kg-day, suggesting that a subclinical hyperthyroid response was induced by the low selenium diet. T_3 was significantly different between groups from day 45 onward, and by day 64, body weight began to show differences and was significantly different between groups from day 92 onward (Hawkes *et al.*, 2001, 2003; Hawkes and Keim, 2003; Hawkes and Turek, 2001). No effects of dietary selenium at 0.0048 mg/kg-day on mood were reported. No hematological, endocrine, immunological, or reproductive effects were observed at 0.0039 mg/kg-day in these healthy men (Hawkes and Turek, 2001).

Selenium supplementation at 100 µg/day in 50 British subjects in the United Kingdom in a double-blind cross-over randomized controlled trial was associated with a significant improvement of self-reported mood on the Profile of Moods States questionnaire and in particular, a decrease in anxiety after both 2.5 and five weeks of treatment. The effects were most notable in those with a selenium-deficient diet at baseline based on a food frequency questionnaire (). However, the study could not differentiate whether people with mood symptoms have lower selenium status than the general population, or whether people with mood symptoms have a higher need for selenium. The lower the level of selenium in the diet the more reports of anxiety, depression, and tiredness decreased following five weeks of selenium therapy.

Developmental and reproductive effects

Reproductive effects

Between September 1972 and September 1988, a few thousand residents in Reggio Emilia, in northern Italy, were accidentally exposed to drinking water containing selenate levels of 7 to 9 µg/L, higher than the usually low levels of selenium, through a local public water supply system. Vinceti *et al.* (2000a) studied reproductive measures in 18 women exposed to this tap water. No deleterious effect was found on overall body weight and length of newborns and stillborns delivered by the women. The rate of spontaneous abortions, however, was increased slightly (rate ratio 1.73, 95 percent confidence interval 0.62 to 4.8), compared with the rate among unexposed women from the same municipality. From 1980 through 1988, the prevalence of congenital malformations at birth among the exposed population was similar to that expected, but estimates were imprecise owing to small numbers. These results do not suggest marked effects on human reproduction from chronic exposure to selenate in drinking water at levels lower than 10 µg/L.

High concentrations of seminal plasma selenium were suspected to be harmful to male fertility (Bleau *et al.*, 1984). Supplementation of the diet with selenium compounds has been associated with both beneficial and deleterious effects on sperm function, depending on the dose and chemical form of selenium. A mean selenium concentration of 71.3 ± 29.7 ng/mL in semen was measured in 125 men from couples consulting for infertility, with a range of 7 to 230 ng/mL. Sperm motility was maximal at semen selenium levels between 50 and 69 ng/mL; motility decreased and the incidence of asthenospermia increased above or below this range of selenium levels. A follow-up 4.5 to five years later revealed that semen selenium levels below 35 ng/mL were associated with male infertility. Semen selenium levels greater than 80 ng/mL were associated with a high abortion rate and signs of ovarian dysfunction in the partner with similar diet and environmental exposure. A selenium level between 40 and 70 ng/mL was optimal for reproductive performance with a high pregnancy rate and low abortion rate.

The Hawkes and Turek (2001) 120-day selenium dietary study (described in more detail above) associated decreased sperm motility with high selenium in food. Dietary selenium was 47 µg/day for the first 21 days, then either 14 µg/day or 297 µg/day for 99 days, resulting in significant changes in selenium concentrations in blood and semen. Seminal plasma selenium concentration increased 50 percent with the high selenium ingestion, and decreased 40 percent with the low selenium diet. The fraction of motile sperm in the high selenium group decreased by 32 percent by week 13 and ended at 18 percent below the baseline. Selenium concentrations changed in seminal plasma but not in sperm, and serum androgen concentrations were unchanged in both groups, indicating this effect was neither androgen dependent nor caused by a change in the selenium supply to the testes. Serum T_3 decreased and thyroid-stimulating hormone increased in the high selenium group, suggesting that altered thyroid hormone metabolism may have some effects on sperm motility. Although this decrease in sperm motility does not necessarily predict decreased fertility, the increasing frequency of use of selenium supplements suggests the need for larger studies to more fully assess this potential side effect.

Noack-Fueller *et al.* (1993) found significant positive correlations between semen selenium levels and sperm concentration ($r = 0.51$, $p < 0.05$), and percentage of normally formed sperm ($r = 0.46$, $p < 0.05$), respectively. In a Croatian study, serum selenium blood levels in 23 normospermatic men (71.0 ± 17.7 µg/L) and in 18 men with low sperm counts (57.7 ± 12.0 µg/L) differed significantly ($p < 0.01$) (Krsnjavi *et al.*, 1992).

In 221 Singapore men, positive correlations were observed between concentrations of selenium in seminal plasma and sperm density in normospermic men but not in oligozoospermic men (Xu *et al.*, 1993). The correlation coefficient with sperm density for selenium in seminal plasma was 0.35 ($p < 0.05$). However, no significant correlation between selenium level in the seminal plasma and sperm count or motility was reported in semen samples from 211 normozoospermic, oligozoospermic, asthenozoospermic, and azoospermic men examined (Roy *et al.*, 1990).

In healthy Chinese adults, semen selenium concentrations were associated with sperm quality and sperm 8-hydroxy-2'-deoxyguanosine levels (Xu *et al.*, 2001). Mean semen concentration of selenium was significantly higher in fertile subjects at 0.74 µmole/L than in infertile subjects at 0.56 µmole/L, while there was no significant difference in serum selenium concentration between the groups. Semen volume (3.91 mL versus 2.79

mL), sperm density (46.4×10^9/L versus 36.5×10^9/L), and sperm count (168×10^6 versus 102×10^6) were significantly higher in fertile subjects than in infertile subjects. A significant positive correlation was observed between semen selenium concentration and sperm density ($r = 0.48$, $p < 0.01$ for fertile subjects; and $r = 0.32$, $p < 0.05$ for infertile subjects) as well as sperm count ($r = 0.32$, $p < 0.05$ for fertile subjects; and $r = 0.26$, $p < 0.05$ for infertile subjects), sperm motility ($r = 0.46$, $p < 0.01$ for fertile subjects; and $r = 0.65$, $p < 0.01$ for infertile subjects) and viability ($r = 0.33$, $p < 0.05$ for fertile subjects; and $r = 0.64$, $p < 0.01$ for infertile subjects). Serum concentrations of selenium did not correlate with sperm quality and oxidative DNA damage in human sperm.

Foresta *et al.* (2002) collected sperm from 75 infertile men and 37 controls and analyzed for fertility-related parameters according to WHO criteria, in a study in Italy. They reported a correlation between the sperm content of GPx4 (a selenoprotein), measured as rescued activity, and human fertility. Rescued GPx4 activity of infertile men at 93.2 ± 60.1 units/mg sperm protein was significantly below that of controls, at 187.5 ± 55.3 units/mg. The activity was 61.93 ± 45.42 units/mg in oligoasthenozoospermic specimens ($p < 0.001$) compared with controls and asthenozoospermic samples. Rescued sperm GPx4 activity was correlated positively with viability, morphological integrity, and most profoundly, with forward motility ($r = 0.35$, 0.44, and 0.45, respectively). In isolated motile samples, motility decreased faster with decreasing sperm GPx4 content. Thus in humans, sperm GPx4 appears to be indispensable for structural integrity of spermatozoa and to codetermine sperm motility and viability.

Zhao *et al.* (2001) studied selenium levels in the vein blood of ovary tissue of the chocolate cyst wall and chocolate cyst fluid in 32 operated patients with endometriosis and 23 operated patients with endometrioma in China. Selenium levels were significantly lower than those in a control group ($p < 0.01$) of 30 patients with hysteroma. The selenium level in uterine muscle tissue of endometriomin vein blood was significantly higher than in the control group ($p < 0.01$). The endometriosis and endometrioma were considered to be associated with a metabolism disturbance of oxygen free radical in which selenium may play a role.

In a rural Colorado community exposed to 50 to 125 µg selenium/L drinking water (Tsongas and Ferguson, 1977), fifty-eight women having a history of miscarriage were reported to have higher urinary selenium levels, with a mean of 158 µg/L, than did reference women, with a mean of 80 µg/L. A preliminary study was performed in China on pregnant women with high risk factors of pregnancy-induced hypertension (Han and Zhou, 1994). One hundred women were given 100 µg/day of selenium supplement for six to eight weeks during late pregnancy, and 48 controls were given placebo. The results suggest that the selenium supplement may reduce the incidence of pregnancy-induced hypertension and gestational edema.

Developmental effects

According to ATSDR (2003), no studies have demonstrated that selenium compounds are teratogenic in humans by oral exposure. Anecdotal reports from Colombia, South America, suggested that women living in seleniferous areas gave birth to malformed babies, but there is a lack of reliable studies (Rosenfeld and Beath, 1964). A review by

Jaffe (1973) identified no observations of teratogenic action of dietary selenium in human beings. Human acute and endemic chronic selenium intoxication cases have not been associated with teratogenic effects (Fan, 1992).

Levels of selenium in the drinking water of 453 Nebraska communities in 1986 were compared with health data including the rate of birth defects. Water samples from 42 communities exceeded the 0.01 mg/L state standard for selenium. Statistical analysis using Pearson's correlation coefficient method showed no significant relationship between selenium and any of the health effects studied. However, this type of analysis cannot be interpreted as proving or disproving a cause and effect relationship (Bednar and Kies, 1991). No correlation could be demonstrated between the selenium level in the urine of school children in different Venezuelan states and the incidence of infant mortality due to congenital malformations (Jaffe and Velez, 1973).

Robertson (1970) reported on the outcome of pregnancies in a laboratory in which eight females handled sodium selenite to prepare microbiological media over a five-year period in England. Of the five pregnancies among six females, four ended in spontaneous abortion before term and the only one which went to term resulted in an infant with bilateral clubfoot. Simple clinical factors could possibly account for two of these miscarriages. The urinary selenium levels in all subjects were similar to those in other individuals in the area. Another laboratory employed 10 women over a five-year period to handle selenite; only one was married and she had a miscarriage in early 1969. Inquiries by the author at other laboratories carrying out comparable work did not show evidence of similar problems. The limited number of cases, possible exposure to other toxic agents, and other confounding factors leave the relationship between sodium selenite and developmental effects inconclusive. However, other studies (Jaffe and Velez, 1973; Shamberger, 1971) did not find an association between selenium exposure and teratogenicity.

No significant difference was found in concentrations of serum selenium in 19 pairs of pre-eclamptic women (median 55.6 ng/mL) and matched controls (median 48.5 ng/mL) of average gestation 230 days or 33 weeks in the United Kingdom (Rayman *et al.*, 1996). The serum selenium levels for both groups were less than half those of pregnant women in the U.S., but were not in the deficiency range identified with Keshan Disease (21 ng/mL) in China. Infant birth weights were significantly lower in the pre-eclamptic group. Interpretation of serum selenium measurements from the third trimester of a pre-eclamptic pregnancy is complicated by the reduced fetal growth and probable lower selenium uptake by the fetus in such a pregnancy.

Zierler *et al.* (1988) performed a case control study of 270 children born in Massachusetts with severe congenital heart disease and 665 controls randomly selected from birth certificates. The study compared the selenium concentrations in the public drinking water supply used by the mothers close to the time of conception to the selenium concentrations in the water consumed by the controls. The results indicate that selenium exposure via drinking water appeared beneficial, but many variables are unknown. Possible confounders included no adjustment for age, parity, tobacco, alcohol, drug use, or socioeconomic status, other sources of selenium in the mothers' diet and environment, the amount of drinking water consumed, and the selenium concentrations in the water within the 25-day period of cardiogenesis (Zierler *et al.*, 1988). Another study showed

that premature infants with higher plasma selenium levels had less respiratory morbidity than premature infants with lower plasma selenium. The difference between the two groups was about 7.9 pg selenium/L (Darlow *et al.*, 1995).

Eisenmann and Miller (1990) studied selenium transport across the human placenta in a dually perfused human placental lobule *in vitro*. Following a two-hour control period, [^{75}Se]-labelled sodium selenite at an initial selenium level of 2 or 20 nmole/mL was added to the maternal circulation, and both fetal and maternal perfusates were recirculated for four hours. Time to peak selenium in the maternal artery, maternal vein, fetal vein and artery were 5 ± 1, 11 ± 2, 121 ± 17 and 159 ± 32 minutes, respectively. Fetal perfusate selenium was 70 ± 17 percent of maternal concentration following four hours of exposure, with 47 ± 11 percent of the recovered selenium in the placenta. At an initial selenium concentration of 20 nmole/mL, the perfused placenta contained 10 times the selenium found in the maternal perfusate. Although selenium concentrated in the placenta and rapidly appeared in the fetal circulation, exposure to selenium for four hours did not affect fetal volume, fetal arterial pressure, net oxygen transfer, placental oxygen and glucose consumption, lactate production, and human chorionic gonadotropin (hCG) secretion. Additional studies at an initial selenium concentration of 40 nmole/mL in maternal perfusate were used to examine the toxicity on the placenta, but the result was not reported in this abstract (Eisenmann and Miller, 1990). Selenate exposure at 40 µmole for 24 hours increased thromboxane B2 production. Three exposures to selenite at 6 µmole for 32 hours significantly decreased 6-keto-prostaglandin F1 α production with no effect on thromboxane B2 production or tissue GPx activity. Following two exposures to selenite at 20 and 40 µmole for 24 hours, thromboxane B2 production was significantly increased, while tissue 6-keto-prostaglandin F1 α production and tissue GPx activity were significantly decreased. These results indicate that selenite, but not selenate, can directly affect the human placenta by producing changes in the thromboxane B2 to 6-keto-prostaglandin F1 α ratio, the inactive hydrolysis products of two vasoactive substances thromboxane A2 and prostacyclin, which may be related to increased vasoconstriction and blood coagulation (Eisenmann and Miller, 1995).

Chronic toxicity

Chronic selenium exposure produces external manifestations of hair loss and nail deformity as well as systemic adverse effects in gastrointestinal and neurological systems (ATSDR, 2003; WHO, 1987). Occupational chronic exposure to selenium is mainly through the air and in some cases by direct dermal contact. In India, a 31-year-old male working in manufacture and maintenance of photocopy drums using selenium alloy for four years suffered from brittle nails and total hair loss including eye brows and lashes, starting after about six months on the job (Srivastava *et al.*, 1995). His blood selenium was 0.5 mg/L and nail selenium was 2.04 mg/kg compared to a mean serum selenium of 0.046 ± 0.013 mg/L from five coworkers without selenium exposure.

Epidemiological studies and case reports have shown chronic exposure to environmental selenium is associated with adverse health effects such as dermal, cardiovascular, hematological, musculoskeletal, hepatic, endocrine, neurological, and immunological effects in humans (Vinceti *et al.*, 2001). Some probable human cases of chronic selenium

intoxication with coarse skin and brittle hair were described in 1560 by a Spanish missionary, Father Pedro Simon, in Colombia, South America. Reported symptoms were similar to those observed in animals suffering from alkali disease (Reilly, 2006).

Selenosis-associated skin lesions in humans were most often related to food intake in the 1940s in South and North Dakota, Montana, Nebraska, and Wyoming. The selenium concentration in drinking water is rarely high enough to prduce toxic effects in humans. However, reports in 1955 described toxic corn and streams that had no animal life; men and animals using the streams for drinking water showed loss of hair and horses suffered hoof damage (Rosenfeld and Beath, 1964). Although the specific selenium compounds in these cases have not been completely identified, the water-soluble and bioavailable ones discussed in this document are considered as the relevant toxic agents.

NRC (2000) recommended a safe and adequate selenium intake range of about 50 to 200 µg/day for adults. Health Canada (1992) identified a minimum toxic intake level for adults as about 500 to 700 µg/day. An intake less than about 20 to 30 µg/day has been considered inadequate for selenium essentiality (Health Canada, 1992). Selenium daily requirement was estimated as about 50 to 200 µg/day or a minimum of 1 µg/kg-day, based on studies on its essentiality in human nutrition as a part of the GPx system (Tato Rocha *et al.*, 1994). Lo and Sandi (1980) reported a maximal acceptable selenium intake for each person as about 500 µg/day. The estimated normal dietary daily intake for man in most parts of the world ranges from 4 to 35 µg/day in infants and from 60 to 250 µg/day in adults (Lo and Sandi, 1980).

The extensive work by Yang and colleagues on selenium in China, both on deficiency and toxic levels, has been discussed elsewhere in this document. Based on the Yang *et al.* (1987) value of 40 µg/day to support maximal GPx activities, Combs (1993) summarized the conclusion of a workshop as a minimum selenium requirement of about 0.87 µg/kg-day or 70 µg/day for typical American adult males weighing 79 kg and 55 µg/day for adult females weighing 63 kg. Based on the Yang *et al.* (1989a,b) studies, Combs (1993) summarized the conclusion of a workshop as a maximum selenium intake of about 395 to 600 µg/day for typical American adult males and about 315 to 475 µg/day for adult females. Combs (1997) summarized the conclusion of a workshop as a maximum selenium intake of about 350 to 400 µg/day for typical American adult males weighing 70 kg.

Selenosis resulting in hair and nail loss as well as nervous system and skin disorders has been reported in humans in several areas in China (Yang *et al.*, 1983). Yang and Xia (1995) estimated the maximum safe dietary selenium intake adjusted with an uncertainty factor to be about 400 µg/day or 7 µg/kg for adult males based on selenosis (Xia, 1996;Yang *et al.*, 1989a,b; Yang and Zhou, 1994). This is comparable to the 5 µg/kg proposed as the maximum safe daily oral doses over extended periods (Olson, 1986). The mean safe intake of selenium was estimated to be 11 µg/kg (Yang and Xia, 1995). A blood selenium level of about 952 µg/L (Yang *et al.*, 1989a,b; Yang and Xia, 1995; Yang and Zhou, 1994), corresponding to a mean maximum safe selenium intake of about 800 µg/day or 15 µg/kg, is comparable to the 813 µg/L blood selenium level found among Venezuelan children without clear signs of selenium poisoning (Jaffe *et al.*, 1972).

Sakurai and Tsuchya (1975) estimated that consumers of large amounts of fish may ingest as much as 500 µg/day of selenium. This was proposed as the tentative maximum acceptable daily intake of selenium for protection of human health in Japan. The normal food intake of selenium was estimated as about 100 µg/day, half from fish and shellfish in an average adult Japanese with negligible intake from other sources. The range of the margin of safety was estimated as 10 to 200 times the normal level on the basis of human and animal toxicity data. Wada *et al.* (1993) in Japan estimated the selenium daily requirement as approximately 100 µg/day, ranging from 40 to 230 µg/day. They estimated an excessive level of about 200 µg/day, a toxic level with potential symptoms of approximately 1,000 µg/day (240 to 1,500 µg/day), and a highly toxic level with selenosis of approximately 5,000 µg/day (3,200 to 6,700 µg/day).

Field investigations in the mid-1930s in seleniferous areas of eastern Wyoming, southern South Dakota, and northern Nebraska found that the average dietary intake of selenium in the region was about 10 to 200 µg/kg, or 700 to 1,400 µg/day for a 70-kg adult male, without major symptoms of chronic selenium poisoning. Higher intake levels were associated with dizziness, clouding of the sensorium, extreme lassitude, depression, and moderate emotional instability (Lemley and Merryman, 1941; Smith and Westfall, 1937). Longnecker *et al.* (1991) found that some American adults had chronic selenium intakes as high as 724 µg/day without symptoms.

One clinical trial with 1,312 patients aged 18 years to 80 years (a mean of 63 years) using supplement at about 200 µg selenium/day showed a decrease in total cancer mortality and incidence. However, 35 patients, 21 in the selenium group and 14 in the control group, complained about gastrointestinal adverse effects and withdrew from the study (Clark *et al.*, 1996b, 1998). The use of selenium doses three- to six-fold above the Recommended Dietary Allowance of 55 µg/day (NRC, 2000) as an antineoplastic or cancer preventive agent for humans has been reported (Combs, 2005; Combs and Lu, 2001; Finley, 2005a; Klein, 2004; Lipinski, 2005; Meuillet *et al.*, 2004; Rayman, 2004; Taylor *et al.*, 2004; Trumbo, 2005; Whanger, 2004; WHO, 1996b; Willett, 1986) without indication of selenium toxicity. Rayman (2004) summarized human studies in Arizona, China, United Kingdom, and Denmark showing that the chronic administration of selenium yeast, which is less acutely toxic than inorganic selenium in animal studies (Spallholz and Raftery, 1987; Vinson and Bose, 1987), at about 100 to 800 µg selenium/day, provides no evidence of selenium toxicity. A small group of patients with rheumatoid arthritis receiving 250 µg/day as organic selenium for six months in addition to diet had decreased somatomedin C levels in serum compared to the controls (Thorlacius-Ussing, 1990).

In a supplement study in Norway, selenium, as selenite or selenomethionine , was given at 450 or 500 µg/day to 32 healthy women for three months. No toxicity was observed, but half of the women reported depression and extreme tiredness during the month following termination of the study (Meltzer and Haug, 1995). Similarly, no toxic effects were observed when selenium doses of 100, 200, or 300 µg/day as selenium wheat were given to healthy Norwegian volunteers for six weeks (Meltzer *et al.*, 1993). Similarly, no toxicity was observed with doses of 68 to 724 µg/day as natural selenium to healthy Northern Americans (Salbe *et al.*, 1993); with 200 µg/day as selenium-rich wheat bread to six healthy males for six weeks (van Dokkum *et al.*, 1992); or doses of 32.4, 206, or

388 µg/day as selenium-rich wheat bread to four healthy males for six weeks with a prior intake of 80 µg/day (Longnecker *et al.*, 1993).

Excessive intake of organic selenium in the diet (Rosenfeld and Beath, 1964) or of sodium selenite tablets (Jensen *et al.*, 1984) produces a garlic breath odor from exhalation of dimethyl selenide, and disorders of the skin and its appendages (Combs, 2001). Alopecia, skin rashes, and brittle and malformed nails are characteristic signs of selenium toxicity in humans (Jensen *et al.*, 1984). The selenosis studies in China used loss of hair and finger and toe nails for the diagnosis of selenosis. Slow-healing skin lesions mainly on the limbs and the neck, mottled teeth, and abnormalities of the nervous system including sensory and motor dysfunctions have also been noted in some cases (Yang *et al.*, 1983; Yang and Xia, 1995; Yang and Zhou, 1994).

Little information is available on the human toxicity of selenium in drinking water alone. Rosenfeld and Beath (1964) reported selenosis in an American Indian family consuming well water from the Wasatch geological formation containing 9 ppm selenium. All food consumed by the family was free of selenium. The five boys and girls ranging in age from six months to 10 years suffered total or partial loss of hair, discoloration of fingernails which became brittle and dropped off, and lassitude. The parents and the family dog also showed symptoms of selenium poisoning. Upon discontinuation of use of the well water, regrowth of nails and hair began. The children showed increased mental alertness by doing better in school.

Valentine (1997) reported selenium levels in a well used by an Ute Indian family near Ignacio, Colorado as high as 9 ppm. After consuming the water for more than three months, hair loss, weakened nails, and listlessness was reported in the family members; hair loss was also observed in the family dog (Rossoff, 2002).

In a population living on selenium-rich ranches in the western U.S., whole blood selenium concentrations ranged from 0.18 to 0.67 mg/kg (Longnecker *et al.*, 1991). Doses were calculated as 0.0098 to 0.01 mg/kg-day, and an estimated maximum intake of 724 µg/day (for only one or a few individuals). No evidence of musculoskeletal, hepatic, or dermal effects was observed (Longnecker *et al.*, 1991), in contrast to the observations in China for similar exposure levels (Yang *et al.*, 1989a).

Table 14, adapted mainly from ATSDR (1996, 2003), lists the NOAEL and LOAEL from human studies with chronic oral exposure to organic selenium compounds from foods. The highest NOAEL value is listed for various toxic effects as well as the reliable LOAEL value following chronic oral exposure to organic selenium compounds.

Chronic selenium toxicity was reported in individuals ingesting selenium at 3.2 to 6.7 mg/day. Ingestion of approximately 5 mg selenium/day has caused hair and nail damage, neurological and gastrointestinal symptoms, occasionally jaundice and dermatitis (Hall and Rumack, 2004). A blood selenium concentration under 1 mg/L would probably predict no serious damage, and levels over 2 mg/L are probably predictive of serious toxicity (Gasmi *et al.*, 1997).

Table 14. NOAEL and LOAEL of Chronic Oral Exposure in Humans

Exposure duration and route	Chemical species	System endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
years, food	organic selenium	dermal, neurological	0.022 0.027	0.046 0.058	U.S. EPA, 1990; Yang *et al.*, 1983
years, food	organic selenium	cardiovascular, hematologic, hepatic, dermal	0.025 0.015 0.025 0.015		Yang *et al.*, 1989a
lifetime, food	organic selenium	dermal	0.015	0.023	Yang and Zhou, 1994
more than 2 years, food	organic selenium	musculoskeletal, hepatic, dermal	0.0098 0.0098 0.0098		Longnecker *et al.*, 1991

Yang *et al.* (1983) described a case of a 62-year-old male who took a 2 mg sodium selenite tablet for two years until he was worried that he had became intoxicated. The individual had no symptoms of indisposition but had thickened, fragile nails and garlic odor. After he stopped taking the tablets, the surface of the new nail growth became smooth and he gradually recovered. Yang *et al.* (1983) considered the daily intake of about 1 mg of selenium from the sodium selenite tablet to be sufficient for chronic intoxication. This toxic dose is different from the estimated chronic toxic dose for intake of organic selenium compounds of about 5 mg selenium/day as discussed later in this document.

Hira *et al*. (2004) evaluated selenium exposures leading to selenosis symptoms in a rural population in the Punjab area of India. Mean selenium intake in the endemic area was reported as 632±31 (SE) µg/day in men and 475±53 µg/day in women (n = 40 each sex), versus 65±2 µg/day in men and 52±1 µg/day in women (n = 40 each) in a non-endemic area. The authors reported that in the endemic area 17.5 percent of the men and 15 percent of the women showed loss of hair, while women showed more of the other symptoms, including discolored or lost fingernails and tooth staining; men and women had about the same concentrations of selenium in hair (~250 µg/100 g) and nails (~400 µg/100 g). The authors suggested that 600 µg/day is the maximum limit of acceptable selenium intake and that more than 819 µg/day results in metabolic changes (selenosis).

A decade-long Nutritional Prevention of Cancer trial in the U.S. was conducted with 1,312 patients, mean age 63 years (range 18 to 80 years), whose baseline selenium intake was about 90 µg/day. Participants with a history of basal cell or squamous cell carcinomas of the skin were treated with either 0 or 200 µg selenium/day supplied as a selenium-enriched brewer's yeast tablet for 4.5 ± 2.8 (mean ± standard deviation) years and a follow-up of 6.4 ± 2 years. Twenty-one subjects in the selenium group and 14 controls complained of gastrointestinal upset and withdrew from the study (Clark *et al.*, 1996b, 1998). In the treated group, plasma selenium increased up to about three months,

reaching a plateau at about 190 µg/L by one year (Rayman, 2004). This plasma selenium level is below that associated with selenosis at 1,054 to 1,854 µg/L in whole blood, which is generally 23 percent higher than in plasma, as reported by Yang *et al.* (1989a,b).

Cancer mortality was evaluated among the few thousand residents in Reggio Emilia, northern Italy, who were exposed by accident to tap water contaminated with 7 to 9 µg/L selenium as selenate between 1972 and 1988. Vinceti *et al.* (1998, 2000b,c) analyzed the 12-year mortality in a cohort of 2,065 residents in this area from 1986 to 1997 compared to mortality in the remainder of the local population with less than 1 µg/L of selenium exposure. Mortality from malignant neoplasms appeared to increase as shown by a standardized mortality ratio of 1.17 with a 95 percent confidence interval of 0.96 to 1.42, mainly due to an excess mortality from melanoma and colonrectal cancer in both sexes, kidney cancer in men, and lymphoid malignancies in women (Vinceti *et al.*, 2000b).

The association reported by Vinceti *et al.* (1998) between malignant melanoma and exposure to selenium in drinking water is hampered by lack of information about potential life style confounders, the fact that the exposure could only be characterized by a simple dichotomization, and the inconsistencies of most estimates between the two genders. Their conclusion was challenged by Clark and Jacobs (1998) as data were not presented to indicate the cases actually had higher selenium intake than controls. In addition, an earlier case control study with 101 cases in Germany, a region with a selenium status similar to that of Italy, observed a significant inverse association between malignant melanoma and serum selenium levels (Reinhold *et al.*, 1989). These results do not suggest marked effects on human cancer from chronic exposure to selenate in drinking water at levels lower than 10 µg/L (Vinceti *et al.*, 2000b,c).

There was no significant increase in overall cardiovascular mortality in the Italian population exposed to selenium (standardized mortality ratio [SMR] of 1.05, 95 percent confidence interval 0.89 to 1.23), despite a higher cerebrovascular mortality (SMR 1.43, 95 percent confidence interval 1.03 to 1.93). Coronary disease mortality decreased slightly as shown by an SMR of 0.87 (95 percent confidence interval 0.63 to 1.16), due to a low mortality among women (Vinceti *et al.*, 2000b). Vinceti *et al.* (1994) also analyzed the seven-year temporal distribution of deaths for coronary disease and for stroke in 4,419 individuals exposed for at least five years to the drinking water. From January 1986 until August 1988, when tap water selenium was 7 µg/L, deaths for coronary disease were one in males and two in females. After the decrease in drinking water selenium to less than 1 µg/L, 21 and 10 coronary deaths were observed, respectively, in males and in females from September 1988 to December 1992. No significant difference in the temporal distribution of stroke deaths was observed in males or females.

Cancer incidence was evaluated versus selenium levels in drinking water from 34 water treatment plants in Aomori Prefecture in Japan. A significant standardized partial regression coefficient ($p < 0.01$) was observed for selenium versus age-adjusted rectal cancer incidence (Kikuchi *et al.*, 1999) and gastric cancer mortality rate (Nakaji *et al.*, 2001). These results indicate a need for further study of selenium in drinking water and food, and relationship to rectal and gastric carcinogenesis.

Eye effects

In contrast to the results of cataracts developed within days of selenium exposures reported in animal studies (Flohe, 2005) discussed earlier in this review, Knekt *et al.* (1992), in a case control study nested within a cohort study, found that serum selenium levels of 47 patients with senile cataracts, collected over a 15-year period, were not different from 94 controls matched for age, gender, and municipality.

Tooth effects

Selenium can replace sulfur in collagen, which is the most important component of the organic matrix of the tooth, and the resulting bond is stronger than a sulfur bond. In earlier field studies by Hadjimarkos (1965), there appeared to be a relationship between urinary levels of selenium and increased risks of dental caries in children in Oregon and Wyoming. Similar effects were suspected in studies in India of selenium in drinking water (Gauba *et al.*, 1993), and tooth decay was also a consideration in the Chinese selenosis cases (Yang *et al.*, 1983).

However, these tooth effects were not confirmed in studies on populations in New Zealand, and the World Health Organization Task Force Group (WHO, 1987) concluded that there is no clear-cut evidence of an association between excess selenium ingestion and caries in humans because the index of selenium exposure was inadequate in the population studies and because of the possibility of other interfering environmental factors. Lafond and Calabrese (1979) also reviewed this topic and concluded that selenium has effects on tooth formation in animals and humans but only at toxic levels that affect nutrition.

On the other hand, Parko (1992) reported that selenium supplementation has reduced the incidence of caries in young people in Finland, which is naturally low in environmental selenium. Selenium has been added in Finland to some animal foodstuffs since 1962, to all animal foodstuffs since 1968, and to fertilizers starting in 1984. Intake of selenium by man and cattle today is roughly twice what it was before selenium supplementation. Since selenium supplementation, the conditions of the teeth of children and young people are considered to have improved (Parko, 1992).

Thyroid effects

Bratter and Negretti de Bratter (1996) reported that higher selenium intakes in Portugesa, Venezuela, a mean of 552 µg/day (250 to 980 µg/day), were associated with a decrease in free T_3 serum levels, compared to a mean selenium intake of 205 µg/day (90 to 350 µg/day) for controls in Yaracuy, Venezuela, although the T_3 values in the high-intake area were within the normal range. It was hypothesized that the inverse relationship between blood selenium level and T_3 was related to inhibition of the selenoprotein DI1, which catalyzes the 5'-deiodination of L-thyroxine T_4 to L-triiodothyronine T_3.

Occupational exposures

Reports of occupational exposure to selenium compounds are scarce, in part because this element is not much used in industry. No reports of disabling chronic disease or deaths from industrial exposures were cited by Glover (1967, 1970), who reviewed his long experience with workers exposed to selenium. The compounds encountered in industrial exposure are principally elemental selenium, hydrogen selenide and selenium oxide. These chemical forms of selenium do not occur in drinking water.

Glover (1967, 1970) described the common occurrence of garlic breath odor and the possibility of sociopsychological effects, as manifested by symptoms of lassitude and irritability, in the selenium-exposed worker population. The mortality pattern of selenium-exposed workers in a rectifier plant, 17 deaths over a 26-year period, was not significantly different from what was expected for this group compared to the general population of England and Wales.

Diskin *et al.* (1979) described a 71-year-old man employed in selenium refining for 50 years as having reddish-orange hair and red fingernails, but he denied any respiratory or gastrointestinal symptoms. Physical examination of the abdomen did not reveal organomegaly and his neurologic examination was normal. The individual died from an acute myocardial infarction and upon autopsy, numerous perivascular noncaseating granulomas of the lungs were noted that were suggestive of inflammatory reactions to possible selenium exposure.

Endemic selenosis in China

China possesses one of the world's best epidemiological databases on selenium deficiency and toxicity diseases, which has been used with geochemical data to demonstrate a geological association with human exposure (Fordyce *et al.*, 2000). A low-selenium belt has been defined in the shape of a saddle crossing central China from Heilongjiang Province in the northeast to Tibet Autonomous Region in the west and Yunnan Province in the southwest, covering 14 of the 22 provinces of China (Wang and Gao, 2001). Most parts of China are considered as selenium-adequate, but two small mountainous regions with excessive selenium have been identified, the Enshi area in Hubei Province of central China (Mao *et al.*, 1988, 1990; Yang *et al.*, 1981, 1983) and Ziyang County in Snaanxi Province of central China (Chen and Mei, 1980; Fang and Wu, 2004).

Chronic selenosis was identified in the late 1950s through the 1960s among residents of these two seleniferous areas (Mei, 1985; Yan, 1993); Enshi (Yang *et al.*, 1981, 1983), and Ziyang (Chen and Mei, 1980). The bedrock of both Enshi and Ziyang consists of carbonaceous shales with high-selenium coal (Fang and Wu, 2004; Zhu and Zheng, 2001). High selenium soil is mainly weathered from bedrock. In these regions, soil selenium content is negatively correlated with annual rainfall (Wang and Gao, 2001). In the mountainous slopes, selenate has accumulated in soil through the hydrological and aerial transport of fine particles as well as through evaporation of water. The soluble selenate can also react with organic matter and is retained in the soil.

In Enshi, selenium deficiency disease and selenosis in humans occurred within 20 to 30 km of each other mainly due to uneven distribution of selenium in the soil (Zhu and Zheng, 2001). Burning shale on the ploughing field and covering it with burnt coal ash for soil fertilization as well as using coal for cooking significantly increased the selenium concentrations in soil and in locally grown corn and vegetables (Mao *et al.*, 1988, 1990). Detailed information was provided by Yang *et al.* (1981, 1982a,b, 1983, 1989a,b) of human toxicity from chronic selenium exposure of people living in Enshi, and the information has been reviewed (ATSDR, 1996, 2003; NRC, 2000; SCF, 2000; U.S. EPA, 1985a,b, 1990b, 1991, 2007). Selenium exposure in selenosis Ziyang was mainly from consumption of local crops grown on high selenium soils weathered from carbonaceous slate and volcanic tuff, without the use of high selenium coal (Luo *et al.*, 2004).

The peak prevalence of selenosis in Enshi was between 1959 and 1963, with half or more of the population of several small villages affected. Livestock were also affected in these seleniferous areas, and all the livestock died from selenium poisoning in the most-affected village. The inhabitants of this village were evacuated; most recovered soon after their diets were changed. Those with nervous system symptoms needed more time to recover. The last few cases of chronic selenosis were reported in 1991 (Yan, 1991; Yan and Wu, 1993; Yang and Xia, 1995; Yang and Zhou, 1994).

According to Yang *et al.* (1981, 1983), the human tissues most affected were hair, nails, skin, the nervous system, and teeth. Villagers experienced decreased appetite, fatigue, lack of energy, and itchy scalp before showing symptoms of selenosis. Rashes appeared on the scalp, accompanied by intolerable itching. In victims, hair became dry, lost color, and was easily broken off at the base. Hair was lost from the scalp, brow, beard, armpit, and pubic area. The hair roots appeared normal and new hair grew back gradually, however, new hair had no pigment, lacked luster, and had split ends. Nails were brittle with spots and longitudinal streaks on the surface, sometimes deformed, and fell out and, on regrowth, were rough and thickened, and again fell out. The nails of the thumbs were first to be affected. Symptoms included the presence of distinct transverse or longitudinal ridges on the wall of the nails, and the presence of a white area at the base of the nail wall. Symmetric thickening and stratifying of nails occurred repeatedly over years in chronic selenosis. In more severe cases, fluid effused from around the nail bed. Skin lesions including erythema, edema, eruptions and intense itching mainly on the back of the hands and feet, on the outer side of legs and thighs, the forearms, and on the back of the neck. Affected skin became red and swollen, then blistered, and became eruptive, ulcerative and took a long time to heal. These lesions resemble the toxicity seen in keratinized appendages of the skin in animals (Yang *et al.*, 1989b).

Abnormalities of the nervous system were observed in one heavily affected village with 18 cases of selenosis among 22 inhabitants (Yang *et al.*, 1983). These 18 patients complained of peripheral anesthesia, acroparesthesia, sensations of "pins and needles," and pain in the extremities. Hyperreflexia of the tendons was common, followed by numbness, convulsions, paralysis, and motor disturbance at a later stage, and even hemiplegia and polyneuritis.

Some villagers showed hepatomegaly and gastrointestinal disturbances (Yang *et al.*, 1983). Nearly one third of 66 cases had mottled teeth in one study, and in a few cases there was tooth erosion and pitting. Since some fluorosis was reported in this geographic

area, it could not be decided if the tooth damage was caused by excess fluoride or selenium, or by a combination of the two elements.

Local crops contained high concentrations of selenium, such as 6.33 µg/g in corn and 1.48 µg/g in rice. Some corn samples contained selenium as high as 44 µg/g. A favorite local diet item, turnip greens, had an exceptionally high concentration of selenium of 457 µg/g (Yang *et al.*, 1983). Due to a drought that caused failure of the rice crop, the villagers were forced to eat more high-selenium vegetables and maize, and fewer protein foods that may protect against selenium poisoning. The major chemical form in the local corn and rice was selenomethionine (Beilstein *et al.*, 1991). Selenium content of drinking water averaged 54 µg/L for 11 samples. In a small area with a high prevalence of selenosis, the average of four drinking water samples was 139 µg/L. Whanger (1989) noted that inhalation of smoke from selenium-containing coal may have also contributed to elevated selenium exposure.

The average daily intake of selenium in diet and drinking water was estimated to be 4,990 µg/day, with a range of 3,200 µg/day to 6,990 µg/day. This was more than 40 times the usual intake of residents of the selenium-adequate Beijing region. As might be expected, local residents had unusually high levels of selenium in their blood, hair, and urine as a result of this high dietary intake.

The mean blood selenium level in Enshi villagers with selenosis was 3,200 µg/L, and the mean urine level was 2,680 µg/L. In high selenium areas without occurrence of selenosis in China, the selenium intake was calculated to range from 240 to 1,510 µg/day with a mean of 750 µg/day, yielding blood levels of 350 to 580 µg/L with a mean of 440 µg/L (Yang *et al.*, 1983). The mean individual safe intake of selenium was estimated to be 11 µg/kg (Yang and Xia, 1995). The blood selenium level of about 952 µg/L (Yang *et al.*, 1989a,b; Yang and Xia, 1995; Yang and Zhou, 1994), corresponding to a mean maximum safe selenium intake of about 800 µg/day or 15 µg/kg is comparable to the 813 µg/L blood selenium level found among children in Venezuela without clear signs of selenium poisoning (Jaffe *et al.*, 1972).

In a study on 237 residents of this seleniferous area, 58 of the 60 selenosis cases were older than 18 years of age, and two cases were 13 to 17 years of age. No cases were observed below 12 years of age even though the selenium intakes per kg body weight and blood selenium levels within this age group were higher than or equal to those of affected adults (Yang *et al.*, 1989b).

Yang *et al.* (1989a,b, 1994, 1995) studied a population of about 400 individuals in the Enshi area for clinical and biochemical signs of selenosis using blood levels of selenium to provide an index of selenium intake. A detailed study of selenium intake via various foods and measurements of selenium in tissues allowed more accurate estimates of the dose response relationship (Yang *et al.*, 1989b). The LOAEL was estimated to be 913 µg/day for the most sensitive endpoint, brittle fingernails, based on blood selenium concentrations. The lower limit of the 95 percent confidence interval was about 600 µg/day. Further data analysis suggested that 800 µg/day represented a NOAEL, with a lower 95 percent confidence limit of 600 µg/day.

In a follow-up study, the region was divided into low, medium, and high selenium areas, each with 50 to 75 families. The mean drinking water selenium levels in the three areas

were about 0.37, 1.72, and 12.27 µg/L, respectively; and soil levels were about 370 to 480 µg/kg, 730 to 5,660 µg/kg, and 704 to 12,080 µg/kg, respectively (Yang *et al.*, 1989b). The respective means ± standard error of daily total selenium intake, mainly from food and water, were estimated to be 70 ± 5, 195 ± 23, and 1,438 ± 76 µg/day for males, and 62 ± 4, 198 ± 24, and 1,238 ± 65 µg/day for females in the three areas, with an average body weight of 55 kg in males and 53 kg in females.

No clinical signs of selenosis were observed among those with a blood selenium concentration below 1,000 µg/L, corresponding to an intake level of 853 µg/day (Yang *et al.*, 1989b). Clinical signs were classified less serious, with subjects having mainly finger nail disease or changes alone, and more serious, with subjects having finger nail disease or changes with severe hair loss or skin changes. The prevalence of less serious selenosis varied between 10 percent to 35 percent in three groups with blood selenium concentrations of 1,000 to 1,250 µg/L, ranging up to 1,500 to 2,000 µg/L. The prevalence of serious selenosis varied from three to seven percent in the same three groups. The prevalence of serious selenosis was 45 percent in subjects with a blood selenium concentration above 2,000 µg/L. Blood selenium concentrations in five adult subjects with long-term persistent clinical signs of selenosis ranged from 1,054 to 1,854 µg/L with a mean of 1,346 ± 366 µg/L, corresponding to intakes of 913 to 1,907 µg/day with a mean of 1,270 ± 450 µg/day (SCF, 2000; Yang *et al.*, 1989a).

Yang *et al.* (1989a) noted that whole blood selenium levels above 1 mg/L were associated with prolongation of the prothrombin time. However, this endpoint is not considered by other review groups (NRC, 2000; U.S. EPA, 2009) to be an effect of selenium toxicity. Prolonged bleeding time was observed clinically upon blood collection in the high selenium areas. Prolonged prothrombin time at more than 14 seconds was observed in one of the 20 subjects with a blood selenium concentration ranging from 200 to 990 µg/L, and among 45 percent of those subjects with a blood selenium concentration above 1,000 µg/L, corresponding to an intake level of 853 µg/day. The mean prothrombin time increased marginally without reported ranges.

The prevalences of mottled enamel teeth of school children aged from seven to 14 years were zero, 49 percent, and 95 percent in groups with low, medium, and high blood selenium concentrations, respectively (Yang *et al.*, 1989b). The mean blood selenium concentrations were 130 ± 20 µg/L in the low, 370 ± 320 µg/L in the medium, and 1,570 ± 440 µg/L in the high groups, respectively.

Selenosis in North America and other locations

The health status of families in seleniferous areas of eastern Wyoming, southern South Dakota, and northern Nebraska was surveyed, along with intakes of locally grown food, to determine if individuals exposed to high selenium levels showed selenium poisoning (Lemley and Merryman, 1941; Smith and Lillie, 1940; Smith and Westfall, 1937). Urine samples were analyzed for selenium. No control group was included in the evaluation. Nonspecific signs and symptoms of ill health were noted such as anorexia, indigestion, generalized pallor, carious teeth, a yellowish discoloration of the skin, cutaneous eruptions, chronic arthritis, and dystrophic nails. A causal relationship could not be established in these surveys and the correlation between the signs and symptoms and the

selenium concentration in urine, even in individuals with high urinary levels of selenium, was not clear-cut. The average dietary intake of selenium in the study area was 10 to 20 µg/kg, or approximately 700 to 1,400 µg/day for a 70 kg adult male.

A reawakening of interest in nutritional standards for selenium prompted Longnecker *et al.* (1991) to repeat a study of inhabitants of this seleniferous region. Over a two-year period, 142 male and female ranchers from eastern Wyoming and western South Dakota were evaluated for adverse health effects. Subjects completed comprehensive health questionnaires, underwent physical examinations, provided blood samples for clinical assessment, and provided whole blood, serum, urine, and toenails for selenium analysis. The daily dietary intake was assessed by 48-hour duplicate plate food samples and by diet questionnaires. About half of the subjects had selenium intakes greater than 200 µg/day and 12 individuals were above 400 µg/day, ranging from 68 to 724 µg/L with an average intake of 239 µg/day. Physical findings characteristic of selenium toxicity were absent, as were clinically significant changes in laboratory tests or symptoms related to selenium in the blood, toenails, or diet. Dietary intake and selenium concentrations in body tissues were highly correlated. Blood selenium concentrations in this population were related to selenium intake in a manner similar to that found in the Chinese studies. An association of selenium intake with alanine aminotransferase in serum was observed, however, the values were within the reference range and considered clinically insignificant. Increased prevalence of lethargy was seen with increased selenium intakes, but no clinical evidence of selenium toxicity was found (Longnecker *et al.*, 1991; Salbe *et al.*, 1993).

Reid *et al.* (2004) reported toxic side effects including exhalation of garlicky dimethyl selenide and hair and nail changes in 24 men with prostate cancer receiving selenized yeast as part of a clinical trial of selenium as a chemopreventive agent. Eight subjects with a mean age of 73.6 years took 1,600 µg selenium/day for 14.2 ± 6.4 months with follow-up for 32.6 ± 10.8 months; 16 subjects aged 70.5 years took 3,200 µg selenium/day for 11.5 ± 7.5 months with follow-up for 27.3 ± 13.6 months. Among the 16 subjects in the 3,200 µg/day group, six reported garlic breath, three brittle nails, one brittle hair, two stomach upset, and three dizziness. Among the eight subjects in the 1,600 µg/day group, none reported garlic breath, one brittle nails, none brittle hair, one stomach upset, and one dizziness. The mean plasma selenium levels at the time of the reported toxicity ranged from 25 to 450 ng/mL; peaks in plasma selenium levels did not correspond well to the reports of toxicity. The plasma selenium levels achieved were 492.2 ± 188.3 ng/mL and 639.7 ± 490.7 ng/mL (mean with standard deviation) for the 1,600 and 3,200 µg/day doses, respectively. These intake levels can be compared to the NOAEL of 400 µg/day and the proposed upper tolerated limit for plasma selenium of 1,000 ng/mL established by the NRC (2000). Furthermore, the subjects with the highest plasma levels, exceeding the recommended 1,000 ng/mL, did not exhibit symptoms of selenosis.

In Venezuela regions where selenium intake approaches the upper limits of safety because of soil conditions and consumption of nuts containing high selenium levels, selenosis is not generally observed in the population. Occasional cases of excessive intake have been described by Kerdel-Vegas *et al.* (1966). Local inhabitants were familiar with the depilatory effects of nuts from the *Lecythis ollaria* or "Coco de Mono" tree and avoided consumption, but newcomers consumed these nuts and suffered ill

effects. The prominent signs of toxicity were hair loss and nail damage, accompanied by dizziness, diarrhea and a foul breath odor. The active ingredient in the nuts has been identified as selenocystathione (Olivares *et al.*, 1967).

Selenosis has also been reported in the Punjab area of India (Hira *et al*., 2004). In a study of 40 families in endemic selenosis area, mean selenium intake was reported as 632±31 (SE) µg/day in men and 475±53 µg/day in women. Major symptoms included hair loss, discolored or lost fingernails and tooth staining. The authors suggested 600 µg/day as the maximum limit of acceptable selenium intake and 819 µg/day or above as the dose that results in metabolic changes (selenosis).

Amyotrophic lateral sclerosis

Kilness and Hichberg (1977) reported a cluster of four cases of amyotrophic lateral sclerosis in male farmers living in a seleniferous region of South Dakota. Amyotrophic lateral sclerosis is a disease of the nervous system in which motor neurons of brain and spinal cord degenerate, with progressive wasting of the body and spastic paralysis. It was suggested that the disease was related to high levels of intake of selenium by its victims. One of the patients had elevated urinary levels of selenium (0.45 mg/L versus 0.01 mg/L expected for individuals living in nonseleniferous areas). In subsequent Letters to the Editor commenting on this report, it was noted that the likelihood of this cluster relative to the general incidence of amyotrophic lateral sclerosis in the U.S. was 3/1,000, but the relationship observed by Kilness and Hichberg (1977) was suggested to be a chance occurrence (Kurland, 1977; Lippmann, 1977).

Two studies reported that selenium levels are normal or slightly lower in patients with amyotrophic lateral sclerosis (Norris and Sang, 1978, Moriwaka *et al.*, 1993). However, Nagata *et al.* (1985) reported that the selenium concentration in blood cells was elevated in amyotrophic lateral sclerosis patients compared to control subjects and to another group with neurological diseases such as Parkinson's disease, spinocerebellar degeneration, senile dementia, and cerebrovascular disease. In 40 subjects with mild to severe cases of amyotrophic lateral sclerosis, the levels of selenium were 1.16 ± 0.24 ng/mg versus values of 0.84 ± 0.17 and 0.88 ± 0.16 ng/mg for "control subjects" and "other neurological disease," respectively ($p < 0.01$).

Amyotrophic lateral sclerosis was associated with selenium exposure in a study performed in Italy. Vinceti *et al.* (1996) examined the nine-year incidence of amyotrophic lateral sclerosis in 5,182 residents of Reggio Emilia exposed to increased selenate levels, up to 7 to 9 µg/L in drinking water. Four cases were diagnosed during the follow-up. Using the remainder of the municipal population as the reference group, the standardized incidence ratio was 4.22 with a 95 percent confidence interval of 1.15 to 10.8. The standardized incidence ratio was higher after limiting the analysis to the subcohort with the longest ascertainable exposure period. The findings were interpreted as evidence in favor of an association between exposure to environmental selenium and increased risk of amyotrophic lateral sclerosis. The same group also reported that there was a strong inverse correlation between disability due to the disease with selenium concentrations in patients with sporadic amyotrophic lateral sclerosis and age- and gender-matched controls (Vinceti *et al.*, 1997).

The risk of selenium intake above the 400 µg/day Tolerable Upper Intake Level (NRC, 2000) for the U.S. population appears to be small. It should be noted that this Tolerable Upper Intake Level is based on total dietary and supplement intake, which would likely include a variety of selenium forms. As mentioned above, no conclusive cases of selenosis have been recognized in the high selenium areas of Wyoming and South Dakota (Longnecker *et al.*, 1991). The USDA has identified high selenium areas and proscribed their use for raising animals for food. The extensive food distribution system in the U.S. creates situations such that individuals do not eat diets that originate from one locality. This moderates the selenium content of diets, even in high selenium areas. In high selenium areas, the selenium intake exceeded 400 µg/day in 12 subjects among the 142 male and female ranchers studied, with the highest individual intake being 724 µg/day (Longnecker *et al.*, 1991). There are no reports of teratogenicity or selenosis in infants born to mothers with high intakes of selenium (NRC, 2000).

DOSE RESPONSE ASSESSMENT

Selenium toxicity depends on the chemical form, administration route, and duration of exposure. The water-soluble inorganic salts such as sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) are bioavailable, and appear to be among the more toxic selenium compounds. The organic selenium compounds in grains, plants, and animals which are less soluble in water than the inorganics but are also bioavailable, such as selenomethionine and selenocysteine, have relatively moderate toxicity. The organic selenomethionine is much less toxic acutely, but not chronically, than the inorganic forms. The poorly soluble or insoluble forms that exhibit limited bioavailability, e.g., elemental selenium (Se^0), sodium selenide (Na_2Se), selenium disulfide (SeS_2), and diphenyl selenide [$(C_6H_{13}Se)_2$], are among the least toxic selenium compounds. Inorganic selenium compounds can be methylated by environmental microbes or by enzymes in biological systems. Methylated selenium compounds exhibit lower toxicity than inorganic selenite or selenate salts. Dietary factors such as protein types and levels of heavy metals, methyl group donors, and antioxidants affect the severity of selenium toxicity. Gender and previous selenium exposure may also influence selenium toxicity. In general, toxicity of selenium compounds is less after oral than after parenteral administration (Mihajlovic, 1992).

Studies for Noncarcinogenic Risk in Experimental Animals

As shown in Tables 10, 11, and 13, the lowest NOAEL estimated from the available animal data evaluated in this document is 0.014 mg/kg-day based on hoof cracking in pigs fed higher doses of sodium selenite for 35 days (Mahan and Magee, 1991). However, the study design and quality assurance of the Mahan and Magee (1991) study were not as comprehensive as the NTP studies (Abdo, 1994).

In the NTP studies of sodium selenate (Abdo, 1994), male and female F344/N rats and $B6C3F_1$ mice received 0, 3.75, 7.5, 15, 30, or 60 ppm sodium selenate for 13 weeks. These concentrations were estimated to deliver 0, 0.29, 0.44, 0.92, 1.57, 2.54 mg selenium/kg-day to male rats, 0, 0.31, 0.47, 0.88, 1.57, 2.54 mg selenium/kg-day to

female rats, and 0, 1.07, 1.87, 2.95, 5.45, or 7.17 mg selenium/kg-day to male or female mice. Doses at the estimated LOAEL of 7.5 ppm sodium selenate (0.47 mg selenium/kg-day) or greater were associated with increased incidences of renal papillary degeneration in rats. A NOAEL of 0.31 mg selenium/kg-day was estimated. No lesions related to sodium selenate were observed in mice.

In the NTP studies of sodium selenite (Abdo, 1994), male and female F344/N rats and $B6C3F_1$ mice received 0, 2, 4, 8, 16, or 32 ppm sodium selenite for 13 weeks. These concentrations were estimated to deliver selenium doses of 0, 0.17, 0.29, 0.54, 0.98, 1.59 mg/kg-day to male rats, or 0, 0.15, 0.28, 0.5, 0.86, 1.67 mg/kg-day to female rats, and 0, 0.44, 0.91, 1.61, 3.31, or 3.83 mg/kg-day to male or female mice. Sodium selenite at the estimated LOAEL of 8 ppm (0.5 mg selenium/kg-day) was associated with an increased incidence of renal papillary regeneration in female rats. A NOAEL of 0.28 mg selenium/kg-day was estimated. No selenium-related lesions were observed in mice.

Based on mortality in rats, body weight depression, and renal lesions, sodium selenate and sodium selenite were more toxic to rats than to mice. These chemicals caused increases in estrous cycle length in rats; sodium selenite also caused an increase in estrous cycle length in mice. Based on mortality, body weight depression, decreased water consumption, and renal papillary lesions, the estimated NOAEL in rats was 0.31 mg selenium/kg-day for sodium selenate and 0.28 mg/kg-day for sodium selenite (Abdo, 1994). Based on body weight depression and decreased water consumption, the estimated NOAEL in mice was 1.07 mg selenium/kg-day for sodium selenate and 0.91 mg selenium/kg-day for sodium selenite (Abdo, 1994). However, the adverse effects observed in rats, including mortality, body weight depression, and renal lesions, have not been observed in humans. Conversely, the rat does not manifest the hair loss and dyskeratosis that are characteristic signs of selenium toxicity in humans and several other species.

In terms of the relative susceptibility to natural selenium, Raisbeck (2000) has reviewed selenosis in domesticated livestock grazing on toxic pastures and concluded that swine is the most sensitive species, following by horse and cattle, and sheep is the most resistant to the dermal toxic effect. The manifestations of selenium intoxication in the skin and integument have been ascribed to the replacement of sulfur in keratin or keratin-associated proteins by selenium, which may compromise the structural integrity of keratin (Dudley, 1936). Ekfalck (1990) measured the amino acid content of different layers of horse's hooves and noted that cystine is an important component for hard keratinization of the hoof matrix. The selective lesions produced by selenium in stratified squamous epithelium in most species are consistent with the idea that sulfur amino acid functions are being affected by selenium. Ganther (1999) demonstrated that selenite reacts with protein thiols and suggested that selenium toxicity is due to denaturation of critical subcellular macromolecules, including enzymes.

Extrapolation of the animal data to humans poses considerable difficulties as some species like sheep exhibit exceptional sensitivity to selenium toxicity, but sheep may not be a good animal model for human extrapolation. For example, acute lethality of sodium selenite and sodium selenate is seen at relatively low doses in sheep; however, sheep appear to tolerate relatively high doses in chronic studies. Ruminants such as sheep absorb less inorganic selenium than nonruminants because selenite and selenate may be

reduced to insoluble selenide by microorganisms in the rumen (Butler and Peterson, 1961; Hidiroglou *et al.*, 1968; Spears, 2003). Selenite has a lower relative bioavailability than selenate in ruminant animals (Henry *et al.*, 1988). In addition, multigastric sheep retain nearly three-fold less of an oral dose of inorganic selenium than monogastric swine (Davis *et al.*, 2006; Wright and Bell, 1966), and sheep excrete selenium differently from monogastric humans (Jacobsson, 1966; Krishnamurti *et al.*, 1989; McClure and Mahan, 1988). Sheep have no significant iodothyronine deiodinase activity in the thyroid (Kohrle, 2000, 2002, 2005). The ontogeny of the iodothyronine deiodinases and their tissue distribution in sheep appears to be different from that in humans (Beech *et al.*, 1993). Furthermore, ruminant immune responses differ from those of human beings and laboratory animals, as well as differing among themselves (Finch and Turner, 1996).

In lambs, the estimated oral LD_{50} of sodium selenate was 0.45 mg selenium/kg (Hopper *et al.*, 1985) and of sodium selenite was 1.9 mg selenium/kg (Caravaggi *et al.*, 1970b). The parenteral LD_{50} for sodium selenite in lambs was also estimated to be about 0.46 mg/kg (Caravaggi *et al.*, 1970a; Gabbedy, 1970; Combs and Combs, 1986). The LD_{50} for an intramuscular dose of sodium selenite in female sheep was estimated to be 0.7 mg/kg (Blodgett and Bevill, 1987b). Lambs died within five to 29 hours from pulmonary edema, subcutaneous hemorrhages, and extensive destruction of the renal cortices. These lethal doses are higher than the NOAEL of 0.014 mg/kg-day identified in pigs (Mahan and Magee, 1991). However, no NOAEL or LOAEL can be derived from the available sheep data (Allaway, 1973; Ammerman and Miller, 1975; Blodgett and Bevill, 1987b; Buck and Ewan, 1973; Case, 1974; Caravaggi *et al.*, 1970a,b; Caravaggi and Clark, 1969; Clark, 1995; Clark and Simpson, 1997; Combs and Combs, 1986; Davis *et al.*, 2006; Diplock, 1976; Ekermans and Schneider, 1982; Fessler *et al.*, 2003; Franke and Potter, 1935; Gabbedy, 1970; Gabbedy and Dickson, 1969; Gardiner, 1966; Glenn *et al.*, 1964a,b,c; Hopper *et al.*, 1985; Morrow, 1968; Muth, 1970; Muth and Binns, 1964; Rosenfeld and Beath, 1946; Rosenfeld and Beath, 1964), because of the lack of appropriate study designs.

Mechanisms of Toxicity in Animals

Selenium and many of its compounds are among the most toxic of essential nutrients. Possible mechanisms of selenium toxicity have been suggested, such as substitution of selenium for sulfur in protein synthesis, inhibition of methylation metabolism resulting in selenide accumulation, or membrane and protein damage from selenium-generated reactive oxygen species (ROS) (Spallholz *et al.*, 2004). Other molecular mechanisms of selenium toxicity that have been suggested include redox cycling of autooxidisable metabolites, glutathione depletion, protein synthesis inhibition, depletion of S-adenosylmethionine (the cofactor for selenide methylation), or reactions with critical sulfhydryl groups of proteins and cofactors (SCF, 2000).

Reaction of selenium compounds such as selenite with thiols such as glutathione, producing superoxide and other ROS, has been documented *in vitro* (Spallholz, 1997). This catalytic reaction of selenium compounds with thiols likely accounts for selenium toxicity to cells *ex vivo* and *in vivo* where the major glutathione producing organ, the liver, is also the major target organ of selenium toxicity. Selenium enzymes and

selenoethers that do not readily form a selenide anion are not toxic, and compounds such as Ebselen in which selenium is sequestered are nontoxic. Methylation of selenium by both plants and animals serves to detoxify selenium by generating methylated selenides. Alternatively, reduction of selenium to elemental selenium as done by some bacteria and formation of heavy metal selenides such as mercuric selenide (Hg_2Se), results in a noncatalytic nontoxic form of selenium. The catalytic prooxidant attribute of some selenium compounds appears to account for their toxicity when such activity exceeds plant and animal methylation reactions and antioxidant defenses. This prooxidant activity may also account for cellular apoptosis and anticancer property of some selenium compounds.

As discussed earlier in this document, selenium is considered to be protective against oxidation damage because of its function in GPxs and TRs as well as other antioxidant enzymes. Madeja *et al.* (2005) and Bjorkhem-Bergman *et al.* (2002) showed data suggesting TR is responsible for cell resistance against selenium-induced acute cytotoxicity. Selenite, and selenate at a considerably lower rate, can be reduced to selenide. Selenide can then be excreted or used for selenium-containing protein biosynthesis. Selenide may also, if present in sufficient concentrations, participate in a redox cycle with oxygen and TR and form ROS, a reaction considered to be a mechanism of selenium induced cytotoxicity, DNA fragmentation, and cell death (Bjornstedt *et al.*, 1997). In acute toxicity, selenium may function as a prooxidant where selenium itself is reduced at the expense of reduced glutathione. The toxicity of selenite may arise from glutathione selenotrisulfide (GSSeSG) or factors related to depletion of intracellular glutathione. Selenium is toxic, when prooxidative conditions exceed antioxidant defenses *in vitro* or *in vivo*, because it increases the rate of oxidation of thiols by redox catalysis producing superoxide (Spallholz, 1994, 1997). Growth retardation in chronically exposed experimental animals has been considered to be caused by selective selenium accumulation and toxicity to growth hormone-producing cells in anterior pituitary gland (Thorlacius-Ussing, 1990).

Watanabe *et al.* (1988) showed that acute sodium selenite lethality is enhanced by coinjection with reduced glutathione. This is probably due to selenotrisulfide formation in the extracellular fluid space. Selenite, when mixed with glutathione, immediately forms the glutathione selenotrisulfide complex (Sandholm, 1993). It appears that sodium selenite administered parenterally at high concentrations can damage most vital organs, the particular manifestations being dependent on species. Spallholz (1994) summarized a series of *in vitro* experiments that suggested some chemical forms of selenium produce ROS. For example, selenite reacts with reduced glutathione to produce superoxide anion and elemental selenium, and this reaction initiates hemolysis *in vitro* (Spallholz, 1994). This effect is not seen with sodium selenate or selenomethionine, even in the presence of excess thiol donor groups.

Noncarcinogenic Risk in Humans

Adults

The most common manifestations of selenium poisoning in humans are alterations in keratinized appendages of the skin. There is loss of hair, and the nails of the hands and feet become brittle, discolored, and may be lost (Yang *et al.*, 1983, 1988a, 1989a,b; Yang and Zhou, 1994). Other signs of excessive intake include gastrointestinal disturbances, skin rash, garlic breath odor (caused by organoselenium compounds), fatigue, irritability, and disorders of peripheral nerve sensation. The data on human toxicity from chronic exposure to selenium are based primarily on about 380 cases reported by Yang *et al.* (1983, 1989a,b) in individuals living in the Enshi District of central China. Additional data on human poisoning are from individuals who took a selenium supplement that had abnormal levels of selenium, primarily as sodium selenite (Jensen *et al.*, 1984). Yang *et al.* (1983) also reported a single, well-documented poisoning case of a worker who consumed a 2-mg sodium selenite tablet daily for two years, which provides a relevant perspective.

The studies by Yang *et al.* (1983, 1989a,b) on seleniferous Enshi District residents have provided useful correlations of blood selenium level and estimated intake with toxic manifestations as described in previous sections in this document. From these data, using fingernail disease as the endpoint, a LOAEL was estimated to be 913 µg/day (Yang and Zhou, 1994). The lower limit of the 95 percent confidence interval (CI) was about 600 µg/day. No clinical signs of selenosis were observed in individuals with blood selenium below 1,000 µg/L, corresponding to a selenium intake of about 850 µg/day. This could be considered as a NOAEL for clinical selenosis (SCF, 2000; Yang *et al.*, 1989a; Yang and Zhou, 1994). Toxic effects were considered likely with increasing frequency at selenium intakes over 750 to 850 µg/day (Yang and Zhou, 1994). Hira *et al*. (2004), evaluating selenosis symptoms in a population in India, considered 600 µg/day to be the maximum limit of acceptable selenium intake and suggested that more than 819 µg/day results in metabolic changes (selenosis).

Further data analysis suggested that 800 µg/day, rounded from a range of 750 to 850 µg/day shown in Yang *et al.* (1989a), represented a NOAEL, with a lower 95 percent confidence limit of about 600 µg/day (NRC, 2000; U.S. EPA, 2009). Yang *et al.* (1989a) recommended an uncertainty factor of two be applied to the 800 µg/day level to obtain an acceptable intake level for adults of 400 µg/day. The U.S. National Research Council (NRC, 2000) agreed with Yang *et al.* (1989a) and applied an uncertainty factor of two to the NOAEL of 800 µg/day to derive a Tolerable Upper Intake Level for selenium of 400 µg/day for adults age 19 years and older. This value also applies to females during pregnancy and lactation for adolescents aged 14 to 18 years and for adults 19 years and older (NRC, 2000). The Tolerable Upper Intake Level was for selenium intake from food, water, and supplements, as inhalation and skin exposures do not provide significant amounts of selenium. The NRC (2000) noted that intake above 400 µg/day may be acceptable for controlled clinical trials. The rationale for permitting higher intakes is based on recent findings that selenium supplements may reduce the risk of prostate and colon cancers (Clark *et al.*, 1996b; Nelson *et al.*, 1999). The NRC (2000) considered that

inorganic selenium might be more hazardous than the organic selenium compounds ingested by Enshi District residents. The NRC (2000) stated:

> "A useful data set for determining dose-response of selenium toxicity from food sources was reported by Chinese investigators (Yang and Zhou, 1994). The report consisted of a reexamination (in 1992) of five patients previously found (in 1986) to have overt signs of selenosis: hair loss and nail sloughing. Because the same patients were studied at different times while consuming the same food form of selenium, blood levels of selenium can be compared and dietary intakes can be inferred from blood selenium concentrations."
>
> "The lowest blood level of selenium measured in the five subjects at initial examination was 13.3 µmole/L (105 µg/dL), corresponding to a selenium intake of 913 µg (12 µmole)/day (range: 913 to 1,907 µg [12 to 24 µmole]/day). The average blood selenium level was 16.9 µmole/L (135 µg/dL). At the time of reexamination in 1992, all five patients were described as recovered from selenium poisoning, although their fingernails reportedly appeared brittle. The mean blood selenium level had decreased to 12.3 µmole/L (97 µg/dL), corresponding to a selenium intake of about 800 µg (10 µmole)/day (range 654 to 952 µg [8.3 to 12 µmole]/day). The lower limit of the 95 percent confidence interval was 600 µg (7.6 µmole)/day."
>
> "Yang and Zhou (1994) therefore suggested that 913 µg (12 µmole)/day of selenium intake represents an individual marginal toxic daily selenium intake or LOAEL. They further suggested that the mean selenium intake upon reexamination (800 µg [10 µmole]/day) represented a NOAEL, while 600 µg (7.6 µmole)/day of selenium intake was the lower 95 percent confidence limit for the NOAEL. These values appear reasonable, although the number of subjects was small. Nevertheless, the LOAEL for selenosis in this small data set appears to be representative of the larger data set, and the reexamination of the subjects provides valuable dose-response data. Uncertainty occurs because of the smallness of the data set and because the Chinese subjects may not be typical (e.g., they may be more or less sensitive to selenium than other populations)."
>
> "Longnecker *et al.* (1991) studied 142 ranchers, both men and women, from eastern Wyoming and western South Dakota who were recruited to participate and were suspected of having high selenium intakes based on the occurrence of selenosis in livestock raised in that region. Average selenium intake was 239 µg (3 µmole)/day. Dietary intake and selenium in body tissues (whole blood, serum, urine, toenails) were highly correlated. Blood selenium concentrations in this western U.S. population were related to selenium intake in a similar manner to that found in the Chinese studies, presumably because the form of selenium ingested was selenomethionine. No evidence of selenosis was reported, nor were there any alterations in enzyme activities, prothrombin times, or hematology that could be attributed to selenium intake. The highest selenium intake in the study was 724 µg (9 µmole)/day."
>
> "It thus appears that a UL (Tolerable Upper Intake Level) based on the Chinese studies is protective for the population in the U.S. and Canada. Therefore a NOAEL of 800 µg (10 µmole)/day is selected."

"An uncertainty factor (UF) of 2 was selected to protect sensitive individuals. The toxic effect is not severe, but may not be readily reversible, so a UF greater than 1 is needed."

"The NOAEL of 800 µg/day was divided by a UF of 2 to obtain a UL for adults as follows:

$$\frac{\text{NOAEL}}{\text{UF}} = \frac{800\ \mu\text{g/day}}{2} = 400\ \mu\text{g/day.}"$$

The NOAEL of 800 µg/day is further supported by a review of Rayman (2004) in which it was concluded that none of about one dozen selenium yeast supplementation studies has shown evidence of toxicity at intakes up to 800 µg selenium/day over several years, for example, up to 12 years in the case of the 200 µg/day dose. Furthermore, thousands of South Dakota residents have had organic selenium intakes as high as 724 µg/day for their lifetimes without observed adverse effects. The risk of selenium intake above the 400 µg/day value for the U.S. population appears to be small (Longnecker *et al.*, 1991).

The USDA has identified high selenium areas and prohibited raising animals for food there. However, the extensive food distribution system in the U.S. means that individuals usually do not eat diets from one locality. This moderates the selenium content of diets, even in high selenium areas. There are no reports of teratogenicity or selenosis in infants born to mothers with high intakes of selenium (NRC, 2000; Yang *et al.*, 1989a).

Based on the data of Yang *et al.* (1989a,b) and in consideration of the results of Longnecker *et al.* (1991), the U.S. EPA (1991) derived the oral reference dose (RfD) for selenium in the current IRIS database (U.S. EPA, 2009) and in the U.S. EPA (1991, 2009) document as follows:

"The principal study for the oral RfD of selenium is based on Yang *et al.* (1989b), which is a follow-up of an earlier study (Yang *et al.*, 1983) describing selenosis in Enshi County, China. Approximately 400 individuals living in an area of high environmental concentrations of selenium were evaluated. The studies by Yang *et al.* (1989a,b) provided a large sample size, analysis of tissue selenium levels, and an accurate estimate of the dose response relationships. Selenium levels in soil and approximately 30 typical food types commonly eaten by the exposed population showed a positive correlation with blood and tissue selenium levels. The daily average selenium intakes, based on lifetime exposure, were 70, 195 and 1,438 µg for adult males and 62, 198 and 1,238 µg for adult females in the low, medium, and high selenium areas, respectively. Significant correlations demonstrated between selenium concentrations of various tissues were used to estimate the minimal daily selenium intake values that elicited various clinical signs of selenosis, that is, hair or nail loss."

"Analysis of the results indicated that clinical signs of selenosis were observed only in 5/349 adults. The blood selenium concentration in this group ranged from 1.054 to 1.854 mg/L with a mean of 1.346 mg/L. Clinical signs observed included the characteristic "garlic odor" of excess selenium excretion in the breath and urine, thickened and brittle nails, hair and nail loss, lowered hemoglobin levels, mottled teeth, skin lesions and central nervous system abnormalities (peripheral anesthesia,

acroparesthesia and pain in the extremities). Alterations in the measured biochemical parameters occurred at dietary intake levels of 750 to 850 µg/day. These alterations were described as a prolonged prothrombin time, i.e., increase in blood coagulation time and reduction in blood glutathione concentration. However, these indicators were poorly characterized and are not typically used as an index for clinical selenosis resulting from chronic exposure to selenium (NRC, 1989). Based upon the blood selenium levels shown to reflect clinical signs of selenium intoxication, a whole blood selenium concentration of 1.35 mg/L corresponding to 1.261 mg of daily selenium intake is indicative of the lowest correlative selenium intake causing overt signs of selenosis. The next lowest whole blood selenium concentration of 1 mg/L, corresponding to 0.853 mg selenium/day, produces no clinical signs of selenosis. The NOAEL for this study is 0.85 mg selenium/day and the LOAEL is 1.26 mg selenium/day. The "Conversion Factor" is taken from the legend of Figure 1 in Yang *et al.* (1989b) which shows the correlation between whole blood selenium levels and dietary intake of selenium."

The U.S. EPA estimated health-protective doses of selenium based on the dermal effects of clinical selenosis reported by Yang *et al.* (1983, 1989a,b) and Yang and Zhou (1994). These doses were estimated using conversion factors for the NOAEL (0.853 mg/day) and LOAEL (1.261 mg/day), calculated from the regression analysis (log Y = 0.767 log X – 2.248, where Y = blood selenium and X = selenium intake). This was based upon the correlation (r = 0.962) between dietary selenium intake and blood selenium level for selenosis in the Chinese population studied. The NOAEL and LOAEL were calculated from the blood selenium concentrations using the body weight as follows: 0.853 mg/day ÷ 55 kg = 0.015 mg/kg-day, 1.261 mg/day ÷ 55 kg = 0.023 mg/kg-day (Yang *et al.*, 1989a,b; Abernathy *et al.*, 1993; Poirer, 1994). The conversion factor is from Figure 1 in Yang *et al.* (1989b).

The U.S. EPA (1991) established a reference dose (RfD) of 0.005 mg/kg-day for oral intake of selenium in the current IRIS database (U.S. EPA, 2009, last revised in 1991) using a NOAEL of 0.015 mg/kg-day with an uncertainty factor of three, as shown in the following calculation:

$$\text{RfD} = \frac{0.015 \text{ mg/kg-day}}{3} = 0.005 \text{ mg/kg-day}$$

where,

NOAEL = 0.015 mg/kg-day,

UF = 3 (to account for sensitive individuals).

A total daily oral intake of 350 µg/day is then calculated by multiplying the reference dose times the default adult body weight of 70 kg (0.005 mg/kg-day × 70 kg = 0.35 mg/day or 350 µg/day).

Infants and children

The Tolerable Upper Intake Level for selenium for infants aged zero to six months is estimated by the NRC (2000) to be about 45 µg/day. The committee identified a NOAEL of 47 µg/day based on the data of Shearer and Hadjimarkos (1975) showing that a human milk selenium concentration of 60 µg/L was not associated with known adverse effects. The NRC (2000) also identified an estimated average intake of human milk of 0.78 L/day (Allen *et al.*, 1991; Butte *et al.*, 1984; Heinig *et al.*, 1993) for infants zero through six months of age. The NOAEL was derived by multiplying 60 µg/L times 0.78 L/day, and dividing by an uncertainty factor of one. The NOAEL was adjusted for younger and older infants and children on the basis of relative body weight and rounded down to the nearest five µg/day. The NRC (2000) identified an estimated average intake of human milk of 0.6 L/day (Dewey *et al.*, 1984) for infants 7 to 12 months of age.

Levander (1989b), based on the estimated dietary selenium intake that is toxic to adults in China reported by Yang *et al.* (1989a,b), extrapolated that 47 µg selenium/day intake from breast milk in high selenium areas of the U.S. with no reported human selenosis is the upper limit of dietary selenium intake for six-kg infants in the U.S. The NRC (2000) estimate of average milk intake of 780 mL/day was based on studies of full-term infants weighed before and after each feeding. Patterson *et al.* (1988) found that intake of cow's milk in Brisbane, Australia, ranged from about 203 mL/day in adults, 209 mL/day in adolescents, 399 mL/day in preschool children, to 467 mL/day in infants. Using a daily consumption of 785 mL of breast milk, Yang *et al.* (1989a,b) calculated a selenium intake of 220 µg/day for infants in Enshi District. Based on the same daily consumption of 785 mL of breast milk but assuming the milk contains the highest selenium concentration of 60 µg/L reported from South Dakota (Shearer and Hadjimarkos, 1975), an upper limit of dietary selenium intake of 47 µg/day was derived (60 µg/L × 785 mL/day ÷ 1000 mL/L = 47.1 µg/day), which was rounded down to 45 µg/day (NRC, 2000).

Carcinogenicity of Selenium

Evaluation of potential carcinogenicity of selenium is informed by many studies on anti-neoplastic effects of selenium. For this purpose selenium has been administered mainly in the form of sodium selenite, selenium-enriched yeast, selenomethionine, or organoselenium compounds, in doses three- to six-fold higher than the RDA of 55 µg/day (NRC, 2000). The careful evaluation of cancer incidence in these studies provides credible evidence that moderate to high doses of selenium are not associated with increases in human cancer rates. The weight-of-evidence, if anything, points in the other direction, that selenium may have cancer protective properties. Such effects are presumed to be mediated through selenoprotein actions as direct or indirect antioxidants, regulating enzymes, and controlling apoptosis, angiogenesis, and the cell cycle. Studies on the role of selenium as an antineoplastic or a cancer preventive agent have been repeatedly reviewed (Willett, 1986; WHO, 1996b; Combs and Lu, 2001; Klein, 2004; Meuillet *et al.*, 2004; Rayman, 2004, 2005; Taylor *et al.*, 2004; Whanger, 2004; Combs, 2005; Finley, 2005a; Lipinski, 2005; Nelson *et al.*, 2005; Trumbo, 2005). The Selenium

and Vitamin E Cancer Prevention Trial (SELECT) being conducted by the National Institute of Health may provide more definitive results, which are anticipated in 2013.

The supporting evidence for a role of selenium in reducing the risk of certain human cancers is based primarily on animal studies, inverse geographic correlations between human intake and site-specific cancer incidence, and an inverse association between serum selenium and subsequent risk of cancer. However, the association between selenium status in humans, genotoxic damage, and cancer risk is enigmatic because epidemiologic studies have failed to consistently link selenium status with increased cancer risk in men and women or consistently show that selenium supplementation can reduce human cancer incidence (Willett, 1986). Overall, both the intervention studies and the selenosis studies provide considerable assurance that exposure to environmental levels of selenium, even in selenosis areas, does not increase cancer risk.

U.S. EPA (2009) in its IRIS database (carcinogenicity file last revised on July 1, 1993) has rated selenium and its compounds as class D, not classifiable as to carcinogenicity in humans based on inadequate human data and inadequate evidence of carcinogenicity in animals. IARC has placed selenium and selenium compounds except selenium sulfide in Group 3, not classifiable as to carcinogenicity to humans (IARC, 1975, 1987).

A mixture of selenium monosulfide and disulfide has been shown to be carcinogenic in experimental animals (ATSDR, 1996, 2003; U.S. EPA, 2009). Selenium sulfide and selenium disulfide are classified as B2 probable human carcinogens based on sufficient animal data and inadequate human data by the U.S. EPA (2009), the NTP (2002), and IARC (1987). Selenium sulfide has been listed under the California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) as carcinogenic since 1989. However, selenium sulfide and selenium disulfide are not readily soluble in water (ATSDR, 2003). In addition, the primary routes of exposure for selenium sulfide and selenium disulfide are dermal and inhalation (NTP, 2002). Therefore, their biological properties are not germane to this assessment focused on protection from exposure to selenium in drinking water. OEHHA has not developed a No-Significant Risk Level (NSRL) for selenium sulfide under Proposition 65 based on its carcinogenicity.

Because of the lack of carcinogenic effects of selenium compounds likely to be present in drinking water, the recommended public-health protective concentration for selenium in water is estimated based on non-cancer effects.

CALCULATION OF PHG

Calculation Based on Noncarcinogenic Effects

General Method

Calculations of a public health-protective concentration of a chemical contaminant in drinking water associated with negligible risks for carcinogens or noncarcinogens must take into account the toxicity of the chemical, as well as the potential exposure of individuals using the water. The OEHHA calculations for derivation of a public health-

protective concentration are derived from considerations similar to those for the U.S. EPA maximum contaminant level goal (MCLG) for noncarcinogenic endpoints.

In general, calculation of a public health-protective concentration (C, in mg/L) for organic chemicals or some inorganic chemicals in drinking water for noncarcinogenic endpoints uses the following equation:

$$C = \frac{\text{NOAEL or LOAEL} \times \text{BW} \times \text{RSC}}{\text{UF} \times \text{DWC}}$$

where,

NOAEL	=	no observed adverse effect level in mg/kg-day;
LOAEL	=	lowest observed adverse effect level in mg/kg-day;
BW	=	body weight, a default of 70 kg for a male or 60 kg for a female adult;
RSC	=	relative source contribution, a default range of 0.2 to 0.8 (20 to 80 percent), as explained below;
UF	=	uncertainty factors, as explained below, to account for gaps in our knowledge (uncertainty) about the toxicity of chemicals and for recognized variability in human responses to toxic chemicals;
DWC	=	daily water consumption rate, a default of 2 L/day for an adult or 1 L/day for a child, plus, where appropriate, an equivalent volume to account for additional inhalation and dermal exposures from household uses of drinking water (Leq/day).

Estimation of an appropriate uncertainty factor (UF) considers several common sources of uncertainty, including cross-species extrapolations, variations in human sensitivity, duration of the critical study, and other factors such as data quality. To acknowledge the human variations in response to toxic chemical and drug exposures due to age, disease states, and genetic makeup, particularly in genetic polymorphisms for enzymes (isozymes) for detoxifying chemicals, an uncertainty factor of 10 is generally applied. In determining health-protective levels for chronic effects, it is conventional to apply an uncertainty factor if data are only available from short or medium term exposures of animals. When important data are missing, such as cancer bioassays, developmental toxicity studies, or multi-generation reproductive studies, an additional uncertainty factor may be appropriate.

When conducting a risk assessment for a nutrient, however, traditional uncertainty factors may not be appropriate (Abernathy *et al.*, 1993; Poirer, 1994). For nonnutritive contaminants, uncertainty factors are designed to limit exposure to a chemical to a level that is almost completely without risk of toxicity. However, with a nutrient, adverse effects also occur when total intake is below a minimal level, i.e., the Recommended Dietary Allowance (Olin, 1998). Risk assessment of essential trace elements such as selenium examines higher intakes resulting in toxicity and lower intakes resulting in nutritional deficiencies. For selenium, both a Reference Dose of 0.35 mg/day and a

Tolerable Upper Intake Level of 0.4 mg/day represent the upper level at which there are not likely to be adverse health effects in a population (Goldhaber, 2003). A large uncertainty factor to account for data deficiencies and variability among individuals might reduce the recommended exposure to a nutrient to below the Recommended Dietary Allowance (WHO, 1998). This is particularly true with nutrients such as selenium that have a relatively narrow range between deficiency and excess. Caution must be exercised in these cases to ensure that nutritional needs are balanced against the potential for toxic effects.

The daily water consumption rate (DWC), in Leq/day, represents the amount of tap water consumed as drinking water as well as that mixed with beverages and used in cooking. The default for an adult's intake is two L/day. For children a default value of one L/day is used. For volatile organic chemicals, additional exposures occur via the inhalation and dermal routes during and after showering, bathing, flushing of toilets, washing clothes and dishes, and other domestic uses, and a consumption-equivalent volume of two or more Leq/day may be added (OEHHA, 1996, 2000; U.S. EPA, 1992). Selenium compounds found in water are not expected to volatilize, so additional exposures by inhalation are judged to be insignificant. Selenium salts also penetrate intact skin poorly, so dermal exposure is also expected to be insignificant.

The relative source contribution (RSC) is based on an estimate of the contribution of drinking water exposure relative to other sources such as food, air, etc. U.S. EPA typically applies values in the range of 0.2 to 0.8, with 0.2 as the most common value. OEHHA generally follows the U.S. EPA guidance, but has used relative source contribution factors up to one when the health-protective concentration for drinking water is based on a human study, with drinking water as the actual exposure source.

Alternative Method

An alternative method for calculating an allowable drinking water level involves subtracting exposures to a chemical from non-water sources from a maximum acceptable daily dose and allocating the remainder to exposures from water. The health-protective concentration (C, in mg/L) is then calculated as:

$$C = \frac{\text{total dose } (\mu g/day) - \text{food and air } (\mu g/day)}{\text{DWC } (L/day)} = \mu g/L \text{ or ppb in water.}$$

Selenium dietary intake estimates for adults and children have been discussed in a previous section. Because selenium is a required nutrient, the public health-protective concentrations for selenium should not unduly limit drinking water as a potential source of selenium and also account for dietary exposures to this element. As reported above, the Recommended Dietary Allowance (RDA) for selenium is 55 µg/day for the general adult population, 60 µg/day for pregnant women, and 70 µg/day during lactation (NRC, 2000). Most individuals in the U.S. easily meet their nutritional needs for selenium and do not consume selenium supplements. However, consumption of supplements can substantially increase an individual's selenium exposure. To account for this variability

of intakes, OEHHA considers two dietary intake values for selenium. The first value is for the typical consumer who does not take selenium supplements in excess of the RDA. For these individuals, the mean selenium intake from diet alone at 114 µg/day (NRC, 2000) is used as the background selenium consumption rate. The second value considers consumers who take selenium supplements in excess of the RDA. For these individuals, the 90th percentile selenium intake for diet plus supplements at 175 µg/day (NRC, 2000) is used as the background selenium consumption rate.

The choice of an appropriate upper consumption limit for adults is either the current U.S. EPA total daily oral intake of 350 µg/day (U.S. EPA, 1985a,b, 1990b, 1991, 2007) or the NRC (2000) Tolerable Upper Intake Level of 400 µg/day to be safe from selenium toxicity. The total daily oral intake and Tolerable Upper Intake Level are based on slightly different NOAEL values derived from human exposure data and use a three- and two-fold safety factor, respectively. Because the data were generated from study of endemic chronic human selenium toxicity and the toxic endpoints are not severe (NRC, 2000), the use of an additional safety factor based on toxicity issues would be difficult to justify. While inorganic forms of selenium (found in drinking water) are more acutely toxic than selenomethionine (the major form consumed in the selenosis studies), they are considered to have similar chronic toxicities (NRC, 2000). As recent data suggest that selenium intake levels of approximately 50 to 200 µg/day are antineoplastic (Clark *et al.*, 1996b, 1998), the range between therapeutic and toxic doses of selenium for adults at 350 to 400 µg/day may be narrower than previously appreciated.

An estimate can also be provided for appropriate consumption limits for selenium in drinking water for infants. An Adequate Intake for infants aged zero to six months has been estimated as 15 µg/day, and a Tolerable Upper Intake Level as 45 µg/day by NRC (2000). The NHANES III survey data from 1988 to 1994 (U.S. DHHS, 1997, 2002) used by the NRC (2000) estimated the mean selenium intakes for infants zero to six months of age as 35.3 µg/day. The upper 95th percentile levels for selenium consumption are about 50 percent greater than the means, according to the NHANES data (Moshfegh *et al*., 2005), which would be greater than the Tolerable Upper Intake Level for infants.

NRC (2000) reported no observable adverse effects on infants of consumption of human milk at selenium concentrations up to 60 µg/L, with mean concentration of selenium in human milk of about 18 µg/L. The Tolerable Upper Intake Level was based on the extrapolation by Levander (1989b) from studies by Yang *et al.* (1989a,b) on adults and children without selenosis symptoms in Enshi, and a study by Shearer and Hadjimarkos (1975) on human milk-fed infants without symptoms in seleniferous areas in the U.S. It was supported by a study by Bratter *et al.* (1991) on children without symptoms in seleniferous areas in Venezuela. Since all such exposures are not associated with any effects, it appears that the Tolerable Upper Intake Level may be relatively conservative.

Calculation for Noncarcinogenic Effects

The standard method for calculation of a health-protective concentration of selenium in drinking water utilizes the NOAEL of 0.015 mg/kg-day based on toxic non-cancer effects in the population studies in China. An uncertainty factor of only three is chosen because the NOAEL is based on chronic human data, which should encompass most of the

expected human variability. U.S. EPA (2009; last updated 09/01/1991) also chose a factor of three based on the same study, with the rationale that "A full factor of 10 was not deemed necessary since similar NOAELs were identified in two moderately-sized human populations exposed to selenium levels in excess of the RDA throughout a lifetime without apparent clinical signs of selenosis." Using the default values for adult body weight, drinking water consumption, and relative source contribution in this calculation yields the following estimate:

$$C = \frac{0.015 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{3 \times 2 \text{ L/day}} = 0.035 \text{ mg/L} = 35 \text{ µg/L}$$

A similar calculation for infants exclusively drinking formula made with tap water would use a body weight of 10 kg, a water consumption value of 1 L/day, and an RSC of 0.6 to account for the selenium fortification of powdered formula (18 µg/L). This yields the following estimate:

$$C = \frac{0.015 \text{ mg/kg-day} \times 10 \text{ kg} \times 0.6}{3 \times 1 \text{ L/day}} = 0.030 \text{ mg/L} = 30 \text{ µg/L}$$

Estimation of a health-protective concentration (C, in mg/L) in drinking water by the subtraction method can be based on the current U.S. EPA (1985a,b, 1990b, 1991, 2009) total daily oral intake of 350 µg/day for adults, or a Tolerable Upper Intake Level of 45 µg/day for infants zero to six months of age (NRC, 2000). The adult calculations should consider the selenium exposure of those who do consume supplements (i.e., 175 µg/day from diet plus supplements) or do not (i.e., 114 µg/day from diet only). Infants who consume powdered formula would receive selenium based on the required selenium fortification level, equivalent to 18 µg/L. Water consumption rates could utilize the traditional defaults of 2 L/day for adults and 1 L/day for infants, or could utilize the updated U.S. EPA water consumption estimates (U.S. EPA, 2004b). The default calculations are as follows:

$$C_{w/o} = \frac{350 \text{ µg/day} - 114 \text{ µg/day}}{2 \text{ L/day}} = 118 \text{ µg/L or ppb in water.}$$

$$C_{with} = \frac{350 \text{ µg/day} - 175 \text{ µg/day}}{2 \text{ L/day}} = 88 \text{ µg/L or ppb in water.}$$

$$C_{infants} = \frac{45 \text{ µg/day} - 18 \text{ µg/day}}{1 \text{ L/day}} = 27 \text{ µg/L or ppb in water.}$$

U.S. EPA (2004b) estimates the upper 95th percentile for drinking water intake as 2.85 L/day for all adults (20+), 2.60 L/day for the general population (all ages), and 1.24

L/day for infants less than six months of age. The health-protective concentration for "all adults" who take supplements would be (350 – 175 µg/day) / 2.85 L/day = 61 µg/L and for infants would be [45 µg/day – (18 µg/L × 1.24 L/day)] / 1.24 L/day = 18 µg/L; the other estimates fall within the range above. This range of possible values for a public health-protective concentration represents, in our opinion, the true uncertainty as to appropriate health-protective values. Acknowledging that some people drink considerably more than two liters of water per day on a long-term basis, OEHHA suggests that the higher estimates above are not adequately health-protective. The lowest estimates, those for infants, are quite uncertain because the dermal effects in question were not, in general, observed in children. However, a drinking water concentration at the high end of the range above would apparently be excessive for children who consume formula made up with tap water, considering the low estimated Tolerable Upper Intake Level. Thus OEHHA believes that the best value should lie toward the low end of this range. Accounting for the higher exposures of infants, we therefore establish a PHG for selenium in drinking water of 30 µg/L or 30 ppb.

OEHHA concludes that a PHG of 30 µg/L or ppb is appropriate to protect the public, including sensitive populations, from adverse effects of selenium in drinking water. Estimation of the public health-protective level of exposure to inorganic, soluble forms of selenium in drinking water explicitly acknowledges the major contribution of food to daily selenium intake and the status of selenium as a required essential nutrient.

RISK CHARACTERIZATION

The assessment described in this document is related to levels of soluble selenium in drinking water, which are principally in the form of selenite and selenate ions. These forms are distinct from selenomethionine, the principal form of human exposure to selenium, mainly through the diet. However, no data are available on the detection or measurements of selenomethionine in drinking water, and most of the relevant health effects data are based on findings of dietary exposures to organic selenium compounds in humans. Thus, one of the features of the assessment is the use of information on the biological actions of selenomethionine and on the toxic effects of dietary exposure to selenium to interpret exposure to the inorganic selenium ions in drinking water.

For the normal pH range of drinking water of 6.5 to 8.5, selenate is the more predominant species (Lauchli, 1993; McKeown and Marinas, 1986). Selenite and biselenite ions are likely to be reduced to elemental selenium, to be oxidized to selenate ion, or to form insoluble complexes in the presence of ferric ions. The precise ratio of selenate to selenite ions in a given sample of drinking water would depend on constituents such as hydrogen ion concentration and oxidative potential, but a conservative number that might be used for risk estimates could be a proportion of 60 percent selenate to 40 percent selenite, with the likelihood that the proportion of selenate would be much higher.

The principal manifestations of selenium toxicity in human adults are brittleness and loss of hair and nails, and a skin rash. The underlying lesion may arise from disruption of keratinocyte forming cells in the epidermis. The effects on the human skin and its appendages have been reported for excess exposure to both selenomethionine and sodium selenite, but not for sodium selenate. In domestic species such as the cow and pig,

selenium forage, sodium selenite and sodium selenate have produced dyskeratosis, with multiple toxic effects on hair, hoofs, and horns.

From the limited amount of data on humans, it appears that sodium selenite is more potent than selenomethionine in producing dyskeratosis. Yet, according to NRC (2000): "Incorporation of dietary selenomethionine into protein delays selenium toxicity. This storage has created the impression that protein-bound dietary selenium is less toxic than inorganic selenium. Although inorganic selenium has greater immediate toxicity than does selenomethionine, both forms are likely to have similar toxicities under conditions of chronic intake." As the present assessment is on chronic exposure for development of a PHG, a significant differential between the organic and inorganic forms should not be assumed.

Some data suggest that sodium selenate is slightly less toxic than sodium selenite. It is not clear if any difference in toxic potential between the two ions is due to intrinsic activities or due to toxicokinetic factors. In human and animal studies, sodium selenate is absorbed more readily and excreted more rapidly than sodium selenite, in part, perhaps, because of its chemical similarity to the sulfate ion. Studies by Spallholz (1994) and Shiobara *et al.* (1999) indicate that selenate reduction to selenite does not readily take place even in the presence of reduced glutathione. Selenite ion and its reduction to selenide ion are considered to be the proximate toxic forms of selenium. Further research is required to better estimate the toxic potential of drinking water containing selenate or selenite ions.

The soluble forms of selenium found in drinking water are also distinct from the insoluble sulfide forms. However, selenium sulfide and selenium disulfide are not soluble in water and not found in drinking water, so their biological properties (e.g., carcinogenicity) are not germane to the development of a health-protective level of selenium in drinking water.

Total exposure levels associated with avoidance of toxic effects from exposure to selenium in foods is very well established from the extensive studies in China, as well as corroborating evidence from selenosis areas within the United States. These data sources are well supported by clinical studies on selenium as a potential anti-cancer nutritional supplement. Although the range of doses between deficiency and toxicity is relatively narrow for selenium, there should be no real doubt that exposures up to about 300 µg/day have no observable adverse effects in adults.

Potential effects in infants are less well defined, including the estimated Tolerable Upper Intake Level (NRC, 2000), which has been set within the range of common exposures of U.S. infants. We have defined a health-protective level in drinking water which reflects and acknowledges this uncertainty, without presuming a hazard where none has been observed. There are no reports of teratogenicity or selenosis in infants born to mothers with high intakes of selenium (NRC, 2000), and selenium deficiency has not been shown to be responsible for problems with pregnancy and delivery, or female reproduction or fertility.

Other potentially sensitive subpopulations have also been considered. However, data are inadequate to demonstrate whether any subpopulations are more or less sensitive to selenium toxicity than other groups because of the limited information on differences in

selenium metabolism and distribution as well as differences in selenoprotein expression based on ethnicity, age, gender, health, and dietary factors.

OTHER REGULATORY STANDARDS

Drinking Water

The U.S. EPA (1991) has promulgated an MCLG (and MCL) of 50 ppb or 0.05 mg/L for total water-soluble selenium. Two calculations were used by the U.S. EPA in deriving the MCLG for selenium. These methods are described in the drinking water criteria document on selenium (U.S. EPA, 1990) and the Federal Register (U.S. EPA, 1991). The reference doses in both methods were based on the data of Yang *et al.* (1989a,b) on human exposures to environmental selenium. The U.S. EPA has two LOAEL values for selenium in drinking water. The value listed in the IRIS database (U.S. EPA, 1991, 2009) is 0.023 mg/kg-day based on the report by Yang *et al.* (1988a, 1989) and Yang and Zhou (1994). The value listed in the U.S. EPA (1990) document is 0.046 mg/kg-day based on the report by Yang *et al.* (1983).

In the first calculation, the U.S. EPA (1985a,b; 1991) used the LOAEL of 0.046 mg/kg-day, an uncertainty factor of 15, and a dietary intake of selenium of 0.125 mg/day, to calculate the MCLG as:

$$\text{MCLG} = \frac{[(0.046\ \text{mg/kg-day} \div 15) \times 70\ \text{kg}] - 0.125\ \text{mg/day}}{2\ \text{L/day}}$$

$$= 0.0448\ \text{mg/L, rounded up to } 50\ \mu\text{g/L.}$$

Regarding the uncertainty factor of 15, U.S. EPA (1990) wrote: "This uncertainty factor was based upon a human study identifying the LOAEL. According to the Office of Drinking Water of the U.S. EPA, referring to the guidelines of the National Academy of Sciences, when a human study with a LOAEL is used, the reference dose is calculated using an uncertainty factor of 100. In this instance, however, a judgment was made to use an uncertainty factor of 15 to counterbalance the evidence of the severity of deficiency effects from selenium, as well as the potential anticarcinogenic properties of selenium. Thus, the uncertainty factor of 15 appears to represent an adequate balance between the adverse and beneficial effects of selenium in humans." The U.S. EPA (1985a,b) also noted that if a traditional uncertainty factor of 10 were used, then a health-protective concentration of 100 µg/L would have been derived.

It was reasoned that if the MCL were set at 50 µg/L, the maximum total dose of 225 µg/day would still be below the no effect level of 400 µg/day, and allow a margin of safety for individuals who consumed selenium supplements. NRC (2000) has also used the 400 µg/day-person dose as a Tolerable Upper Intake Level for adults.

The California DHS adopted a California MCL for selenium of 50 ppb in 1994 following the 1991 federal final rules (CCR, Title 22, for inorganic chemicals Section 64468.1) that established the federal MCL of 50 ppb. Other states that have an MCL of 50 ppb for

selenium are: Alabama, Alaska, Arizona, Colorado, Connecticut, Delaware, Florida, Georgia, Hawaii, Illinois, Indiana, Iowa, Maryland, Massachusetts, Michigan, Montana, Nebraska, New Hampshire, New Mexico, New York, North Dakota, South Carolina, Tennessee, Texas, Utah, Vermont, Washington, and Wisconsin (ATSDR, 2003).

Other relevant drinking water standards for selenium in various states include: Alabama, Colorado, Delaware, and Kentucky, primary drinking water standards of 10 ppb; Arizona drinking water guideline of 45 ppb; Kansas domestic water supply standard of 50 ppb; Louisiana groundwater protection MCL of 10 ppb; Maine drinking water guideline of 10 ppb; Massachusetts groundwater protection MCL of 10 ppb; Minnesota drinking water guideline of 30 ppb; Mississippi water quality criterion of 10 ppb; North Carolina groundwater quality standard of 50 ppb; Nevada domestic water supply dissolved selenium standard of 50 ppb; Ohio groundwater concentration limit of 10 ppb; Oklahoma public and private water supplies standard of 10 ppb; Rhode Island preventive action limit of 25 ppb; South Dakota groundwater maximum allowable concentration of 50 ppb; Virginia MCL of 10 ppb; and Wyoming water quality for human health standard of 10 ppb (ATSDR, 2003; U.S. EPA, 2009).

The U.S. EPA (1990) drinking water criteria document proposed a one-day health advisory of 41 ppb for a child with 10 kg body weight, a 10-day health advisory of 41 ppb for a 10 kg child, a long term health advisory of 31 ppb for a 10 kg child, and a long term health advisory of 107 ppb for an adult with a 70 kg body weight.

WHO (2001) has had a 10 ppb drinking water guideline for selenium since 1984. WHO stated that estimates of selenium intake range between 130 and 200 µg/day and the maximum daily selenium intake from drinking water should not exceed 10 percent (or 0.1) of the recommended maximum daily dietary intake of 200 µg/day. Assuming a daily water intake of 2 L/day, WHO (1996a) determined a guideline value of 0.01 mg/L or 10 ppb (200 µg/day × 0.1 ÷ 2 L/day = 10 µg/L), as also cited by the U.S. EPA (1985b). The WHO (1996a) guideline does not take into account the beneficial effects of selenium, and the 0.1 contribution is arbitrary, applied to all chemicals whether or not they are essential nutrients. The most recent version of the WHO drinking water guidelines restates the 10 µg/L standard with the 10 percent allocation to water (WHO, 2008).

European Union countries including Austria, France, Germany, and United Kingdom adopted the WHO water quality standard of 10 ppb. Health Canada (1992) has adopted 10 ppb as the maximum acceptable concentration (MAC) standard in Canada. The MAC is considered to provide a reasonable factor of safety from adverse effects of selenium. Drinking water containing selenium at this level would be the source of between 10 and 25 percent of total selenium intake. Australia, New Zealand, Japan, and Thailand also adopted the 10 ppb standard (Benner, 2004). The Russian limit for selenium in drinking water has been 1 ppb as selenium trioxide (SeO_3) since 1970 (Calabrese, 1978).

The U.S. FDA has a permissible level of 10 ppb selenium for bottled water (ATSDR, 1996, 2003). U.S. FDA (1987, 1993, 2000, 2001) has ruled that feed for major food-producing animals may be supplemented with sodium selenite or selenate at a level not to exceed 300 ppb or 0.3 ppm of selenium. Feed supplements for limited feeding may provide selenium at a rate not to exceed an intake of 0.7 mg/head-day for sheep and 3 mg/head-day for beef cattle.

Ambient Water

The U.S. EPA (1980) established the ambient water quality criterion for human health as 10 ppb for selenium. The value was chosen with the consideration that only five to 10 percent of daily dietary exposure to selenium should come from drinking water sources. This value was calculated based on an acceptable daily intake of selenium from foods of 0.7 mg. A maximum water-related contribution of 35 µg/day was selected as protective, and consumption of 6.5 g of fish was taken into consideration. A final criterion value of 10 µg/L or 10 ppb was proposed; however, this value did not take into account the beneficial effects of selenium (U.S. EPA, 1980, 1985a,b; 1990).

The U.S. EPA (1987) also established the U.S. national ambient water quality criterion for the protection of aquatic life in fresh water based on continuous chronic exposure as 5 ppb for selenium. However, scientists with the U.S. Fish and Wildlife Service have recommended setting the ambient water quality criterion at 2 ppb for both aquatic and wildlife protection (Lemly, 1993a). The salt water quality criterion based on continuous chronic exposure is 71 ppb for selenium, and the maximum salt water quality criterion is 290 ppb. The criterion for human consumption of organisms from water sources is 11,000 ppb, and for human consumption of water and organisms from water sources is 170 ppb (U.S. EPA, 2002; also see http://www.epa.gov/waterscience/criteria/wqctable). The Canadian water quality guidelines for the protection of aquatic life in fresh or marine water is 2 ppb in the water column, 2 µg/g dry weight in the sediments, and 1 µg/g wet body weight in tissue. To protect wildlife from adverse effects, the maximum concentration of total selenium should not exceed 4 ppb. The maximum allowable concentration of total selenium in irrigation water supplies is 10 ppb. To protect livestock, the maximum concentration of total selenium in livestock watering use should not exceed 30 ppb (Nagpal, 2001).

The U.S. EPA drafted a chronic criterion for selenium in ambient water of 5 ppb in March, 2002, using tissue concentrations with the intent of safeguarding fish reproduction. In December, 2004, the U.S. EPA (2004) proposed a draft freshwater chronic criterion of a concentration in whole body fish tissue of 7.91 µg selenium/g dry weight. The U.S. EPA (2004) also states that if fish tissue samples exceed 5.85 µg/g during summer or fall, fish should be monitored during the winter to determine if selenium exceeds 7.91 µg/g.

A site-specific water quality standard for selenium, in a mining effluent discharge located at the south end of the lake, in the high salinity Great Salt Lake in Utah has been developed through examining the bioavailability and acute and chronic toxicity of selenium, as selenate, to biota resident like brine shrimp *Artemia franiciscana*, brine fly *Ephydra cinerea*, and a hypersaline alga *Dunaliella viridis* and the potential for dietary selenium exposure to aquatic dependent birds that might consume resident biota (Brix *et al.*, 2004, 2005; Toll *et al.*, 2005). The resulting acute and chronic toxicity data indicated that resident species are more selenium tolerant than many freshwater species. Applying a dietary selenium threshold of 5 mg/kg dry weight for aquatic birds to this relationship resulted in an estimate of selenium in water at 27 µg/L or 27 ppb as a safe concentration for this exposure pathway and an appropriate chronic site-specific water quality standard for the protection of aquatic birds.

Based on a probabilistic approach to assess water, food chain, and bird egg residues of four bird species from 15 sites, waterborne selenium concentrations of 6.8 to 46 µg/L were calculated to protect birds; a range of values was associated with an effect threshold of 20 mg selenium/kg dry weight in eggs (Adams *et al.*, 1998). The thresholds correspond to an EC_{10} of 16 mg/kg and an EC_{20} of 21 mg/kg mean egg selenium concentration in mallards (Fairbrother *et al.*, 1999).

Other

ATSDR (1996, 2003) recommended an upper estimated safe level of 0.005 mg/kg-day or 5 µg/kg-day for chronic oral ingestion as the Minimal Risk Level (MRL) based on the NOAEL of 0.015 mg selenium/kg-day or 0.85 mg selenium/day derived from Yang *et al.* (1989a,b) with Chinese subjects of an average weight of 55 kg. This is the same as the U.S. EPA (1985a,b, 1990, 1991, 2007) Reference Dose, as discussed by Abernathy *et al.* (1993) and Poirer (1994). Both the ATSDR (2003) and the U.S. EPA (2009) used an uncertainty factor of three for sensitive individuals. ATSDR states that this Minimal Risk Level is approximately six times greater than the Recommended Dietary Allowance (RDA) for selenium at 55 µg/day or approximately 0.8 µg/kg-day established by the NRC (2000). Yang *et al.* (1989a,b) suggested a human dietary selenium intake at 40 µg/day as the minimum and 400 µg/day as the maximum.

Lo and Sandi (1980) reported a maximal acceptable selenium intake for each person as 500 µg/day. The Expert Group on Vitamins and Minerals (EVM, 2003) of the United Kingdom established a safe upper level of 450 µg/day based on the NOAEL of 900 µg/day from Yang *et al.* (1989a,b) and an uncertainty factor of two for the use of a LOAEL for humans that was close to the NOAEL (Renwick, 2006).

The Scientific Committee on Food (SCF, 2000) of the European Commission established a tolerable upper intake level (UL) of 300 µg selenium/day based on the NOAEL of 900 µg/day from Yang *et al.* (1989a) and an uncertainty factor of three (Renwick, 2006). The Scientific Committee on Food (SCF, 2000) of the European Commission extrapolated from adults to children on a body weight basis to derive a tolerable upper intake level (UL) as 60 µg selenium/day for one to three years old, 90 µg selenium/day for four to six years old, 130 µg selenium/day for seven to 10 years old, 200 µg selenium/day for 11 to 14 years old, and 250 µg selenium/day for 15 to 17 years old. The established levels relate to selenium naturally present in food as well as sodium selenate, sodium selenite, and sodium hydrogen selenite, which have been considered as acceptable for use in food.

The U.S. National Research Council (NRC, 1980a) first estimated an adequate and safe intake of selenium for adults of 0.05 to 0.2 mg/day with correspondingly lower intakes for children and infants. In 1989, the NRC established an RDA of 0.07 mg/day for a standard North American 79-kg man and 0.055 mg/day for a standard North American 62-kg woman (Levander, 1987, 1989a). In general, the RDA is 0.00087 mg/kg-day or 0.87 µg/kg-day. The U.S. NAS recommends 50 to 200 µg/day as the adequate and safe ingestion intake of selenium for adults (NRC, 1989). The Institute of Medicine (IOM) of the NRC set the selenium RDA at 55 µg/day for both male and female adults (Burk, 2002; NRC, 2000; Thomson, 2004a). The estimated daily requirement of selenium for

pregnant women has been set at 65 µg/day (NRC, 1989). The U.S. EPA recommends 213 µg/day as the Acceptable Daily Intake (ADI).

In 1994, an Expert Consultation between the Food and Agriculture Organization (FAO) of the United Nations and WHO (Joint FAO/IAEA/WHO Expert Consultation on Trace Elements in Human Nutrition) accepted the NOAEL of the Yang *et al.* (1989a,b) study and their suggestion that a maximal safe dietary selenium intake should be set at 400 µg/day based on an uncertainty factor of two, or 0.7 µg/kg-day for a 55 kg adult. WHO (2001) set an RDA for healthy adults of 50 to 70 µg/day, or 0.9 to 1.3 µg/kg-day for a 55 kg adult.

However, the lower limit of the safe range of dietary selenium intake estimated by WHO (1996b) is 40 µg/day for men and 30 µg/day for women (Thomson, 2004a). The basal requirement refers to the intake needed to prevent signs of deficiency of the nutrient. The population minimum mean intakes of selenium likely to meet basal requirements for adult males and females were 21 and 16 µg/day, respectively. These were derived from the amount needed to protect against Keshan disease plus a body weight correction factor. On the other hand, the population minimum mean intakes likely to meet normative requirements for adult males and females were 40 and 30 µg/day, respectively (Rayman, 2004). These were calculated from the amount needed to achieve two-thirds of the maximal GPx3 activity, assuming an interindividual variability of normal dietary selenium intake of 16 percent (Levander, 1997; WHO, 1996b).

WHO (1996b) established an RDA of 10 µg selenium/day for children aged one to three years, 15 µg/day for children aged four to six years, 25 µg/day for children aged seven to 10 years, 35 µg/day for adolescents aged 11 to 14 years, 45 µg/day for adolescents aged 15 to 17 years, 55 µg/day for adults and seniors, 55 µg/day for pregnant women, and 70 µg/day for lactating women.

Fish consumption advisories to recommend that fish should not be eaten because of high selenium concentrations have been developed for areas in California, Colorado, North Carolina, Texas, and Utah (ATSDR, 1996, 2003).

Selenium-enriched yeast is approved for use in diets for poultry (U.S. FDA, 2001), swine (U.S. FDA, 2003), and cattle (U.S. FDA, 2004). In a complete diet for livestock, the fortification cannot exceed the equivalent of 0.3 ppm selenium in feed (U.S. FDA, 2004). The U.S. FDA (1987) approves the use of sodium selenate and sodium selenite as a dietary supplement for inclusion at 0.3 ppm selenium to all swine feed. NRC (1988) has recommended a dietary level of 0.15 ppm selenium for reproducing sows.

The acceptable chronic daily inhalation exposure level of selenium recommended by the U.S. EPA is 3.5 $\mu g/m^3$. Other guidelines and standards, especially those for the occupational environment through inhalation exposure, are summarized by ATSDR (1996, 2003), Barceloux (1999), and Hall and Rumack (2004).

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