Investigation of the Toxicity and Toxicokinetics of Selenium from the Accumulator Plant Symphyotrichum spathulatum (Western Mountain Aster) in Sheep

Amanda Wilhelm
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INVESTIGATION OF THE TOXICITY AND TOXICOKINETICS OF SELENIUM
FROM THE ACCUMULATOR PLANT SYMPHYOTRICHTUM SPATHULATUM
(WESTERN MOUNTAIN ASTER) IN SHEEP

by

Amanda Wilhelm

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Toxicology

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Logan, Utah

2010
ABSTRACT

Investigation of the Toxicity and Toxicokinetics of Selenium from the Accumulator Plant *Symphyotrichum spathulatum* (Western Mountain Aster) in Sheep

by

Amanda Wilhelm, Master of Science

Utah State University, 2010

Major Professor: Dr. Jeffery O. Hall
Department: Animal, Dairy and Veterinary Science

This study was designed to observe the effects of selenium from plant material in sheep after a single, oral dose. Purified sodium selenite and selenomethionine were given as positive controls. The plant *Symphyotrichum spathulatum* (Western Mountain Aster) was collected, analyzed for selenium content, and administered orally to sheep at varying doses according to body weight. Clinical signs were observed for 7 days during which time whole blood, serum, and expired air were collected. Following euthanasia, tissues were collected for histopathological analysis and mineral analysis.

Clinical signs were less apparent than expected and included depression and mild dyspnea in sheep receiving the highest doses of selenium as plant material, whereas pathologic lesions were prominent. Acute myocardial degeneration and necrosis was most severe in the highest dose animals, but present to lesser degrees as dose decreased.
Pulmonary lesions of edema and congestion were less frequently observed. Thirteen animals died prior to study completion. Selenium concentration in tissues, brain, liver, kidney cortex, atrium, ventricle, and skeletal muscle, increased with increasing dose of plant material. Treatment had a significant impact on selenium concentration in all tissues collected for mineral analysis ($P < 0.01$).

Whole blood and serum were collected to study the toxicokinetics of selenium in these sheep. Serum kinetic parameters that increased significantly with increasing dose included the elimination rate constant, peak selenium concentration, and area under the selenium concentration versus time curve. Serum kinetic parameters that significantly decreased with increasing dose included the absorption and elimination half-lives. Whole blood kinetic parameters that increased significantly with increasing dose included the elimination rate constant, peak selenium concentration, and area under the curve.

Expired air was collected to study the respiratory toxicokinetics of selenium in the sheep. The selenium concentration in expired air from sheep receiving selenomethionine was significantly greater than all other treatments ($P < 0.0001$) at all collection time points. But an intriguing finding was the dramatic differences in elimination profile curves as selenium dose increased with the plant material. The highest dose group elimination curve continually increased through all collection time points. All other groups dosed with plant material saw a decrease in selenium elimination by the last collection time point.
I would like to begin by thanking my major professor, Dr. Jeffery Hall, for giving me the opportunity to work on this project. I appreciate the time, support, and expertise that he provided throughout my graduate work. I also want to thank my committee members, Dr. Bryan Stegelmeier, Dr. Roger Coulombe, and Dr. Paul Grossl. They have been an excellent support to me during my graduate work. I thank the Animal, Dairy and Veterinary Science (ADVS) Department as well as the Interdisciplinary Toxicology Program for providing me with an opportunity to pursue a master’s degree at Utah State University. I am grateful that I was awarded a departmental assistantship from the ADVS Department.

This research was funded by the Utah Agriculture Experiment Station and the Poisonous Plant Research Laboratory (PPRL). I would like to thank the staff of the PPRL and the Utah Veterinary Diagnostic Laboratory for their help and the use of their facilities. I want to specifically mention Dr. Kip Panter, Ed Knoppel, Joseph Jacobson, Al Maciulis, Rex Probst, Danny Hansen, and Seth Lundquist - I could not have done this work without their assistance.

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Finally and most importantly, I want to thank my family and friends for their support. My parents helped label tubes, type and make editorial corrections in my writing, as well as many other ways they will never realize. I could not have made it through without their support. Also I am grateful for the unfailing friendship of Nancie and John Hergert. Thank you all very much; you have been a tremendous help.

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Selenium is an essential trace element that it is required in the diets of mammals and poultry, but can easily be over supplemented due to a narrow range of safety between ideal and toxic concentrations. The toxicity of inorganic selenium, such as selenite and selenate, has been heavily studied and is well reported in the literature. Recently, toxicity studies of organic selenium forms have increased, but not yet to the extent of that for inorganic selenium. An area lacking in the literature is the study of selenium from plants, particularly selenium accumulating plants. This relative lack of knowledge of selenium from plants is a concern because there is a potential that livestock in several parts of the United States could be grazing plants grown on seleniferous soils. Selenium in plants is known to be in a variety of chemical forms, including both organic and inorganic chemical forms. This means livestock could respond differently to selenium found in plants than to purified organic or inorganic selenium alone as reported in the literature.

The purpose of this study was to observe the effects of selenium from an accumulator plant (Symphyotrichum spathulatum-Western Mountain Aster) and compare results with the effects of purified sodium selenite and purified selenomethionine in orally dosed sheep. I looked at the clinical signs, gross and histopathological changes in sheep given selenium in one of the above three forms, over a period of 7 days. During this time whole blood and serum samples were collected to evaluate the toxicokinetics of selenium from this particular plant in sheep. Expired air was collected to evaluate the toxicokinetics of respiratory selenium elimination as respired air is a known elimination
pathway used by mammals after exposure to toxic concentrations of selenium. Tissue samples for analysis of selenium content were collected from sheep that died immediately following their death. The remaining sheep were euthanized at the study completion of 7 days at which time tissue samples were collected for selenium analysis.

Through this study, I hoped to increase our knowledge of selenium, specifically that associated with selenium accumulator plants. Perhaps the data presented herein will answer some existing questions and will increase interest and opportunities for future research on selenium accumulator plants.
Selenium is an essential trace mineral required in the diets of mammals and poultry. It is a metalloid element with an atomic number of 34. Selenium exists in four oxidation states: -2, 0, +4 and +6. Common inorganic forms include selenite and selenate, whereas common organic forms include selenomethionine and selenocysteine. Other organic forms have recently been identified in some plants.

In 1817, selenium was discovered in a chamber used to make sulfuric acid by Jons Jacob Berzelius. He was studying illnesses of Swedish workers at a sulfuric acid plant when he discovered this element that he named selenium after the Greek moon goddess, Selene.

Since its initial discovery as a toxin, selenium has been found to be toxic under a variety of exposure conditions. In 1295, Marco Polo recorded a case of selenium toxicosis in horses with hoof lesions. Pedro Simon recorded toxicoses in chicks and children that resulted in deformities, as well as nail and hair loss of people in Columbia in 1560. T. C. Madison made the first apparent report of selenium poisoning in North America in 1860 when he reported sick cavalry horses at Fort Randall in South Dakota, an area now known to contain toxic amounts of selenium in soil and plants. These animals exhibited lameness and an eventual loss of hooves, which prevented them from getting food and water, causing the animals to die of starvation. Throughout the 1930’s, a South Dakota farm known as the Reed Farm experienced selenium poisoning problems
in livestock.\textsuperscript{61} Chicken eggs did not hatch because the deformed chicks were unable to break the eggshell. The hens producing these eggs were eating a wheat grain that was found to contain high concentrations of selenium. The federal government purchased this land and it is now an agriculture experiment station.

In 1957, a study by Schwarz and Foltz, led to the realization that selenium is an essential element.\textsuperscript{79} Selenium was later discovered to be a necessary part of glutathione peroxidase enzymes as well as other selenoproteins found throughout the body.\textsuperscript{5}

Soil Selenium

Selenium can accumulate in soil due to leaching from seleniferous rock formations, spread of selenium-containing manure and application of phosphate fertilizer containing selenium.\textsuperscript{5,6} The amount of selenium in soil varies between locations due to variable occurrence in different rock formations. Selenites and selenides are found in sulfide minerals and organic selenium is found in areas resulting from plant and animal decay. According to Bennett,\textsuperscript{10} selenium concentrations in the United States soil can vary from 0.1 parts per million (ppm) to over 1000 ppm. Selenium can be found in association with phosphate rocks, which are then used as phosphate fertilizers in agriculture.\textsuperscript{86} This is significant because the use of phosphate fertilizer will increase the soil selenium concentration.

Generally soil selenium content is in the range of 0.1 to 2 ppm.\textsuperscript{86} However, certain areas have more selenium and are referred to as seleniferous. Selenium concentrations in these areas typically range from around 2 to 10 ppm. Seleniferous soils do not always produce toxic concentrations in plants, because the predominant chemical
form taken up by plants is water soluble selenate. Plants also commonly take up lesser amounts of selenite and organic selenium compounds including selenomethionine. Non-toxic seleniferous soils may contain a high concentration of selenium, but not in available forms for plants to utilize. Toxic seleniferous soils often contain much less selenium than non-toxic seleniferous soils. These toxic soils are a problem because the selenium is in readily available chemical forms for plant uptake such as selenate.

The predominant differentials for toxic versus non-toxic potential of seleniferous soils include pH, moisture content and the chemical form of the selenium. Toxic seleniferous soils are typically of an alkaline pH and in areas of low rainfall. Water-soluble selenate is the predominant form found in these soils and is highly available to plants. In the United States, vegetation that can cause selenium poisoning in livestock is commonly seen in areas of North and South Dakota, Wyoming, Montana, Nebraska, Kansas, Colorado, Utah, Idaho, Arizona, and New Mexico. Soils of Hawaii and Puerto Rico contain large concentrations of selenium, but not in a water-soluble form, therefore unavailable for plant uptake. These soils are more acidic ranging from pH 4.5 to 6.5.

Different types of soil contain selenium in different chemical forms. In well-aerated, alkaline soils, selenium forms selenates, which are highly available to plants. In acidic, poorly aerated soils, selenium forms ferric-iron-selenite complexes, which are only slightly available to plants. Elemental selenium is stable in soils and not easily converted to plant available forms. Organic selenium is thought to come mostly from plant and animal decay. Organic forms are water-soluble and also readily available for plant uptake.
Plants are often divided into three groups based on selenium status: primary accumulators, secondary accumulators and those that do not accumulate large amounts of selenium. Primary accumulators can live on, and often require, highly seleniferous soils and can accumulate selenium up to tens of thousands ppm. Selenium in primary accumulators is believed to reach such high concentrations because these plants have the ability to metabolize selenium to organic storage forms rather than incorporating it into plant proteins. Primary accumulators include species of *Astragalus*, *Stanleya*, *Machaeranthera*, *Oonopsis* and *Xylorhiza*. Secondary accumulators or indicators rarely accumulate more than several hundred to a few thousand ppm. Secondary accumulators include *Aster*, *Castilleja*, *Grindelia*, *Atriplex*, *Gaetrieaia* and *Comandra*. Indicators can live on both seleniferous and non-seleniferous soils. The final group includes grasses, trees and some weeds that generally accumulate less than 100 ppm, but can occasionally accumulate a few hundred ppm.

Plant selenium content may or may not be reflective of soil selenium content, resulting in plant concentrations that can range from deficient to toxic. Some areas of the United States have forages with enough selenium to meet livestock requirements. Some forages do not provide enough to meet livestock requirements causing deficiencies and some forages provide too much selenium, resulting in toxicoses. The chemical form of selenium in plants varies with species. Some plants contain mostly organic compounds and some contain mostly inorganic compounds. Other plant species contain a combination of both organic and inorganic in tissues. The most common chemical
form of selenium found in non-accumulator plants is selenate, however plants can contain selenite, selenomethionine, selenocysteine, selenohomocysteine and others. Selenium accumulator plants such as *Astragalus* and *Stanleya* typically contain selenium in organic forms such as Se-methylselenocysteine and selenocystathionine. Selenium is usually absorbed from soil into plants in the form of selenate because selenite is more likely to be bound to soil particles. After inorganic selenium enters certain plants that chemically alter it to organic forms, it is metabolized mostly to selenomethionine and to a lesser amount to selenocysteine and other much less prominent organic forms.

Certain primary accumulators take up large amounts of selenium from soil and convert it to other chemical forms. The most common chemical form found in accumulator plants is Se-methylselenocysteine, followed by selenocystathionine. Other common forms observed in accumulator plants include Se-methylselenomethionine, γ-glutamyl-Se-methylselenocysteine, γ-glutamyl-selenocystathionine and selenohomocysteine. Selenium in accumulator plants is converted from selenocysteine through the action of the selenocysteine methyltransferase enzyme to methylselenocysteine. Once in the form of methylselenocysteine, it cannot be incorporated into proteins allowing accumulator plants to accumulate and tolerate high concentrations of selenium.

Plant uptake and distribution of selenium varies with chemical forms. Uptake of selenate and organic selenium is metabolically driven via sulfur uptake pathways. In contrast, selenite uptake appears to be passive. In the plant, selenium appears to be metabolized by sulfur assimilation enzymes. Due to the similarities of sulfur and selenium, absorption and metabolism undergo competitive interactions between the two
elements. The chemical form affects distribution of selenium within the plant. Leaves accumulate the most when supplied as selenate and roots accumulate the most when supplied as selenomethionine.

After absorption of selenium from soil, selenium is moved to the chloroplast and metabolized by sulfur assimilation enzymes.\textsuperscript{27} It is activated by ATP sulfurylase forming adenosine-5’-phosphoselenate which is then reduced to yield selenide.\textsuperscript{80} Selenide is used to make selenocysteine, which can react to form selenomethionine. Selenomethionine can be methylated to various products including volatile dimethyl selenide and dimethyl diselenide. Selenium-accumulating plants metabolize selenomethionine to products such as Se-methylselenocysteine, selenohomocysteine or Se-methylselenomethionine. Accumulator plants can accumulate large amounts of Se-methylselenocysteine as well as S-methylcysteine, which indicate they share pathways.

The amount of selenium taken up by plants differs with chemical form of selenium. In a study by Gissel-Nielsen and Bisbjerg\textsuperscript{34} of mustard, clover and barley, elemental selenium uptake from soil was very minimal, selenite uptake was \textasciitilde1\% of the added amount and selenate uptake was 12-60\% of the added amount. Another study on broccoli, Indian mustard, sugar beet and rice showed the maximum accumulation of selenium in shoots occurred with selenate supplementation except in the case of sugar beet which had the greatest accumulation with selenomethionine.\textsuperscript{95} The highest accumulation of selenium in roots occurred with selenomethionine followed by selenite then selenate in all four plants species studied. Sulfate can inhibit uptake of selenate, but not selenite or selenomethionine. When sulfate was added in increasing amounts to the growth medium, selenate uptake was progressively inhibited, while sulfate addition had
no effect on selenite or selenomethionine. This results from the selenate having the same uptake mechanism as sulfate. Non-selenium accumulators preferentially take up sulfate over selenate. Selenium accumulating plants preferentially take up selenate over sulfate from the soil.

In plants, absorbed selenate can stay in the same chemical form while absorbed selenite is metabolized to organic selenium compounds and to a lesser extent, selenate. Selenate tends to accumulate in old leaves and organic selenium tends to accumulate in young plant tissues. Selenomethionine is the predominate selenium containing amino acid in non-selenium accumulating plants, but is only made in small amounts in accumulators and leads to the production of other organic selenium products.

Selenoproteins

Selenium is an essential element in mammals and poultry. These animals and humans use selenium as a necessary component of many selenoproteins found throughout the body. There may be over 30 selenoproteins, but only a few major ones are discussed here. G. C. Mills discovered the first glutathione peroxidase in 1957. The enzyme, in conjunction with reduced glutathione, protected erythrocytes against oxidation and hemolysis by peroxide. The 80 kDa enzyme contains four identical subunits that each contain selenocysteine in the active site. There are at least four known glutathione peroxidase enzymes which all appear to function as antioxidants in the body. The known enzymes are Cytosolic Glutathione Peroxidase, Plasma Glutathione Peroxidase, Gastrointestinal Glutathione Peroxidase and Phospholipid Hydroperoxide Glutathione Peroxidase. All are found in cattle, human, swine, sheep and rat tissues.
Cytosolic Glutathione Peroxidase was the first selenoprotein discovered.\textsuperscript{3} It metabolizes potentially toxic hydrogen peroxide as well as lipid hydroperoxides to non-toxic products. Mice have been shown to develop normally without this enzyme, but are more susceptible to oxidative damage.\textsuperscript{28} Non-virulent Coxsackie virus can mutate to a virulent form in mice devoid of glutathione peroxidase.\textsuperscript{6} The increased mutation rate occurs due to an increase in hydroperoxides, which cause mutations in the viral genome, leading to a virulent strain. These mice are similar to Chinese patients with Keshan Disease, a disease caused by selenium deficiency.

The specific function of Plasma Glutathione Peroxidase is not known.\textsuperscript{28} It may reduce lipid hydroperoxides in low-density lipoproteins or serve as a detoxification enzyme. It appears to be synthesized mostly in kidneys and lungs and may have a specific function to these locations.\textsuperscript{3}

Gastrointestinal Glutathione Peroxidase is found in the intestine and is therefore assumed to function against ingested hydroperoxides.\textsuperscript{3,28,82} Phospholipid Hydroperoxide Glutathione Peroxidase prevents lipid peroxidation of biomembranes. This particular glutathione peroxidase also appears to possibly have a role in regulation of enzyme activity.

Each glutathione peroxidase is found in high concentrations in different areas of the body. Cytosolic Glutathione Peroxidase is found in high concentrations in the liver, kidney, lung, red blood cells and placenta as well as other tissues.\textsuperscript{28} Plasma Glutathione Peroxidase is located in extracellular body fluids (e.g. blood plasma, chamber water of the eye and amniotic fluid). Gastrointestinal Glutathione Peroxidase is located in epithelial lining of the gastrointestinal tract from the esophagus to the rectum.
Phospholipid Hydroperoxide Glutathione Peroxidase is located in endocrine tissues, testes and brain.

Selenoprotein P was the second selenium containing protein discovered.\textsuperscript{47,82} It is located in the plasma and over 60\% of rat plasma selenium is part of Selenoprotein P. This protein has been shown to incorporate selenium more efficiently than glutathione peroxidase. Approximately 7.5 atoms of selenium are present per Selenoprotein P molecule. One possible function of Selenoprotein P is as an antioxidant. In times of selenium deficiency, Selenoprotein P is more resistant than glutathione peroxidase to the lack of selenium. The amount of Selenoprotein P usually does not fall until after liver glutathione peroxidase concentrations have fallen. In selenium deficient rats, diaquat leads to lipid peroxidation and liver necrosis. In rats given a small dose of selenium, enough to raise Selenoprotein P but not glutathione peroxidase content, peroxidation and liver damage did not occur following administration of diaquat. Another possible function of Selenoprotein P is as a transport mechanism of selenium from the liver to other tissues.

Another selenium containing protein is Selenoprotein W.\textsuperscript{77} It is found in high concentrations in human and sheep cardiac and skeletal muscle. Selenium deficient animals develop White Muscle Disease therefore Selenoprotein W has been proposed to contribute to muscle health in sheep and humans due to its location. A possible antioxidant function has also been proposed for Selenoprotein W.
Selenium Absorption

Ingested selenium is absorbed mostly in the small intestine of most animals. Vendeland et al. observed rapid absorption of selenomethionine from all three segments of the small intestine in rats. Selenate was absorbed to the greatest extent in the ileum, followed by the jejunum and then duodenum. Selenomethionine was absorbed better than selenite or selenate. Selenate absorption was greater than selenite in jejunum and ileum, however selenite absorption was 60% better in the duodenum compared to selenate. Overall, uptake of selenate from all segments of the small intestine was 40% greater than selenite and absorption of both inorganic compounds increased with progression down the small intestine. In contrast, selenomethionine absorption was roughly equal from all three segments of the small intestine.

A study in guinea pigs supplemented with organic selenium at 0.1, 0.2, or 0.3 ppm selenium showed an increase in overall absorption of selenium with each increase in dose. The pigs receiving 0.1 and 0.2 ppm selenium had a higher average daily gain over those supplemented with 0.3 ppm and those in the control group, which were not supplemented with selenium. The authors conclude that selenium can increase body weight gain, but there is a threshold where selenium supplementation no longer impacts daily gain.

Ruminants do not absorb as much selenium as monogastrics because it is also utilized by microbes in the rumen. Hidiroglou and Jenkins reported a small amount of selenomethionine absorption from the rumen of sheep. About 90% of the dose was recovered in the rumen following the completion of the experiment. After 2 hours, the
amount of ruminally absorbed $^{75}$selenium in blood peaked, most of which was bound to plasma proteins.

Organic selenium compounds are usually absorbed better than inorganic selenium. Selenate is absorbed better than selenite, which is absorbed better than elemental selenium. Organic selenium species are absorbed through active transport, selenite is absorbed through passive diffusion and selenate is absorbed through a sodium-mediated carrier with sulfate. Selenomethionine and methionine are absorbed by the same amino acid transport therefore they can inhibit each other resulting in nonspecific uptake and distribution into proteins. In an experiment by Awadeh et al. beef cows were supplemented with one of the following: 20, 60, or 120 μg selenium/g of salt mix as sodium selenite or 60 μg selenium/g of salt mix as selenized yeast which contained mostly selenomethionine. Cows supplemented with organic selenium as selenized yeast had significantly increased selenium concentration in whole blood compared with cows given 60 μg selenium as selenite. Selenized yeast supplemented cows at 60 μg selenium/g of salt mix had similar whole blood selenium concentrations as selenite supplemented cows at 120 μg selenium/g salt mix. A study by Steen et al. also showed that ewes fed organic selenium, mostly as selenomethionine, had higher whole blood selenium concentrations than ewes fed inorganic selenium as sodium selenite. It was also noted that lambs of ewes fed organic selenium had higher concentrations in whole blood compared with lambs of ewes fed inorganic selenium.

Selenium can be absorbed through inhalation. The rate of absorption depends on the chemical form. Radiolabelled $^{75}$selenium was found in the blood of rats exposed to
selenious acid or elemental selenium through inhalation. Selenious acid was found in the blood sooner than elemental selenium.

Echevarria et al. performed a study to observe tissue selenium concentrations in lambs following administration of selenium as sodium selenite. Lambs received one of the following: basal diet, basal + 3 mg selenium/kg, basal + 6 mg selenium/kg or basal + 9 mg selenium/kg. Serum and tissue selenium concentrations increased linearly with each increase in selenium supplementation. Serum, kidney and liver selenium concentrations increased the most after exposure to selenium.

Koenig et al. studied the effects of chemical form and diet on selenium absorption, retention and excretion. Sheep were fed either a forage or concentrate-based diet. Organic selenium was administered as selenium labeled yeast and inorganic selenium was administered as selenium labeled selenite. Absorption of selenium was significantly better for sheep fed the concentrate diet compared to forage. Most of the selenium was absorbed in the intestine. Selenite absorption was greater for both diets presumably due to bacterial use of organic selenium. Bacterial protein from sheep on both diets contained greater concentrations of organic selenium.

Selenium Distribution

Once in the blood, selenium is bound to plasma proteins. A study by Beilstein and Whanger on humans, rhesus and squirrel monkeys, rats and sheep showed significant differences for selenium distribution in blood proteins between species. Most of rat, squirrel monkey and sheep erythrocyte selenium was associated with glutathione
peroxidase, whereas, most of rhesus monkey and human erythrocyte selenium was associated with hemoglobin.

From the blood, selenium is distributed to body tissues. Kidneys and liver contain the highest concentrations generally followed by spleen, pancreas, cardiac and skeletal muscle. Overall muscle contains the largest pool of selenium due to its size. Lungs also can have high concentrations as well as hair and wool.

In a study by White et al. of copper and molybdenum interactions with selenium, skeletal muscle contained the highest portion of selenium. Copper supplementation decreased the amount in skeletal muscle. Supplementation of either molybdenum or copper and molybdenum produced skeletal muscle concentrations between that of the control and copper supplemented groups. The treatments had no significant effects on other tissues studied such as liver, kidneys, heart and testes. Janghorbani et al. also observed skeletal muscle to contain the largest portion of selenium followed by liver, plasma, kidneys, heart and brain following a 30 day selenite supplementation of rats. Supplementation increased liver selenium concentration 136%, kidney concentration 132%, skeletal muscle concentration 38%, heart concentration 32% and brain concentration 8% compared with control animals.

Echevarria et al. studied the effects of sodium selenite in sheep fed varying concentrations of selenite for 10, 20, or 30 days. They observed liver selenium concentration increased as supplemental selenium increased and more selenium accumulated in the liver in the sheep fed selenite for 30 days. Kidney selenium concentration also increased with supplementation and with time. Muscle, heart and spleen selenium concentrations each increased with increased supplementation, but time
had no affect on these tissue concentrations. Brown and Burk\textsuperscript{11} recorded maximum uptake of selenium by the brain at 2-3 days following \textsuperscript{75}selenite injection in rats. The brain selenium concentration declined slower than all other tissues studied. They recorded a small uptake of selenium by the eye.

Hawkes et al.\textsuperscript{44} provided \textsuperscript{75}selenite in drinking water of rats for 5 months. Muscle, liver, plasma, erythrocytes, kidney, testes, epididymides, lung, and heart accounted for 85\% of the total body selenium. Seventy-three percent of the total body selenium was located in skeletal muscle, blood, and liver. Kidney, testes and liver contained the highest concentrations of selenium in the body. Liver, erythrocytes and skeletal muscle also were observed to account for the highest glutathione peroxidase activity. Supplemented selenite was mostly converted to selenocysteine which is protein bound, however, there appeared to be some conversion of selenite to selenomethionine. Furchner et al.\textsuperscript{31} studied the effects of selenite supplementation in mice, rats, dogs, and monkeys. Of the tissues collected at death, they found the highest selenium concentrations in the kidneys and liver, whereas the highest amount of selenium was observed in muscle of mice exposed to \textsuperscript{75}selenite.

Organic selenium results in higher tissue selenium concentrations than inorganic selenium.\textsuperscript{56,86} Behne et al.\textsuperscript{7} observed higher selenium concentrations in liver and muscle of selenomethionine supplemented rats than selenite supplemented rats. Selenite and selenomethionine were assumed to incorporate into specific selenoproteins. However, selenomethionine also was observed to incorporate non-specifically into body proteins in place of methionine and was influenced by selenomethionine and methionine concentrations in the body.
Goehring et al.\textsuperscript{37} fed pigs diets containing 0, 3, 6, or 9 μg selenium/g from seleniferous wheat and oats. These were normal wheat and oat grains grown in soil of high selenium content. Liver, kidney, heart, spleen, diaphragm muscle, hair, and blood selenium concentrations increased linearly with an increase of dietary selenium. Dietary selenium did not significantly change cellular or plasma glutathione peroxidase concentrations. A second experiment was performed supplementing pigs with selenium as selenite instead of wheat or oats. Liver, kidney, heart, spleen, diaphragm muscle, hair, and blood selenium concentrations all increased with an increase of dietary selenium. Cellular and plasma glutathione peroxidase concentrations increased with each increase in dietary selenite. The authors believe the different response of the pigs to organic versus inorganic selenium relates to different metabolism pathways, as selenomethionine (the likely organic form found in these plants) can be non-specifically incorporated into tissue proteins in place of methionine.

Salbe and Levander\textsuperscript{78} observed tissue concentrations were greater in rats supplemented with selenomethionine than in rats supplemented with selenate. Hair and nail selenium concentrations were higher when fed selenomethionine than with selenate. In a second experiment they observed methionine deficiency usually resulted in more tissue selenium, especially in selenomethionine fed rats. The muscle retained more selenium when methionine deficient rats were fed selenomethionine. Selenate had no such effect.

Qin et al.\textsuperscript{76} fed sheep one of four diets: a control diet and ones supplemented with sodium selenite, selenized yeast or selenium enriched probiotics. All selenium supplemented animals had higher concentrations of selenium in liver, kidney, and muscle
than the control group. The organic selenium sources of selenized yeast and enriched probiotics increased the concentration of selenium in liver, kidney, and muscle more than did selenite.

Selenium accumulation in male reproductive organs demonstrates an important function of selenium in male reproduction. Brown and Burk observed that selenium uptake by the testes and epididymis in males reached a maximum at two weeks and four weeks, respectively. They observed the selenium in the sperm accumulated in the midpiece of the tail. Calvin studied selenium accumulation in rat sperm tails with selenite. He also observed most of the labeled selenium was located in the midpiece of the sperm and proposed the name selenoflagellin for the selenopolypeptide in the sperm tail. Behne, et al. observed a significant increase in selenium concentration in testes of rats as they matured. Glutathione peroxidase also increased in the testes with rat maturation. These researchers found that between 20 and 55 days, testis selenium concentration rose 500%. Selenium appears to be important in normal sperm development.

Selenium supplementation in hens increases selenium concentration in eggs. Cantor and Scott studied selenium supplementation in hens and found that even as little as 0.015 ppm sodium selenite significantly increased egg production. After 7-9 weeks, selenium supplementation also significantly increased hatchability. Selenium supplementation significantly increased the concentration of selenium in the eggs. In a second experiment comparing selenite and selenomethionine, selenite supplementation resulted in 80% of selenium in egg yolk and 20% in albumen. When supplemented with selenomethionine, 60% of egg selenium was in yolk and 40% in albumen. The increase
in albumen selenium when supplemented with selenomethionine appears to be because egg white has higher methionine content than egg yolk, so more selenomethionine becomes incorporated in place of methionine in egg white proteins.

Selenium can cross the placenta and also enter milk. Van Saun et al. recorded that fetal liver selenium concentrations were greater than maternal liver selenium concentrations in dairy cattle even when the dam is low on selenium. In contrast, maternal serum selenium concentration was found to be higher than fetal serum concentration. The authors suggested there is possible selenium storage and concentrating ability in fetal liver. Fetuses deficient in selenium result in White Muscle Disease. Maus et al. observed that milk and plasma selenium concentrations increased as dietary selenium increased in dairy cows supplemented with selenite. Steen et al. observed the concentration of selenium in newborn lamb blood to be higher in lambs from ewes who received organic selenium as mostly selenomethionine compared to newborn lambs from ewes fed inorganic selenium as selenite.

Ammerman et al. studied the effects of selenium supplementation on beef cows and their calves fed either a linseed meal or soybean meal diet. Selenium was provided to the cows in the form of selenite. Plasma selenium concentration in calves increased with selenium supplementation due to higher milk selenium concentration. The highest plasma and milk selenium concentrations occurred in selenium-supplemented cows fed linseed meal. Calves of these linseed-fed cows had more selenium in muscle than calves of soybean meal-fed cows.
Selenium Metabolism

Inorganic selenium compounds can be converted to organic forms and can be incorporated into amino acids and proteins. Inorganic selenium can also be reduced to form selenide. Selenocysteine is cleaved to form selenide. Because the methionine-tRNA cannot identify the difference between methionine and selenomethionine, selenomethionine can be substituted non-specifically for methionine in body proteins. Selenomethionine can also be metabolized to selenocysteine and be cleaved to form selenide. Selenide can either be methylated or be used for selenoprotein synthesis. If selenide is methylated, it results in excretable products. When selenide is used for selenoprotein synthesis, it is used to create selenophosphate. Selenophosphate converts the serine-tRNA to selenocysteine-tRNA which inserts selenocysteine into polypeptides, forming selenoproteins.

Gasiewicz and Smith found that selenite metabolism to selenodiglutathione and eventually to selenide by rat erythrocytes depends on the amount of reduced glutathione in erythrocytes. As selenite concentration increased, reduced glutathione became depleted due to the use of it in the first step of the selenite metabolism reaction, yielding selenodiglutathione. Glutathione reductase is necessary in the conversion of selenodiglutathione to selenide, again yielding reduced glutathione. When given chromate, an irreversible inhibitor of glutathione reductase, the amount of selenite metabolized was reduced due to a lack of reduced glutathione.

Behne et al. administered selenite orally and found that it followed a similar metabolic pathway as intravenously injected selenite reported in an earlier paper. They
found selenomethionine incorporated non-specifically into muscle proteins ten times more than selenite at the same dose. At high doses, selenite was recorded to incorporate non-specifically into proteins but at a much smaller scale than selenomethionine.

Rumen microbes can utilize selenium for proteins or they can metabolize it to insoluble forms, which are then passed out in the feces by ruminants because the ruminant cannot absorb them. 86 Koenig et al. 57 fed sheep either a concentrate or forage diet supplemented with either organic or inorganic selenium. Rumen bacteria of concentrate fed sheep tended to have higher selenium concentrations. Selenium concentrations in rumen bacteria were greater when sheep were supplemented with organic rather than with inorganic selenium on both diets.

Selenium Elimination

The major elimination pathways include either urine or feces depending on the type of animal. Monogastrics excrete most selenium in urine in the form of trimethylselenonium ion regardless of administered route. 86 Selenide is first methylated to methyl selenide, then dimethyl selenide and then to trimethylselenonium ion. 27,82 Feces is the major pathway of loss for ingested selenium in ruminants due to the metabolism of selenium to insoluble forms by rumen microbes. The ruminant cannot absorb these insoluble metabolites so they are passed in the feces. If selenium is administered to ruminants via intravenous injection, then urine is the major route of excretion. Young ruminants excrete more selenium in urine than in feces, presumably due to fewer rumen microbes.
Janghorbani et al.\textsuperscript{50} supplemented rats with 4 μg selenium/ml water as selenite. Urinary excretion of control animals was in the range of 3.5 to 6.9 μg selenium/48 hours. Selenite supplemented animals had a urinary excretion of 37 μg selenium/48 hours for the first two days and 80 μg selenium/48 hours for the rest of the experiment. Trimethylselenonium ion accounted for about 35-40\% of urinary selenium in supplemented animals and accounted for only about 2\% of urinary selenium in control animals. Urinary excretion did not account for total selenium therefore a second experiment was performed identically to the first experiment, except feces was collected as well as urine. Selenium supplemented animals in experiment 2 consumed about 1830 μg selenium and excreted about 980 μg selenium in urine and 210 μg selenium in feces. About 94 μg selenium was found in the carcasses of the rats. The remaining ingested selenium was unaccounted for in this study; however, the authors suspect the loss may have been through respiratory excretion and possibly a small amount lost to skin and hair.

Cousins and Cairney\textsuperscript{22} dosed pigs with selenite and surprisingly found a higher fecal excretion than urinary excretion. They also observed that lambs lost about half the daily ingested dose in urine and feces, most of which was in the feces. In sheep, fecal loss was 3-4 times greater than urinary excretion regardless of the chemical form, either organic selenium in feed or inorganic selenite. The authors suggest the likely reason for a higher fecal loss is due to conversion of both organic and inorganic selenium to an insoluble form in the rumen.

Langlands et al.\textsuperscript{63} found fecal loss was higher than urinary excretion in sheep. They found a significant amount of selenium was reabsorbed in the lower sections of the
small intestine. Fecal elimination increased with higher selenium status in sheep presumably due to selenium turnover of tissues as they reached equilibrium.

Elimination rates depend on the amount of supplemented selenium. Burk et al. fed rats diets with increasing selenium content as selenite and observed excretion. Fecal excretion was about 10% for all groups and therefore appears to be unrelated to dose. Urinary excretion increased from 6% in the control group to about 67% in the highest selenium treatment group. Respiratory elimination was not measured in the experiment but a small amount appears to have been eliminated in expired air in all groups except the control group.

The majority of selenium elimination in most animals occurs within 1-3 days of exposure. Furchner et al. observed that urine is the predominant excretory pathway of selenium during the first day following administration but later decreases. They found fecal loss to be consistent and not related to dietary factors. Although respiratory elimination was not measured, there appeared to be a small amount of loss not attributable to urine or feces therefore was assumed to be due to respiratory elimination.

At extremely high doses of selenium, respiration becomes an important excretion pathway. Dimethyl selenide is the predominant chemical form eliminated through respiration. Methylation of dimethyl selenide to trimethylselenonium ion appears to be rate-limiting so respiratory elimination of dimethyl selenide increases at high doses.

Tiwary et al. noted garlic odor to the breath of sheep which received selenite or selenomethionine. Air samples were collected at 4, 8, and 16 hours after selenium administration. The highest concentration of selenium in expired air was observed at 4 hours, followed by 8 and then 16 hours. The amount of selenium expired through
respiration increased with increasing dose. Selenomethionine produced higher respired air selenium concentrations than did selenite at equal selenium doses. No selenium was detected from control animals in expired air. Garlic odor was first detected one hour after administration and was stronger in the selenomethionine treated sheep.

Jiang et al.\textsuperscript{54} found dimethyl selenide and dimethyl diselenide to be the major selenium species in expired air from rats given selenite, selenomethionine or selenocystine in drinking water. Dimethyl selenide was the main compound observed when rats were given selenocystine or selenite. Dimethyl diselenide and an unidentified selenium product were primarily observed when rats were given selenomethionine.

Selenium Deficiency

Selenium deficiency is a widespread problem in livestock in the United States. A survey of state veterinarians and veterinary diagnostic laboratories in 1993 reported selenium deficiency as an important problem in 37 states.\textsuperscript{25} Only four states reported no observed deficiency problems. Areas of the United States where significant selenium deficiency problems exist include the Northwest, Southeast and Northeast.\textsuperscript{2} Generally, 0.3 ppm of selenium is recommended in the diets of domestic livestock to prevent deficiency.\textsuperscript{55} Deficiency causes many problems that result in economic losses to livestock producers.

Cattle and sheep experience nutritional muscular dystrophy known as White Muscle Disease (WMD).\textsuperscript{55} Signs of WMD include stiff hindquarters, arched backs, pneumonia and death. Calves and lambs with WMD may experience muscle tremors, have hard, swollen muscles or die from myocardial dystrophy.\textsuperscript{58} Observed lesions
include calcification and degeneration of myocardium and skeletal muscle.\textsuperscript{86} Lesions occur predominantly in the most active skeletal muscles. Stress and exercise compound deficiency symptoms.\textsuperscript{66} Deficient ewes can produce affected lambs that are often born dead or die within a short period of time due to physical exertion.\textsuperscript{86} Godwin and Fraser\textsuperscript{36} found severe lesions in the left ventricle of the heart and the rear and fore limbs in selenium deficient lambs. They noticed a drop in blood pressure in deficient lambs over time. Many of the deficient lambs eventually died. But, Ullrey \textit{et al.}\textsuperscript{87} did not see an increase in weight gain in selenium supplemented versus deficient sheep and cattle.

Cousins and Cairney\textsuperscript{22} observed up to 50\% of lambs died soon after birth due to muscular dystrophy in areas of selenium deficiency in New Zealand. Ewe fertility was reduced in affected areas; however, selenium supplementation increased fertility. Kott \textit{et al.}\textsuperscript{59} observed no difference in reproductive performance or fertility in Vitamin E and selenium supplemented ewes compared with marginally deficient ewes. But, they saw an increase in number of lambs weaned per ewe in supplemented ewes.

Reproductive deficiencies occur in ruminants that are selenium deficient.\textsuperscript{86} Spermatozoa from selenium deficient animals may have lower viability and motility.\textsuperscript{25} Fertilization is often reduced but can be increased with selenium and Vitamin E supplementation.\textsuperscript{86} Retained placenta is a common problem that has been associated with selenium deficiency in cattle. Selenium and possibly Vitamin E supplementation have been shown to significantly reduce retained placenta in selenium deficient areas. Deficiency can cause abortion, with aborted fetuses showing signs of muscular dystrophy.\textsuperscript{66}
Poultry are also susceptible to selenium deficiency. Exudative diathesis is a common problem seen in chicks fed deficient diets. Subcutaneous edema, especially of breast and abdomen indicates exudative diathesis. Chicks are anemic and do not grow as well as selenium sufficient chicks. Hemoglobin break down produces a green-blue color of the skin as a result of the disease. Exudative diathesis is seen by 2 to 3 weeks of age in selenium deficient chicks and by 6 to 10 days of age in chicks hatched from deficient hens. Death will occur within 3 to 4 weeks in selenium deficient chicks and by 10 to 14 days of age in chicks hatched from deficient hens. Both Vitamin E and selenium appear to prevent exudative diathesis. Exudative diathesis also occurs in turkeys and is prevented with Vitamin E or selenium supplementation. Cantor and Scott observed a significant increase in egg production of selenium supplemented hens. Hatchability also significantly increased with selenium supplementation. Chicks from supplemented hens had better growth rates than chicks from non-supplemented hens. Chicks receiving either selenium or Vitamin E supplementation had reduced incidence of exudative diathesis.

Chicks are also susceptible to nutritional muscular dystrophy when fed selenium deficient diets. Vitamin E supplementation is required to prevent muscular dystrophy in chicks, but if selenium is supplemented in conjunction with Vitamin E, the required amount of Vitamin E is reduced. Turkeys are also susceptible to muscular dystrophy, which can be prevented completely by selenium supplementation. Calvert et al. found selenium alone was not effective at preventing muscular dystrophy in chicks. Vitamin E alone was only effective in preventing muscular dystrophy at 10 mg Vitamin E/kg diet. Vitamin E and selenium together prevented muscular dystrophy even at much lower concentrations of Vitamin E supplementation.
Selenium deficient rats often have liver degeneration, which can result in death. Offspring of deficient rats may have poor growth, failed reproduction and thin hair coat, all of which are reversed with selenium supplementation. Hafeman and Hoekstra fed rats selenium and Vitamin E deficient diets and compared them to selenium, Vitamin E or selenium/Vitamin E supplemented rats. By the 35th day, the unsupplemented rats showed depressed growth. Selenium and Vitamin E increased the growth rates. Lesions in the deficient rats included kidney damage, liver necrosis and lung hemorrhage. Increases in evolution of ethane were used to indicate lipid peroxidation in the deficient rats. During ethane collection, the rats were forced to fast. The stress of fasting caused death in about half of the deficient rats. Vitamin E supplementation alone prevented ethane evolution in this study. Selenium supplementation alone did not prevent ethane, but a combination of selenium and Vitamin E supplementation also prevented ethane production.

Muscular dystrophy and steatitis can be seen in selenium deficient swine and horses. Deficient swine exhibit signs of nutritional myopathy, hepatosis dietetica and mulberry heart disease. Muscular dystrophy, steatitis and azoturia in horses respond to selenium and Vitamin E treatment. Selenium deficient foals are weak, stiff, and reluctant to move, experience difficulty nursing and often die soon after birth. A foal was submitted to Washington State University Veterinary Hospital because of severe emaciation, but had a continued appetite. It was weak, needed assistance standing and had a rough hair coat. Some of the muscles were hard and swollen. The serum selenium concentrations were low. After the foal died, the diagnosis of generalized steatitis with mineralization was made. Vitamin E concentrations were normal. The authors
concluded generalized steatitis can result from selenium deficiency as well as Vitamin E deficiency.

Myxedematous cretinism, Keshan disease and Kashin-Beck disease are seen in humans suffering from severe cases of selenium deficiency. These diseases are restricted mostly to parts of China and Africa low in selenium and in people whose diets are restricted to locally grown foods. Myxedematous cretinism occurs in areas of selenium and iodine deficiency. Symptoms include dry, scaly skin, distorted growth of long bones, cranial plates that do not fuse, hypothyroidism and delayed sexual maturation.

Keshan disease is named because of its occurrence in Keshan County, China. Like myxedematous cretinism it occurs in areas deficient in both selenium and iodine. It occurs in children and women of childbearing age. Symptoms of congestive heart failure and sometimes death and stroke occur as a result of this disease. This occurs in peasants eating mostly locally grown corn with no diversity. A study of children 1-9 years of age was performed during 1974-1975. Half the children received sodium selenite orally at doses of either 0.5 mg or 1.0 mg sodium selenite depending on their age. The other half of the children received an oral placebo to serve as a control group. Selenite supplementation resulted in a significantly lower incidence of Keshan disease compared to controls. The control group consisted of 9,430 children during 1974-1975. A total of 106 cases of Keshan disease occurred. During 1974-1975, the treatment group consisted of 11,277 children of which only 17 cases of Keshan disease occurred. Because supplementation reduced the incidence, all children received selenite in 1976 and onward. Out of 12,579 children supplemented in 1976, only four cases of Keshan disease
occurred. During 1977, no cases of Keshan disease occurred in 12,747 selenite supplementation children.

Kashin-Beck disease is also a result of selenium and iodine deficiency. Possibly other factors are involved because the disease does not occur in selenium and iodine deficient areas of Africa, only in China. This disease affects bones and joints of growing children. One symptom is enlargement or cracking of small joints while a more serious symptom is distorted growth of long bones that leads to shorter stature. Recent supplementation of salt with selenium has reduced the occurrence of the disease.

Selenium Toxicosis

Selenium toxicosis can occur from either acute or chronic exposure. Acute poisoning occurs after a large single dose of selenium. Chronic selenium poisoning is often referred to as “Alkali Disease” due to its common occurrence in association with alkaline soils, although other disease conditions are occasionally erroneously included under this name. Chronic poisoning usually occurs after a long-term exposure to selenium for many weeks or months. In a 1993 survey, seven states reported selenium toxicoses problems due to grazing of native plants. The states included California, Colorado, Idaho, Oregon, South Dakota, Utah and Wyoming. Acute toxicosis is more often due to over supplementation and it is more common than toxicosis due to grazing accumulator plants and reported to occur in 15 states.

Initially, animals suffering from chronic toxicosis show hair loss and a loss of vitality. The hooves become deformed and can slough off, reproduction may be reduced or result in abnormal offspring and finally death may result. Chronic selenium
poisoning results from consumption of grains or grasses containing 5-40 ppm selenium over several weeks to months. The deformed hooves cause lameness, which prevents the animal from moving to food or water leading to starvation. Calves and foals may be born with deformed hooves because selenium crosses the placenta. Pigs are especially susceptible to growth reduction when affected by chronic poisoning. Reduced growth, feed efficiency and egg production occurs in poultry.

The toxic affect of selenium on hair, nails, horns and hooves is due to damage of keratin. Keratin contains many disulfide bridges. As selenium replaces sulfur in seleno-amino acids, these bridges or bonds are lengthened and weakened. Overall, keratin becomes weakened so these keratin-containing tissues become more susceptible to damage.

Animals affected with acute toxicosis develop a distinct odor to the breath due to the release of volatile selenium compounds. Other symptoms of acute toxicosis can include vomiting, dyspnea and death due to respiratory failure. Common lesions can include congestion and necrosis of the liver, congestion of the kidneys, and edema and hemorrhage of the lungs. Lesions also include endocardial, myocardial, and epicardial damage with hemorrhage. Acute toxicosis usually occurs after an accidental over supplementation of selenium compounds or after an animal consumes a large quantity of accumulator plants over a short period of time. Generally, however, animals avoid accumulator plants unless there is no other forage available.

Humans are also susceptible to selenium poisoning. Signs of acute poisoning are similar to those observed in animals including tachycardia, nausea, vomiting, pulmonary edema and neurologic problems such as muscle tremors and delirium and death.
garlic odor can also be present in humans following selenium intake. One selenium poisoning incident involved a man who drank selenite powder in water at a dose later calculated to be about 10 g selenium. The man experienced abdominal pain and diarrhea. He was taken to the hospital where he experienced cardiac arrest and died after resuscitation attempts failed. A post-mortem examination revealed congestion and hemorrhage of the lungs and death was attributed to acute selenium toxicity. Several other cases of selenium poisoning have been reviewed and include incidences of suicides and attempted suicides and accidental poisonings due to gun-blueing (a selenium containing metal stain) and selenium mineral supplements. These suicides and accidental over supplementations are alarming because selenium in mineral supplements is readily available to the general population under no supervision. The availability could continue to lead to more incidences of over supplementation of selenium.

The information regarding chronic selenosis in humans is limited and variable. Common symptoms include brittle fingernails and hair, red, swollen skin that can blister easily, breath odor, tooth decay, numbness and paralysis. These symptoms depend upon the severity of toxicosis. These symptoms occur mostly in areas of high selenium content of soils and plants. Humans may also be at risk to occupational exposures. As occupational exposure usually occurs through inhalation, the symptoms commonly affect the respiratory tract. Mucous membrane irritation, wheezing, dyspnea, broncho-pneumonia and pulmonary edema can result after inhalation of hydrogen selenide.

The subacute or subchronic form of selenium toxicosis is sometimes called “Blind Staggers” because affected animals are reported to wander aimlessly away from the herd due to reduced vision. The front legs of affected animals become weak as the
symptoms worsen. Animals show complete blindness, tongue paralysis and increased respiration and salivation. Death usually occurs soon after clinical signs appear. This subacute or subchronic form occurs after a limited exposure of animals to selenium-accumulator plants over weeks or months. Other factors, such as plant alkaloids, possibly contribute to the syndrome referred to as blind staggers as this has not been repeated in studies involving pure selenium compounds alone.

The mechanism of selenium toxicity has not been well established. One possible mechanism being studied is that of oxidative damage caused by the formation of reactive oxygen species from an over exposure of selenium. A recent study in fish exposed to high doses of selenium as selenite showed oxidative damage in the hepatocytes. They observed that with increasing doses of selenium, cellular lipid peroxidation increased. This leads to cellular membrane oxidative damage. It is believed that at high doses of selenium, reactive oxygen species form to such high levels that the antioxidant systems become overloaded and are therefore unable to metabolize all the damaging reactive oxygen species which lead to cellular damage as seen by Misra and Niyogi.

The severity of selenium toxicosis depends on the chemical species involved. Selenite appears more toxic than selenate. Selenomethionine seems to be less toxic than selenite and selenocysteine appears to be similar to selenite. Organic selenium caused toxicosis appears more severe in cattle and horses than in sheep. The methylated products formed in the body such as dimethyl selenide and trimethylselenonium ion are much less toxic than selenite. Halverson et al. found selenite to be more toxic than selenate in chick embryos.
Tiwary et al.\textsuperscript{84} dosed sheep with either sodium selenite or selenomethionine at varying concentrations of each. Sheep which received 2, 3, or 4 mg selenium/kg body weight as selenite or 4, 6 or 8 mg selenium/kg body weight as selenomethionine were depressed, tachypnic and had reduced feed intake between 12-24 hours following administration. All animals from the 6 and 8 mg/kg selenomethionine and 4 mg/kg selenite groups showed pulmonary edema and foam in the trachea and bronchi at necropsy. Histological lesions included myocardial necrosis, pulmonary edema and hemorrhage. The authors concluded that selenomethionine can be more bioavailable than selenite, but is less toxic than selenite.

Glenn et al.\textsuperscript{35} found no effect of selenium treatment on reproduction or body weights of ewes. A few ewes developed signs of toxicosis and died within a few days. Signs included anorexia, inability to stand and respiratory distress. Caravaggi et al.\textsuperscript{18} dosed lambs with sodium selenite to cause acute toxicosis. Affected lambs developed signs including difficult respiration, convulsive spasms and respiratory failure resulting in death. The nostrils and mouth contained froth and fluid. The pleural cavity contained excess fluid, the lungs showed congestion, edema and hemorrhaging. The liver was swollen with congestion. Fessler et al.\textsuperscript{26} observed myocardial degeneration, hemorrhage, and edema of heart tissue and pulmonary edema in a ewe allowed to graze on an area of high selenium content in the forage.

Gabbedy and Dickson\textsuperscript{32} observed pulmonary edema in a case of acute selenium poisoning in lambs given sodium selenite. Hopper et al.\textsuperscript{48} observed pulmonary edema with hydrothorax and destruction of renal cortices in lambs poisoned with selenium in the form of sodium selenate. An accidental case of selenium poisoning resulted in death of
many sheep as recorded by Kyle and Allen. Sodium selenite was given instead of the intended sodium selenate. Froth was found in the trachea of sheep with pulmonary edema and congestion. Lambourne and Mason recorded another incident of accidental poisoning of lambs with selenite. Prior to death, the animals were lethargic with increased respiration. Foam was found in the trachea and bronchi, edema and congestion were found in the lungs and some damage to the kidneys was observed.

Chick embryos are very sensitive to selenium poisoning. Selenium toxicosis results in deformed chicks, which may lack wings, beaks, toes or eyes. Poor hatchability occurs as a result of deformed chicks that are unable to break out of the egg. Ort and Latshaw found 5 ppm selenium as selenite in laying hens lowered hatchability of eggs. In a second experiment, 9 ppm selenium as selenite decreased egg production. Seven or 9 ppm selenium reduced egg weight and hatchability. Although percentage of infertile eggs was not statistically affected by dietary selenium, the authors noted that infertility percentage was twice as high in 7 and 9 ppm selenium groups compared with hens receiving no selenium supplementation.

Cantor et al. administered selenite in the drinking water of 9 day old chicks. They found selenite significantly reduced body weight gain and feed intake. Water intake was related to the amount of selenium in the water. As the amount of selenium in water increased, water intake of the chicks decreased.

Harrison et al. performed a feeding trial using two feed rations that had been identified as a possible cause of paralysis to several pigs at local farms. Although a chemical form was unidentified, selenium was present in the rations at toxic concentrations and was later believed to be the cause of paralysis. In the trial, paralytic
pigs were observed. Lameness developed in the pigs because the hoof separated from the coronary band. These pigs developed a rough hair coat. Poliomyelomalacia was observed in the spinal cord of affected pigs. Goehring et al.\textsuperscript{38} observed reduced growth and feed intake with increasing amounts of selenite in pigs. Hoof lesions developed in two pigs. The hoof was inflamed around the coronary band and eventually the hoof wall sloughed off. A pig with paralysis had bilateral symmetrical malacia of gray matter of ventral horns at necropsy.

Herigstad et al.\textsuperscript{45} compared selenite and selenomethionine toxicosis in pigs. Pigs that developed toxicosis had reduced daily weight gains compared to controls. Selenite was found to be slightly more toxic than selenomethionine. Pigs suffering from peracute and acute toxicosis showed vomiting, increased respiration, coma and eventually death. Pigs suffering from subacute toxicosis refused to eat resulting in emaciation. These animals had rough hair coat, yellow skin, muscular atrophy and weakness. Pulmonary edema was found in pigs suffering peracute toxicosis. A few hemorrhages were seen on the heart and lungs of pigs with acute toxicosis.

A dog was accidentally poisoned with selenium when given a Vitamin E supplement.\textsuperscript{51} The organs appeared normal at necropsy except foam was found in the trachea and bronchi. A guinea pig receiving the same solution died within two hours of injection. Prior to death, the guinea pig experienced severe dyspnea. Foam was found in the trachea and bronchi and the lungs were congested and edematous.

Other elements given in conjunction with selenium can be effective in reducing the severity of selenium toxicosis. Donaldson and McGowan\textsuperscript{23} studied the interaction of selenium and lead on toxicosis in chickens. The group which received 40 ppm selenium...
without lead showed 85% mortality. The addition of 2000 ppm lead along with 40 ppm selenium reduced mortality compared with those which received selenium alone; however this group had lower body weights. Overall growth was better when selenium was supplemented with lead compared with diets of selenium supplementation alone. Lead supplemented alone reduced growth compared to controls but not as significantly as selenium supplemented alone.

Lowry and Baker\textsuperscript{65} observed that arsenic can reduce selenium toxicosis in chicks. \textit{As}_2\text{O}_3 was the most effective by reducing growth depression caused by selenium. Not all forms of arsenic were as effective. Arsanilic acid was totally ineffective while phenylarsonic, phenylarsine oxide and \textit{As}_2\text{O}_3 were moderately effective in reducing growth depression caused by excess selenium. \textit{As}_2\text{O}_3 appears effective in reducing both selenite and selenomethionine caused toxicosis.

Jensen\textsuperscript{53} noted that the addition of 5 ppm selenium as selenite reduced weight gain in chicks, but the addition of silver with selenium increased growth with increasing amounts of selenium. Copper had no such effect on growth. However, both silver and copper reduced mortality caused by selenium. Thus, silver and copper appear to reduce selenium toxicosis.

Halverson and Monty\textsuperscript{42} observed a large growth reduction in rats supplemented with 10 ppm selenium as selenite or selenate. However, a small addition of sulfate helped to prevent growth reduction. A high concentration of sulfate given helped reduce liver damage in rats receiving selenate. Halverson \textit{et al.}\textsuperscript{40} observed sulfate to reduce growth depression, mortality and liver damage caused by selenium poisoning in rats given selenate. Sulfate had no effect on rats receiving toxic wheat or selenite.
Jensen and Chang\textsuperscript{52} recorded that 10 ppm selenium in diets of chicks reduced growth and 20 ppm selenium reduced feed efficiency. However, 10 ppm selenium supplemented to a diet containing 20\% linseed meal did not affect the rate of gain. Linseed meal supplemented with 20 ppm selenium only slightly reduced growth and did not affect feed efficiency. Linseed meal also reduced mortality. Linseed meal appears to reduce the effects of selenium toxicosis, possibly due to sulfur or other mineral constituents.

Conclusions

Selenium is an essential mineral required by mammals and poultry. It is essential as part of numerous selenoproteins. The most common functions of selenoproteins are to act as antioxidants. However, there may be other functions to be discovered through future research. Care must be taken to prevent both deficiency and toxicosis when supplementing livestock with selenium. Animals grazing areas known to be selenium deficient need to receive an appropriate selenium supplement before losses are incurred. Extra management and care must be taken when grazing animals where selenium accumulates in soil and plants to prevent toxicosis. There is a fine line between too much and too little selenium but an optimum must be found to achieve the best overall performance in livestock. In addition, differences in selenium effects are significantly associated with the chemical form to which an animal is exposed.
References


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CHAPTER 3

PATHOLOGICAL EFFECTS OF SELENIUM IN SHEEP FROM
THE ACCUMULATOR PLANT SYMPHYOTRICUM
SPATHULATUM (WESTERN MOUNTAIN ASTER)

Abstract

Thirty-four lambs were used to study the effects of selenium (Se) from the accumulator plant *Symphyotrichum spathulatum* (Western Mountain Aster). Six animals were randomly assigned to each of the following treatments: 0, 2, 4, 6, or 8 mg plant-Se/kg body weight (BW). Four animals were used as positive controls. Two received 4 mg Se/kg BW as purified sodium selenite and two animals received 8 mg Se/kg BW as purified selenomethionine (Se-Met). All treatments were administered orally. Sheep were observed for 7 days, following which all remaining live animals were euthanized and necropsied. All animals that died prior to 7 days were necropsied immediately following death. Tissues were collected for mineral analysis and histopathology.

Clinical signs were less apparent than expected and observed in some animals in the 6 and 8 mg plant-Se/kg BW groups. Most showed no desire to move and depression while some had mild dyspnea. Upon increase in activity level, startled animals would fall over and die. Myocardial degeneration and necrosis were most prominent in affected animals. Pulmonary edema and congestion was also observed in some animals. Treatment was highly significant on all tissue Se concentrations (*P* = 0.0015 in skeletal muscle and *P* < 0.0001 in all other tissues). Se concentration in all tissues collected for mineral analysis
increased with increasing dose for sheep receiving plant material. No significant effects were observed on the body weight change of the animals over 7 days. A significant effect was seen on the weight of the lungs ($P = 0.0420$). Lung weight in the Se-Met group was significantly greater than all except the 8 mg plant-Se/kg BW group.

Introduction

The element selenium (Se) is required for proper health of mammals. There are numerous Se containing proteins (known as selenoproteins) necessary in the body of mammals and poultry. These proteins are unique because they contain selenocysteine. There may be over 30 selenoproteins.\textsuperscript{2} Glutathione peroxidase was the first of such enzymes to be discovered.\textsuperscript{23} There are now known to be at least four different glutathione peroxidase enzymes functioning in the body.\textsuperscript{8} These include Cytosolic Glutathione Peroxidase, Plasma Glutathione Peroxidase, Gastrointestinal Glutathione Peroxidase and Phospholipid Hydroperoxide Glutathione Peroxidase.\textsuperscript{2} The common function of these enzymes includes active reduction in damage caused by peroxides in the body, however, there may be more functions discovered in the future.\textsuperscript{8} Selenoproteins P and W are also recently discovered Se-containing proteins. There may be multiple uses for these two proteins, but recent research leads to the conclusion that they serve as antioxidants.\textsuperscript{11,18}

Although required, too much Se in the diet can prove to be fatal. This is an important concern for livestock producers as they strive to reach the optimum concentration of Se in livestock diets. Another concern is that certain species of plants can accumulate large amounts of Se.\textsuperscript{23,25} When Se concentrations are high enough in
these plants, ingestion can be fatal to livestock. Plants are commonly categorized into three groups based on their ability to accumulate Se. The first group, primary accumulators, can accumulate tens of thousands parts per million (ppm) Se. Species in this group include Astragalus, Stanleya, Machaeranthera, Oonopsis and Xylorhiza genera. The second group, secondary accumulators, can reach Se concentrations between several hundred to several thousand ppm Se. These include species of Aster, Castilleja, Grindelia, Atriplex, Gatierreaia and Comandra genera. The final group includes those that only accumulate Se passively. This group includes grasses and some weeds which normally do not accumulate more than 100 ppm, but can accumulate a few hundred ppm.

Acute Se toxicosis is a major problem following ingestion of these accumulator plants by livestock. Generally, however, livestock avoid the primary accumulator plants. Symptoms of acute poisoning can include vomiting (in monogastrics), dyspnea and death due to respiratory failure. A distinct odor is often observed on the breath of acutely poisoned animals due to the respiratory elimination of volatile Se compounds. Lesions associated with acute poisoning include pulmonary edema and hemorrhage, myocarditis and endocarditis, liver congestion, liver necrosis, and congestion of the kidneys. Several cases of accidental Se poisoning have reported pulmonary edema and/or congestion. Although livestock typically avoid accumulator plants, this is not always the case as evidenced by a report from Fessler et al., who reported that over 500 sheep died in a period of about 4 years from Se poisoning. These sheep grazed on reclaimed phosphate mine sites in Idaho and appeared to have been poisoned by high Se concentrations in both forage and drinking water.
There are numerous different chemical forms of Se. Selenate and selenite are common inorganic forms. Selenite is the form commonly used as a supplement in livestock diets. Organic forms include selenomethionine (Se-Met) and selenocysteine. Se-Met is thought to be the most common form found in most plant tissues. However, recent research shows that Se-accumulating plants convert absorbed Se to a variety of organic forms for storage. Common chemical forms found in Se-accumulators include selenate, selenite, Se-methylselenocysteine, selenocystathionine, Se-methylselenomethionine, γ-glutamyl-Se-methylselenocysteine, and selenohomocysteine. Accumulator plants can survive and sometimes require large amounts of Se. All plants take up Se in common forms such as selenite and selenate. Reactions in the plants cause the conversion of selenite and selenate to Se-Met and selenocysteine. These organic compounds are incorporated into protein structure. Plants can tolerate only a small amount of Se incorporation into proteins. Se accumulator plants deal with this problem by converting Se into other organic forms, which do not incorporate into proteins. The most common organic form of Se in accumulator plants is Se-methylselenocysteine. This particular form accumulates to a large extent in the young leaves of these plants.

A recent study was performed comparing orally-dosed selenite and Se-Met in sheep. They observed a higher accumulation of Se in tissues, whole blood and serum when sheep were dosed with Se-Met, due to better absorption of Se-Met. The current study was designed to study varying doses of Se from the Se-accumulating plant *Symphyotrichum spathulatum* (Western Mountain Aster), in sheep and to compare the results with those previously reported with purified Se-Met and sodium selenite.
Materials and Methods

Animals

Thirty-four sheep ranging in weight from 22.7 to 36.4 kg were group housed with *ad lib* access to alfalfa hay and water. Prior testing of the alfalfa hay found Se concentrations of 0.11 to 0.13 ppm. The sheep were allowed to acclimate for 2 weeks prior to the study. This study was approved by the Institutional Animal Care and Use Committee of Utah State University.

The study proceeded for 7 days during which time animals were observed for any unusual symptoms. Clinical observations were recorded with the date and time of occurrence. Death date and time was recorded for all animals that died prior to the completion of the study. Surviving animals were euthanized after 7 days. Necropsies were performed on all animals immediately after death or euthanasia.

Dosing Material

Western Mountain Aster (*Symphyotrichum spathulatum*) was collected from an area of southeastern Idaho. A plant specimen is found in the Intermountain Herbarium at Utah State University in Logan, Utah under the accession number 246175. The Aster used for the treatment groups was found to have 4782 ppm Se. The Se content of the low Se Aster used as a negative control was found to be 1.59 ppm. The concentration of the plant material was determined as described in the Analytical Methodologies and Sample Collection section. Purified sodium selenite compound was purchased from United States Biochemical Corporation, Cleveland, Ohio. Purified Se-Met compound was purchased from Sigma-Aldrich, St. Louis, Missouri.
Thirty-four sheep selected for use in this study were randomly assigned to one of seven treatments. The groups consisted of 6 negative control animals, 2 sodium selenite animals, 2 Se-Met animals, and 6 animals to each of the plant treatment groups. Each animal was weighed prior to the study and the treatments were prepared for each individual according to body weight. The treatments were administered through an intraruminal gavage tube. A flush of water was used to ensure each animal received the full assigned dose. The treatments with Se containing Aster (4782 ppm Se) consisted of one of the following: 2, 4, 6, or 8 mg plant-Se/kg BW from Symphyotrichum spathulatum. Two negative control animals did not receive plant material and were given only a flush of water. The remaining four negative control animals received low Se Aster plant material (1.59 ppm Se), in order to eliminate other plant compounds as the potential cause of any observed effects. Three of these four negative control animals were dosed with about 3 times as much plant material, according to individual body weight, as they would have received if they were assigned to the 8 mg plant-Se/kg BW group. An average of 42.7 g plant material was given to the animals in the 8 mg plant-Se/kg BW group compared with an average of 148.4 g plant material given to three of the control animals. This resulted in Se doses of 0.0077 mg/kg BW, which is less than a typical daily recommended Se intake. The last of the negative control animals received plant material at about 1.5 times as much plant material, according to body weight, as the 8 mg plant-Se/kg BW group due to a lack of total low Se plant material. The selenite animals received 4 mg Se/kg BW as purified sodium selenite and the Se-Met animals received 8 mg Se/kg BW as purified Se-Met. The selenite and Se-Met groups served as positive
controls. These doses were chosen for comparison to a previously performed study with selenite and Se-Met.

Analytical Methodologies and Sample Collection

The plant sample was ground and dried overnight in an oven at 100°C. After drying, the sample was mixed well and 0.5 g was weighed into a 30 ml Oak Ridge Teflon Centrifuge Tube (Nalge Nunc International, Rochester, NY). Acid digestion using nitric acid was performed on the plant material to eliminate organic material leaving only elemental Se for analysis. Nine milliliters of trace mineral grade nitric acid (Fisher Scientific, Pittsburgh, PA) was added to the centrifuge tube. The tube was then capped (not tightly) and placed on a heat block set at 90°C. The plant material was allowed to digest on the heat block for 1.5 h, during which time, the tubes were closely observed to prevent boil over of nitric acid. Following digestion, the tubes were allowed to cool at room temperature. The total sample was transferred to a 10 ml graduated cylinder and nitric acid was carefully added until the total volume was 10 ml. This was then transferred to an empty, labeled 15 ml trace metal free polypropylene tube (CPI International, Santa Rosa, CA) and vortexed. Then, 0.5 ml was transferred to another labeled 15 ml trace metal free polypropylene tube containing 9.5 g ultra pure water and vortexed. Sequential 1:10 dilutions with 5% nitric acid water were made as necessary for quantification. These dilutions were then used to determine Se content as well as the content of 28 other elements of the plant material using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Perkin Elmer, Shelton, CT).
Tissues were collected and immersed in neutral buffered formalin for histopathological evaluation. Liver, kidney cortex, brain, skeletal muscle, ventricle and atrium of the heart were collected and stored frozen at -20°C until ICP-MS Se analysis. Weights of heart, liver, lungs, spleen, left and right kidney were recorded at necropsy. Gross pathological observations were recorded at necropsy. Whole blood and serum samples were collected during the 7 day study in the event that further testing was warranted.

Tissues collected for mineral analysis were prepared using the following acid digestion procedure to eliminate organic material. One gram of sample was weighed and added to a 10 ml Oak Ridge Teflon Centrifuge Tube (Nalge Nunc International, Rochester, NY). Two ml of trace mineral grade nitric acid was added to the tube. Tubes were then placed on a heat block set at 90°C with loosened caps. The tubes were allowed to digest for 1 h under close observation to prevent boil over. If tubes began to boil, they were temporarily removed from the heat block and allowed to cool and were then replaced to the heat block. After digestion, the tubes were removed from the heat block and allowed to cool with tightened caps. The tubes were then carefully rolled to collect material from the sides of the tube and then the contents were transferred to a 10 ml graduated cylinder. Nitric acid was carefully added to bring the sample volume to a total of 3 ml. This was then transferred to an empty, labeled 15 ml trace metal free polypropylene tube and vortexed. One half milliliter was transferred to another labeled 15 ml trace metal free polypropylene tube containing 9.5 ml ultra pure water. Following vortexing, 0.5 ml was transferred to another labeled 15 ml trace metal free polypropylene tube containing 9.5 ml of 5% nitric acid solution. These preparations created final,
matrix matched, 5% nitric acid solutions that were vortexed and used for mineral analysis using ICP-MS. All samples were processed in this manner except those samples from negative control animals. For negative control samples, 3 g tissue was weighed and digested in 6 ml nitric acid. Following digestion, nitric acid was added to the sample in a 10 ml graduated cylinder to bring the total volume up to 9 ml. All other procedures were performed as stated above. This change was made in order to use the excess negative control digest material to create the standard addition Se standards.

In order to make standard addition Se standards for each individual tissue, 5% stock digest solutions needed to be made using the excess digests from the control animals. Two milliliters of the pooled, excess tissue digest was added to a labeled 50 ml trace metal free polypropylene tube (CPI International, Santa Rosa, CA) containing 38 g ultra pure water. Three standards and a blank for each tissue were prepared using the 5% stock digest solutions as follows. Standard #3 was made first by adding 200 µl multielement standard (SPEX CertiPrep Inc., Metuchen, NJ) to a labeled 50 ml trace metal free polypropylene tube. Then 5% stock digest solution was added until the total weight was 40.84 g. Standard #2 was made by adding 20.00 g Standard #3 to a labeled 50 ml trace metal free polypropylene tube followed by 5% stock digest solution until the total weight was 40.00 g. Standard #1 was made by adding 8.00 g Standard #2 to a labeled 50 ml trace metal free polypropylene tube followed by 5% stock digest to bring the total weight up to 40.00 g. Each tube was vortexed between steps. This resulted in a standard addition blank, as well as 0.1 ppm, 0.05 ppm, and 0.01 ppm standard addition standards. Standard preparation was done separately for each individual tissue collected.
Statistical Methods

Means and General Linear Models (GLM) procedures of SAS (SAS Inst., Inc., Cary, NC) were used to evaluate data. A setting of $P < 0.05$ was used to establish significance. The Means function was used to separate significance versus insignificance between the treatment groups.

Results

Clinical Signs and Gross Lesions

Between 10 and 24 h post dosing, sheep in the 6 and 8 mg plant-Se/kg BW groups stood with their heads down, ears that drooped and were reluctant to move. They would often stand with their neck extended with mild dyspnea. No respiratory sounds were noted. Spontaneously or when startled, affected animals would fall over and die. Clinically this was suggestive of sudden cardiac failure as one would see with cardiac arrhythmias, though this is speculative as no animals were monitored with electrocardiography. The hearts of the animals that died suddenly were soft with areas of pallor and hemorrhage. The livers were red and mildly swollen. The hearts of animals that developed dyspnea were grossly normal.

Most of the animals that survived 24 h recovered. However, two developed clinical signs between 18-24 h similar to those seen in Se-Met and sodium selenite treated animals. These animals developed severe dyspnea. They stood with their heads down and necks extended taking short rapid shallow breaths. Their condition continued to deteriorate until they died. At necropsy these sheep had severe pulmonary edema.
characterized by marked interlobular edema and accumulations of serosanguinous fluid and foam in the trachea, bronchi, and bronchioles.

All animals in the Se-Met and the 8 mg plant-Se/kg BW groups as well as one from the 4 mg plant-Se/kg BW group and 4 from the 6 mg plant-Se/kg BW group died prior to the seven day study completion. One animal in the 8 mg plant-Se/kg BW group died at just under 12 h, followed by another death at 12.5 h of an animal in the 6 mg plant-Se/kg BW group. Four animals in the 8 mg plant-Se/kg BW group died between 14 and 14.5 h. The next death, an animal in the Se-Met group, occurred at 15.5 h. Then two animals, one in the Se-Met and one in the 6 mg plant-Se/kg BW group, died between 17 and 17.5 h. The final animal of the 8 mg plant-Se/kg BW group died at just over 21 h. The next deaths of one 4 mg and one 6 mg plant-Se/kg BW group animals occurred at 29 and 30 h, respectively. The final animal that died early was in the 6 mg plant-Se/kg BW group and died at almost 56 h. A total of 13 animals died prior to the end of the study period.

Histologic Findings

The histologic changes in the animals that died suddenly (6 and 8 mg plant-Se/kg BW) were extensive involving the myocardium (60-80%). The necrosis was characterized by acute myocardial degeneration and necrosis (Figure 3.1). The affected myocytes were swollen with clumping of sarcolemma and loss of striations. The vasculature was dilated and congested. Inflammation was minimal and perivascular, being composed of small numbers of lymphocytes, macrophages and rare neutrophils. The lungs were congested and there were increased numbers of neutrophils and
lymphocytes in the pulmonary interstitium (Figure 3.2). The skeletal muscle of these animals had minimal and sporadic multifocal myocyte degeneration (Figure 3.3). This was characterized by loss of striation, clumping of proteins and minimal inflammation. The liver and spleen were mildly congested and there was extensive lymphoid necrosis of the lymph tissues in the spleen, lymph nodes and gastrointestinal system. The animals that developed pulmonary edema had less severe and less extensive acute multifocal myocardial necrosis (< 25% of the myocardium was affected) than the sheep that died suddenly. These sheep also had extensive pulmonary alveolar vasculitis and pulmonary edema with hemorrhage. The myocardial lesions were multifocal randomly distributed zones of acute monophasic necrosis. The necrosis was characterized by myofibers swelling with coagulation of sarcoplasmic proteins; however, these lesions were of longer duration with fibrosis and focal accumulations of macrophages and lymphocytes (Figure 3.4). There was no evidence of mineralization. The pulmonary lesions were those of edema with multifocal perivascular edema and accumulations of neutrophils and fibrin within some alveolar septae. In some areas there were small hemorrhages that occasionally filled the alveoli.

Many of the animals (in the 4 plant-Se/kg BW treatment group) that did not develop clinical signs and survived the 7 day study period had mild multifocal interstitial pneumonia characterized by focal vasculitis with mild edema and congestion. Some of these animals also had mild multifocal swelling and separation of myocardial myofibers (involving 1-2% of the myocardium). In some areas there was mild fibrosis with small accumulations of lymphocytes in the adjacent myocardial interstitium.
All of the animals that survived until necropsy in 6 mg plant-Se/kg BW group had extensive inflammation and early myocardial fibrosis (~30% of the myocardium). The inflammatory cell population was composed of macrophages with smaller numbers of lymphocytes and plasma cells. There was some myocardial regeneration with increased numbers of myocyte nuclei.

No significant histologic lesions were found in sections of brain, spinal cord, liver, kidney, adrenal gland, pancreas, thyroid, rumen, abomasum, duodenum, jejunum, ileum, colon or cecum. All of the control animals, both those treated with alfalfa and low Se Aster were clinically and histologically normal.

Tissue Analysis

Treatment had a highly significant effect on Se concentration in all tissues. Animals that died early were not included in statistical analysis, due to lack of time for elimination that occurred in the other animals. All tissues collected for mineral analysis (brain, atrium, ventricle, skeletal muscle, kidney cortex and liver) showed a trend that Se concentration increased with each increase in dose from plant material (Table 3.1). The mean Se concentration of the selenite group fell between the 2 and 4 mg plant-Se/kg BW groups in all tissues except the kidney and skeletal muscle. In the kidney, the selenite group mean fell between the 4 and 6 mg plant-Se/kg BW groups and in the skeletal muscle, the selenite group mean fell between the control and 2 mg plant-Se/kg BW groups.

Treatment group had a significant effect on Se concentration in the brain ($P < 0.0001$) (Figure 3.5). The mean Se concentration of the 6 mg plant-Se/kg BW group was
significantly greater than all other treatment groups. The control group mean was significantly lower than all other treatments. The selenite group mean in brain was not significant from either the 2 or 4 mg plant-Se/kg BW group means, however, the 2 and 4 mg plant-Se/kg BW group means were significantly different from each other.

The treatment group had a significant effect on the Se concentration in the kidney (\(P < 0.0001\)) (Figure 3.6). As in the brain, the mean Se concentration in the 6 mg plant-Se/kg BW group was significantly greater than all other groups. The control group mean Se concentration was also significantly lower than all other treatment groups. The selenite, 2 and 4 mg plant-Se/kg BW group means were not significantly different from each other.

Treatment group had a significant effect on Se concentration in the atrium of the heart (\(P < 0.0001\)) (Figure 3.7). The 6 mg plant-Se/kg BW mean Se concentration was significantly greater than all other groups and the control mean Se concentration was significantly lower than all treatment groups. The selenite group was not significantly different from either the 2 or the 4 mg plant-Se/kg BW groups. However, the 2 and 4 mg plant-Se/kg BW group mean Se concentrations were significantly different from each other.

Treatment had a significant effect on the Se concentration in the ventricle of the heart (\(P < 0.0001\)) (Figure 3.8). The control, 6, and 4 mg plant-Se/kg BW group means were significantly different from all other treatment groups. The selenite and 2 mg plant-Se/kg BW group means were not significantly different from each other.

Treatment had a significant effect on the Se concentration in the liver (\(P < 0.0001\)) (Figure 3.9). The control and the 6 mg plant-Se/kg BW groups were
significantly different from all other treatment groups. The selenite group was not significantly different from the 2 or 4 mg plant-Se/kg BW group. However, the 2 and 4 mg plant-Se/kg BW groups were significantly different from each other.

Treatment had a significant effect on the Se concentration in the skeletal muscle ($P = 0.0015$) (Figure 3.10). The Se concentration of the 6 mg plant-Se/kg BW group was significantly greater than all other treatment groups. The 4 mg plant-Se/kg BW group was significantly different from the control group, but not the selenite or 2 mg plant-Se/kg BW group. The control, selenite and 2 mg plant-Se/kg BW group means Se concentrations were not significantly different from each other.

Table 3.2 shows the Se concentrations in the collected tissues of the 13 animals which died prior to the study completion of 7 days. Both treatment group and time from dose till death had a statistically significant effect on the mean Se concentration in the brain ($P = 0.0032$ for treatment and $P = 0.0432$ for time) and atrium ($P = 0.0333$ for treatment and $P = 0.0286$ for time) of animals that died prior to the 7 day study completion. Treatment had a significant effect on the mean Se concentration in the liver ($P = 0.0111$) and skeletal muscle ($P = 0.0010$) of animals that died early. Time from dose till death did not have a significant effect on either the liver or skeletal muscle Se concentrations. Neither treatment nor time from dose till death had a significant effect on the mean Se concentration in the ventricle ($P = 0.0741$ for treatment and $P = 0.5730$ for time) or the kidney ($P = 0.6220$ for treatment and $P = 0.0785$ for time) of animals that died early.

Of animals that died early, the mean Se concentration of the Se-Met group was greater than all other groups in the kidney, brain and skeletal muscle. The 8 mg plant-
Se/kg BW group mean was greater than other groups in the liver, atrium and ventricle. The mean concentration of the 8 mg plant-Se/kg BW group was almost double that of Se-Met in the atrium and ventricle. This corresponds with the results of the histopathology. Heart muscle showed more lesions when Se was dosed as plant material, whereas lung lesions were more prevalent when Se was given in the form of purified Se-Met.

There were no statistically significant differences in the pre-dose or post-dose weight of the animals. There were no significant differences in the amount of weight gained or lost between the treatment groups. After 7 days, treatment group did not have a significant effect on the weight of the heart, liver, spleen, right or left kidney. A significant effect was observed on the weight of the lungs ($P = 0.0420$). The Se-Met group mean lung weight (620.85 g) was significantly greater than all other treatment groups except the 8 mg plant-Se/kg BW group mean lung weight (477.58 g).

Discussion

Although Se is required in the body to prevent deficiency syndromes such as White Muscle Disease, the research reported here shows a great need to monitor Se intake in grazing livestock as well as when supplementing livestock diets with Se. A small error could result in poor performance and even death.

Though Se-Met was believed to be the most common form of Se found in plants in the past, recent research show Se accumulator plants store Se in other organic chemical forms.\textsuperscript{19,24} Like recent research shows, the data presented here shows that Se found in Western Mountain Aster is not stored as Se-Met. The sheep described here responded differently to Se provided in plant material compared with Se provided as purified
selenite or Se-Met. This suggests that the majority of Se in this particular plant species was in a form(s) other than Se-Met or selenite. Although all plants take up Se in common forms such as selenite and selenate reactions in accumulator plants covert these to Se storage forms.\textsuperscript{10,21}

The doses of Se from plant material were chosen to be similar to the doses of Se-Met and sodium selenite given orally to sheep as reported by Tiwary \textit{et al.}\textsuperscript{22} In the Tiwary\textsuperscript{22} study, only two sheep proved too ill and were euthanized prior to the completion of the study. We believed the current choices of 2, 4, 6 or 8 mg Se/kg BW from plant material, 4 mg Se/kg BW from selenite and 8 mg Se/kg BW from Se-Met would cause similar responses as described by Tiwary \textit{et al.}\textsuperscript{22} This did not prove to be the case. Both animals in the Se-Met group and all animals in the 8 mg plant-Se/kg BW group died within 21 h of dosing. The two sick sheep from the Tiwary\textsuperscript{22} study were euthanized at 24 and 36 h from the Se-Met group at doses of 6 and 8 mg Se/kg BW, respectively. Tiwary \textit{et al.}\textsuperscript{22} first observed clinical signs 12-24 h after dosing. These signs included reluctance to move, tachypnea, dyspnea and general depression. Of the sheep reported here that died before 24 h, clinical signs observed prior to death were less apparent and less severe than reported earlier. Weakness and depression were observed in animals that died later in the week. Some of these animals experienced tachypnea while others experienced dyspnea. Most of the clinical signs were exhibited within 1-3 days post dosing. The severity of acute Se poisoning, leading to death, appeared to be worse for animals given plant material compared with those receiving an equivalent dose of Se-Met by Tiwary \textit{et al.}\textsuperscript{22} The onset of poisoning also was more rapid in the current study. The differences in response of the Se-Met lambs of this study and those of the
Tiwary study are hard to explain; however, Tiwary et al.\textsuperscript{22} reported their sheep seemed to have a greater resistance to Se poisoning compared with earlier reports. Of an interesting note is the fact that the Se concentrations in the control animal tissues from this study were lower than those reported for the Tiwary et al. study.\textsuperscript{22} Thus, the prior Se status could have played a role in the differences observed. Smith et al.\textsuperscript{20} also noted a variation of susceptibility in individual animals of the same species. The histological and gross lesions seen in the Se-Met animals of this study were similar to those seen by Tiwary et al.\textsuperscript{22} These lesions involved the lungs and included pulmonary edema, hemorrhaging and alveolitis as well as froth and foam in the trachea and bronchi. However, those receiving Se from plant material exhibited mostly heart lesions; another indication that the chemical form of Se in this plant was not likely to be predominantly Se-Met.

Ewan\textsuperscript{6} collected study data relating to the amount of Se fed to sheep and the amount of Se found in tissues. The data collected included only diets with Se in the feed and not added as a supplement. He stated that much of the data was from diets that were low in Se. Through his research, he noted that the kidney typically contains more Se than liver. This is in contrast to this research as the liver had higher concentrations of Se in all treatment groups except the control group, in which the kidney had a higher concentration of Se. The concentration of Se in the diet of the data collected by Ewan\textsuperscript{6} was typically under 1 ppm. Of those instances where he observed that the concentration in the diet exceeded 2 ppm, the concentration of Se in the liver was greater than the concentration of Se in the kidney. Our sheep followed a similar pattern. The negative control animals had higher Se content in the kidney than in the liver. All treatment groups were given at least 2 mg Se/kg BW, which would far exceed 2 ppm in the diet. It
was observed that the liver Se concentration was higher than in the kidney for all treatment groups. Qin et al.\textsuperscript{17} observed that kidney had a higher concentration of Se than liver after sheep were given selenite, selenized yeast or Se enriched probiotics, but they noted that other studies recorded the opposite effect when selenium doses were high.

Ewan\textsuperscript{6} noted that skeletal muscle and heart muscle contain similar concentrations of Se. This research shows that at the lowest dose of Se (2 mg plant-Se/kg BW), skeletal muscle Se concentration was almost 66\% of the concentration of Se in the atrium and 56.5\% of the concentration of Se in the ventricle. However, as the dose increased, the difference between the concentrations of Se in the ventricle and atrium, as compared to skeletal muscle, increased. At 8 mg plant-Se/kg BW, skeletal muscle Se concentration was just 22\% of the Se concentration in the atrium and almost 14\% of the Se concentration in the ventricle.

A study by Echevarria et al.\textsuperscript{5} showed a large increase in liver and kidney Se concentration after feeding sheep for 10, 20, or 30 days at 3, 6, or 9 mg Se/kg diet on a fed basis. They described seeing a linear increase in Se tissue concentrations as was observed in our study. They found liver and kidney to be the most sensitive of observed tissues to dietary Se. Our study indicates that liver, kidney and heart muscle are all highly sensitive to dietary Se. Both this study and that reported by Echevarria et al.\textsuperscript{5} show that Se concentration in tissues was affected significantly by ingested Se after either a single oral dose or a long term dose for 10-30 days. Following our acute, single dose of Se, liver and heart Se concentrations of the highest dose sheep were much greater than the control compared with that seen following the chronic dose of Se over 10-30 days. Liver Se concentration from this acute study was observed to be over 53 times greater in
the 8 mg plant-Se/kg BW group compared to the control group. Liver Se concentration from the chronic study in the 9 mg group was observed to be almost 10 times higher than the control group after 30 days. Se concentration in the ventricle of the heart from this acute study was over 41 times greater in the 8 mg plant-Se/kg BW group compared with the control group. Se concentration in the atrium of the heart from this acute study was just over 44 times greater in the 8 mg plant-Se/kg BW group compared to the control group. Heart muscle Se concentration from the chronic study was almost 2 times higher in the 9 mg group compared with the control group after 30 days. The kidney Se concentration also increased significantly following an acute and chronic dose, though the animals from our acute study did not show as large a difference as was seen in the liver and heart. Kidney Se concentration from this acute study in the 8 mg plant-Se/kg BW group was observed to be almost 7 times greater than the control group. Kidney Se concentration from the chronic study in the 9 mg group was observed to be 9 times higher than the control group after 30 days.

Our acute tissue concentration comparisons above reflect the negative control group compared to the 8 mg plant-Se/kg BW group, in which all animals died before 24 h. A comparison of the negative control group with the highest dose animals that survived the 7 day study period (6 mg plant-Se/kg BW) showed smaller differences in all tissues except the liver. The kidney concentration was just over 4 times greater in the 6 mg animals compared to the control animals. The concentration of Se in the ventricle and atrium was only 4 and 5 times greater in the 6 mg animals, respectively. The tissues of the sheep in the 8 mg plant-Se group did not have the time to eliminate Se that some of the other animals had, accounting for the smaller differences in the comparisons of the
negative control animals with the highest dose of surviving and non-surviving animals. An opposite effect was observed in the liver. The difference was actually greater in the 6 mg animals compared to the control animals, at almost 63 times greater. The liver is a storage organ for Se$^1$ thus the animals in the 6 mg plant-Se group that survived to the end of this study were likely accumulating and storing Se in the liver while the other tissues were continuously eliminating Se during the study period.

Fessler et al.$^7$ performed a study grazing sheep on reclaimed phosphate mine sites. There were three treatment groups, control, low Se and high Se. One ewe that died in the high Se group had myocardial degeneration, hemorrhaging and edema of heart tissue and pulmonary edema. These lesions are similar to those seen in the sheep reported in this study. Heart and lung lesions are associated with Se poisoning and can be observed separately or together in the same animal.$^7,12-14,16$

Se has a detrimental impact on both the heart and lung tissues. The severity seems to be related to both the dose and chemical species of Se. The lesions worsen with dose in both tissues. The type of Se found in the plant material appears to affect the heart muscle early on and if the animal survives the dose, it affects the lungs as well, possibly as a result of the heart damage causing reduced heart function overall, which can lead to a buildup of fluid in the lungs. However Tiwary et al.$^{22}$ only observed mild effects on the heart muscle when dosing with purified compounds of Se-Met or selenite. This indicates that this plant (Symphyotrichum spathulatum) contains Se in another chemical form and potentially several different chemical forms that impact heart muscle and to a lesser extent, lung tissue.
Conclusions

The plant-associated Se causes more severe and extensive myocardial necrosis than Se-Met or sodium selenite. Some of these animals had pulmonary lesions similar to those described in animals dosed with Se-Met and sodium selenite in this study. However, the plant treated animals did not develop pulmonary edema unless they survived the initial day post dosing. The animals that developed severe clinical disease died suddenly had severe myocardial necrosis and degeneration. Two of the plant treated animals died at about 24 h post dosing. These animals had extensive pulmonary edema with less severe myocardial necrosis. These changes are similar to those seen in the study by Tiwary et al.\textsuperscript{22} and in the sheep in this study that died in the Se-Met group and in the sodium selenite group. Those that survived to the end of the study had less severe cardiac lesions with fibrosis and chronic inflammation. The plant treatment groups also had significant skeletal muscle necrosis that was not evident in the other groups dosed with purified Se compounds.

As shown by the data collected through this study, sheep seem to be more sensitive to Se when ingested through plant material than as ingested in common purified compounds. This is significant because areas of high Se content in soil and plants can lead to over ingestion of Se as was noted by Fessler et al.\textsuperscript{7} Depending on the amount ingested of such plant material, illness and even death can result causing substantial financial losses. Future studies are needed to identify the chemical form of the Se in the plant \textit{Symphyotrichum spathulatum}. 


Table 3.1. Mean selenium (Se) concentration ± 1 standard deviation (SD) in parts per million (ppm) of tissues collected at necropsy on day 7 of the study. Data does not include animals that died prior to the 7 day completion of the study.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Number of Animals</th>
<th>Liver (mean ± 1 SD)*</th>
<th>Kidney cortex (mean ± 1 SD)*</th>
<th>Brain (mean ± 1 SD)*</th>
<th>Skeletal Muscle (mean ± 1 SD)*</th>
<th>Atrium (mean ± 1 SD)*</th>
<th>Ventricle (mean ± 1 SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>0.288 ± 0.089a</td>
<td>0.890 ± 0.091a</td>
<td>0.129 ± 0.018a</td>
<td>0.121 ± 0.016a</td>
<td>0.095 ± 0.027a</td>
<td>0.159 ± 0.019a</td>
</tr>
<tr>
<td>Selenite</td>
<td>2</td>
<td>6.712 ± 0.232bc</td>
<td>2.292 ± 0.361b</td>
<td>0.181 ± 0.004bc</td>
<td>0.149 ± 0.004ab</td>
<td>0.266 ± 0.037bc</td>
<td>0.315 ± 0.018b</td>
</tr>
<tr>
<td>2 mg/kg</td>
<td>6</td>
<td>4.420 ± 1.093b</td>
<td>1.757 ± 0.172b</td>
<td>0.164 ± 0.005b</td>
<td>0.155 ± 0.016ab</td>
<td>0.235 ± 0.022b</td>
<td>0.274 ± 0.034b</td>
</tr>
<tr>
<td>4 mg/kg</td>
<td>5</td>
<td>8.323 ± 3.511c</td>
<td>2.164 ± 0.334b</td>
<td>0.204 ± 0.012c</td>
<td>0.175 ± 0.038b</td>
<td>0.329 ± 0.036c</td>
<td>0.400 ± 0.046c</td>
</tr>
<tr>
<td>6 mg/kg</td>
<td>2</td>
<td>18.129 ± 3.560d</td>
<td>3.662 ± 1.230c</td>
<td>0.254 ± 0.050d</td>
<td>0.222 ± 0.037c</td>
<td>0.481 ± 0.175d</td>
<td>0.641 ± 0.045d</td>
</tr>
</tbody>
</table>

*Means with different letters indicate significant differences within each tissue (P < 0.05).

Table 3.2. Mean Se concentrations ± 1 SD in ppm of tissues collected from 13 animals which died prior to completion of the study (7 days). Due to death of only one animal in the 4 mg plant-Se/kg body weight (BW) group, SD was not calculated.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Number of Animals</th>
<th>Time of Death (hours)</th>
<th>Liver (mean ± SD)*</th>
<th>Kidney cortex (mean ± SD)*</th>
<th>Brain (mean ± SD)*</th>
<th>Skeletal Muscle (mean ± SD)*</th>
<th>Atrium (mean ± SD)*</th>
<th>Ventricle (mean ± SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se-Met†</td>
<td>2</td>
<td>16-18</td>
<td>8.768 ± 1.766a</td>
<td>7.026 ± 0.132a</td>
<td>0.823 ± 0.072a</td>
<td>1.137 ± 0.073a</td>
<td>2.576 ± 0.130ab</td>
<td>3.458 ± 0.214ab</td>
</tr>
<tr>
<td>4 mg/kg</td>
<td>1</td>
<td>29</td>
<td>7.760a</td>
<td>6.607a</td>
<td>0.367b</td>
<td>0.394b</td>
<td>2.056a</td>
<td>2.176a</td>
</tr>
<tr>
<td>6 mg/kg</td>
<td>4</td>
<td>13-56</td>
<td>10.410 ± 2.583ab</td>
<td>6.326 ± 1.447a</td>
<td>0.459 ± 0.113bc</td>
<td>0.622 ± 0.132b</td>
<td>2.468 ± 1.256ab</td>
<td>3.547 ± 1.229ab</td>
</tr>
<tr>
<td>8 mg/kg</td>
<td>6</td>
<td>12-21</td>
<td>15.319 ± 2.085b</td>
<td>6.087 ± 0.744a</td>
<td>0.580 ± 0.097c</td>
<td>0.925 ± 0.138a</td>
<td>4.192 ± 1.075b</td>
<td>6.625 ± 2.259b</td>
</tr>
</tbody>
</table>

*Means with different letters indicate significant differences within each tissue (P < 0.05).
†Se-Met, selenomethionine.
Figure 3.1. Photomicrograph of myocardium of a sheep dosed with ground aster to obtain a dose of 8 mg plant-selenium (Se)/kg body weight (BW). Note the shrunken, eosinophilic myofibers that compose about 60% of the myocardium. The inset is a higher magnification demonstrating the extensive myofiber degeneration with hypereosinophilia, nuclear pyknosis and vacuolation (arrowhead). Bar = 100 µm H&E.
Figure 3.2. Photomicrograph of the lung of a sheep dosed with ground aster to obtain a dose of 6 mg plant-Se/kg BW. Note the congestion with focal small accumulations of neutrophils within the alveolar capillaries. Bar = 100 µm H&E.
Figure 3.3. Photomicrograph of semitendinosus skeletal muscle of a sheep dosed with ground aster to obtain a dose of 8 mg plant-Se/kg BW. Note the focal swelling and myocyte hypereosinophilia. Bar = 100 µm H&E.
Figure 3.4. Photomicrograph of the heart of a sheep dosed with ground aster to obtain a dose of 6 mg plant-Se/kg BW. Note the focal myocyte degeneration with accumulations of macrophages and lymphocytes. Bar = 100 µm H&E.
Figure 3.5. Mean Se concentration in brain at 7 days ± 1 SD. Control, selenite (4 mg Se/kg BW) and three doses of Se from Aster are presented. Data does not include animals that died prior to the 7 days of study completion.
Figure 3.6. Mean Se concentration in kidney at 7 days ± 1 SD. Control, selenite (4 mg Se/kg BW) and three doses of Se from Aster are presented. Data does not include animals that died prior to the 7 days of study completion.
Figure 3.7. Mean Se concentration in atrium at 7 days ± 1 SD. Control, selenite (4 mg Se/kg BW) and three doses of Se from Aster are presented. Data does not include animals that died prior to the 7 days of study completion.
**Figure 3.8.** Mean Se concentration in ventricle at 7 days ± 1 SD. Control, selenite (4 mg Se/kg BW) and three doses of Se from Aster are presented. Data does not include animals that died prior to the 7 days of study completion.
Figure 3.9. Mean Se concentration in liver at 7 days ± 1 SD. Control, selenite (4 mg Se/kg BW) and three doses of Se from Aster are presented. Data does not include animals that died prior to the 7 days of study completion.
Figure 3.10. Mean Se concentration in skeletal muscle at 7 days ± 1 SD. Control, selenite (4 mg Se/kg BW) and three doses of Se from Aster are presented. Data does not include animals that died prior to the 7 days of study completion.
CHAPTER 4 

TOXICOKINETICS OF SELENIUM IN SHEEP GIVEN THE 

ACCUMULATOR PLANT SYMPHYOTRICUM 

SPATHULATUM (WESTERN 

MOUNTAIN ASTER) 

Abstract 

Whole blood and serum samples were collected from sheep given the selenium (Se) accumulator plant, Symphyotrichum spathulatum (Western Mountain Aster), to study the toxicokinetics of plant accumulated Se. Six animals per treatment group were gavaged with ground plant material resulting in doses of 0, 2, 4, 6, or 8 mg plant-Se/kg BW. Purified sodium selenite and selenomethionine (Se-Met) were given as positive controls. Two animals received 4 mg Se/kg BW as sodium selenite and two animals received 8 mg Se/kg BW as Se-Met. Whole blood and serum samples were collected prior to dosing and at 10 min, 30 min, 1 h, 3 h, 6 h, 12 h, 18 h, 24 h, 48 h, 72 h, 120 h, and 168 h. Following Se analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), curve stripping was performed on the serum and whole blood data. In serum, the dosage of Se in the plant treatments had a significant effect on the absorption and elimination half-lives and the elimination rate constant. In whole blood, Se dose caused a significant effect on the distribution and elimination rate constants and half-lives, but no such effects were seen for the absorption rate constant or half-life in the plant. The elimination half-life of the 2 mg plant-Se/kg BW group was significantly greater than other treatments in both serum and whole blood ($P = 0.0002$ in serum and $P = 0.0100$ in
whole blood). In serum, the elimination half-life decreased with increasing dose of plant derived selenium. In whole blood, the elimination half-life of the 2 mg plant-Se/kg BW group was 430.1 h compared with 117.6 and 117.1 h in the 6 and 4 mg plant-Se/kg BW groups, respectively. The absorption half-life in serum decreased as dose increased. Treatment had a significant effect on time to peak Se concentration in whole blood, but not in serum. Treatment had a significant effect on the peak Se concentration and area under the curve in both whole blood and serum. Both the peak Se concentration and area under the curve increased with each increase in plant Se dosed in both whole blood and serum.

Introduction

Selenium (Se) is a required nutrient in mammals, but can easily be over supplemented causing serious health concerns, possibly even death. Only a small amount is required to fulfill the dietary needs of animals and a small amount over that requirement can be toxic. Due to the ease of over supplementation, several cases of sick and/or dead animals have been reported to be caused by accidental over supplementation of Se. Deaths have also been reported following livestock ingestion of certain Se-accumulating plants. Plants that assimilate large amounts of Se have become known as Se-accumulator plants. Based on Se content, plants have been categorized into three types: primary accumulators, secondary accumulators and those that do not accumulate significant concentrations of Se. Se can reach concentrations as high as several thousand parts per million (ppm) in primary accumulators. Plant species categorized as primary accumulators.
accumulators include *Astragalus, Stanleya, Machaeranthera, Oonopsis* and *Xylorhiza*.

Primary accumulators thrive in soils high in Se, but usually do not grow well in soils with little Se. Secondary accumulators tend to have Se concentrations between 100-1000 ppm. Secondary accumulators include species of *Aster, Castilleja, Grindelia, Atriplex, Gatierreaia* and *Comandra*. The final group consists of plants that do not significantly accumulate Se and generally contain less than 100 ppm Se, but can occasionally have a few hundred ppm in the tissues. This group includes grasses, trees and some weeds.

Toxicokinetics of Se in mammals has not been well reported in the literature. Tiwary\textsuperscript{17} studied the kinetics of Se given orally to sheep in the forms of purified selenomethionine (Se-Met) and purified sodium selenite. They saw that the concentration of Se in both the whole blood and serum followed a basic dose-response curve. The objective of this study was to evaluate oral-dose Se kinetics in sheep for the chemical form(s) found in the Se accumulator plant *Symphyotrichum spathulatum* (Western Mountain Aster).

**Materials and Methods**

**Animals**

Thirty-four sheep ranging in weight from 22.7 to 36.4 kg were group housed with *ad lib* access to alfalfa hay and water. Prior testing of the alfalfa hay found Se concentrations of 0.11 to 0.13 ppm. The sheep were allowed to acclimate for 2 weeks prior to the study. This study was approved by the Institutional Animal Care and Use Committee of Utah State University.
Dosing Material

Western Mountain Aster (*Symphyotrichum spathulatum*) was collected from an area of southeastern Idaho. A plant specimen is found in the Intermountain Herbarium at Utah State University in Logan, Utah under the accession number 246175. The Aster used for the treatment groups was found to have 4782 ppm Se. The Se content of the low Se Aster used as a negative control was found to be 1.59 ppm. Purified sodium selenite was purchased from United States Biochemical Corporation, Cleveland, Ohio. Purified Se-Met was purchased from Sigma-Aldrich, St. Louis, Missouri.

Administration and Sample Collection

Thirty-four sheep were randomly assigned to the following groups: control, sodium selenite, Se-Met, 2, 4, 6, or 8 mg plant-Se/kg BW. Two animals were assigned to each of the selenite and Se-Met groups. Six animals were assigned to each concentration of plant treatment and received *Symphyotrichum spathulatum* plant material (4782 ppm Se) according to body weight. Six animals were assigned to the negative control group. Two of the negative control animals received equivalent amounts of water by intraruminal gavage. The other four negative control animals received low Se Aster plant material (1.59 ppm Se), in order to eliminate other plant compounds as the potential cause of any observed effects. Three of these four control animals were dosed with about 3 times as much plant material, according to individual body weight, as they would have received if they were assigned to the 8 mg plant-Se/kg BW group. An average of 42.7 g plant material was given to the animals in the 8 mg plant-Se/kg BW group compared with an average of 148.4 g plant material given to three of the control animals. This resulted
in Se doses of 0.0077 mg/kg BW, which is less than a typical daily recommended Se intake. The last of the control animals receiving plant material at about 1.5 times as much plant material, according to body weight, as the 8 mg plant-Se/kg BW group due to a lack of total low Se plant material. The selenite group received 4 mg Se/kg BW as purified sodium selenite. The Se-Met group received 8 mg Se/kg BW as purified Se-Met. These two groups served as positive controls in the study. The treatments were prepared for each individual animal and administered through an intraruminal gavage, followed by a flush of water to ensure each animal received the full assigned dose.

Whole blood and serum samples were collected into trace metal free Vacutainer tubes (Becton, Dickinson & Co., Franklin Lakes, NJ) prior to dosing and at 10 min, 30 min, 1 h, 3 h, 6 h, 12 h, 18 h, 24 h, 48 h, 72 h, 120 h, and 168 h post dosing. Following collection, whole blood samples were stored at 4°C until digested for analysis. Serum was separated through centrifugation and removed to labeled 15 ml trace metal free polypropylene tubes (CPI International, Santa Rosa, CA). Serum was then stored at 4°C until digested for analysis.

Analytical Method

The following acid digestion procedure using nitric acid was followed to digest both whole blood and serum samples to eliminate organic material leaving only elemental Se for analysis. Serum samples were gently mixed with a pipette tip. Then, 750 µl of serum was added to a 10 ml Oak Ridge Teflon Centrifuge Tube (Nalge Nunc International, Rochester, NY) followed by the addition of 750 µl of trace metal grade nitric acid (Fisher Scientific, Pittsburgh, PA). After sealing the digestion tube tightly, the
tubes were placed on a heat block set at 90°C. Samples were allowed to digest for 1 h, during which time the samples were closely observed to prevent pressure build-up. Caps were periodically loosened to relieve pressure and then re-tightened. Following digestion, the tubes were allowed to cool.

After the contents had cooled, the digest was transferred to an empty, labeled, 15 ml trace metal free polypropylene tube and vortexed. One milliliter of the digest was transferred to another labeled, 15 ml trace metal free tube containing 9 ml ultra pure water. This dilution was vortexed and then the sample was ready for Se analysis using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Perkin Elmer, Shelton, CT).

Whole blood samples were prepared and digested similarly to serum; however 750 µl of nitric acid was added to the 10 ml centrifuge tube prior to 750 µl of sample. Nitric acid was added to the tube first so it could be used to clean out any remaining whole blood in the tip to ensure all 750 µl of blood was added. Also, the whole blood dilution tube was centrifuged at 2000 rpm for 5 min to remove any precipitate material that could clog the nebulization system. The supernatant was then transferred to an empty, labeled, 15 ml trace metal free tube and was ready for analysis.

Se standards were prepared separately for both whole blood and serum using the following standard addition procedure. A stock digest solution was first created using the excess digests from all of the pre-dose samples. Analytical standard addition stock was made by adding 5 ml of the whole blood or serum digest stock to a labeled 50 ml trace metal free polypropylene tube (CPI International, Santa Rosa, CA) containing 45 ml ultra pure water. Standard #3 was made by adding 200 µl multielement standard (SPEX CertiPrep Inc., Metuchen, NJ) to a labeled 50 ml polypropylene tube. Stock solution was
added to bring the total weight up to 40.84 g. Standard #2 was made by adding 20.00 g of Standard #3 to a labeled 50 ml polypropylene tube and then adding stock solution until the total weight was 40.00 g. Standard #1 was made by adding 8.00 g of Standard #2 to another labeled 50 ml polypropylene tube and adding stock solution until the total weight was 40.00 g. Each tube was vortexed between steps. This resulted in a standard addition blank, as well as 0.1 ppm, 0.05 ppm and 0.01 ppm standard additions.

Modeling and Statistical Methods

Following ICP-MS analysis, the data were analyzed using PK Solutions 2.0 software (PK, Solutions, Montrose, CO) to evaluate the kinetics through curve stripping using a three-term model of absorption, distribution and elimination unless otherwise noted. Triexponential equations representing the curves were denoted by the following formula; $C = Ae^{-\alpha t} + De^{-\delta t} + Ee^{-\epsilon t}$ where $C$ was the concentration of Se in serum or whole blood at any time ($t$). $A$, $D$ and $E$ designated the Y-axis ($t = 0$) intercepts and $\alpha$, $\delta$, and $\epsilon$ represented the rate constants for the terms representing absorption, disposition/distribution and elimination, respectively. Area under the concentration versus time curve (AUC), a measure of overall bioavailability, was calculated using the trapezoid rule. Finally, the rate constants and half-life data was analyzed using SAS statistical software (SAS Inst., Inc., Cary, NC). Means and General Linear Models (GLM) procedures of SAS were used to evaluate data. A setting of $P < 0.05$ was used to establish significance. The Means function was used to separate significance versus insignificance between the treatment groups.
Results

The mean serum and whole blood Se concentrations at the collection time points are shown in Figures 4.1 through 4.4. Figure 4.1 shows the mean Se concentrations in serum for the positive and negative controls and Figure 4.2 shows the mean serum Se concentrations in the plant treatment groups. Figure 4.3 shows the mean Se concentrations in whole blood for the positive and negative controls and Figure 4.4 shows the mean whole blood Se concentrations in the plant treatment groups.

Whole blood and serum samples were only collected at pre-dose, 12 h and 168 h post dosing from the negative control animals receiving low Se plant material, as the amount of Se dosed is less than dietary. The concentrations at these time points were equivalent to the other negative control animals at the same time points as shown in Table 4.1.

Serum

Due to the early death of both animals in the Se-Met group (16-18 h) and all six animals in the 8 mg plant-Se/kg BW group (12-21 h), curve stripping could not be performed on these animals so both groups were removed from the statistical analysis. One animal from the 6 mg plant-Se/kg BW group was dead by 18 h post dose, which prevented curve stripping so statistical analysis was performed on the remaining five animals in that group. Two other animals in the 6 mg plant-Se/kg BW group died early (13-30 h), however there were enough data points from each animal to perform curve stripping. A two-term model was used on these two animals; therefore the terminal elimination phase was lost. There was enough data from the final animal of the 6 mg
plant-Se/kg BW group that died early (56 h) to perform curve stripping with a three-term model. A two-term model was applied to one animal in the 4 mg plant-Se/kg BW group (died by 29 h) so the elimination phase data was also unable to be calculated for it.

Absorption

Treatment did not have a significant effect on the absorption rate constant ($P = 0.2446$) for the plant Se form(s) (Table 4.2). The mean absorption half-life for the plant treatment groups tended to decrease with each increase in dose, but not significantly (Table 4.3). The selenite half-life was significantly greater ($P = 0.0071$) than all the plant treatment groups. The mean absorption half-life for the selenite group was 8.1 h. The mean absorption half-life for the plant groups ranged from 2.7 h in the 6 mg plant-Se/kg BW group to 4.3 h in the 2 mg plant-Se/kg BW group.

Distribution

The treatment groups showed no significant differences in the distribution rate constant ($P = 0.4200$) (Table 4.2) or distribution half-life ($P = 0.4259$) (Table 4.3). The mean distribution half-life was 10.2 h in the selenite group, 15.8 h in the 2 mg plant-Se/kg BW group, 19.3 h in the 6 mg plant-Se/kg BW group and 29.5 h in the 4 mg plant-Se/kg BW group, but all groups had very broad variability in the results.

Elimination

The treatment had a highly significant effect on the elimination rate constant ($P < 0.0001$) (Table 4.2). The selenite and 6 mg plant-Se/kg BW groups were not significantly different. The 6 and 4 mg plant-Se/kg BW groups were not significantly
different. However, the 2 mg plant-Se/kg BW group was significantly lower than all other groups. Treatment had a significant effect on elimination half-life \((P = 0.0002)\) (Table 4.3). The 2 mg plant-Se/kg BW group was significantly greater than all other groups with a mean half-life of 258.7 h. The half-lives of the selenite, 4 and 6 mg plant-Se/kg BW groups were not significantly different from each other. The mean half-life of the 4 mg plant-Se/kg BW group was 74.9 h, in the 6 mg plant-Se/kg BW group it was 61.9 h and in the selenite group it was 57.6 h.

Other Kinetic Parameters

Treatment did not have a significant effect on time to peak Se concentration \(t_{\text{max}}\) \((P = 0.0604)\) (Table 4.4). The \(t_{\text{max}}\) in the selenite and 2 mg plant-Se/kg BW groups was almost the same at 26.1 and 25.7 h, respectively. The \(t_{\text{max}}\) was 15.3 and 14.4 h in the 6 and 4 mg plant-Se/kg BW groups, respectively.

Treatment had a highly significant effect on the peak Se concentration \(C_{\text{max}}\) \((P = 0.0002)\) (Table 4.4). The \(C_{\text{max}}\) in serum increased with each increase in dose, with the selenite group falling between the 2 and 4 mg plant-Se/kg BW groups. The 2 mg plant-Se/kg BW group was significantly different from all other groups at 0.802 ppm. The selenite group was significantly different from the 6 mg plant-Se/kg BW group at 1.527 ppm. The 4 and 6 mg plant-Se/kg BW groups were not significantly different from each other with \(C_{\text{max}}\) values of 1.828 ppm and 2.360 ppm, respectively.

Treatment had a significant effect on AUC \((P = 0.0301)\) (Table 4.4). AUC increased with increasing dose in serum. The AUC of the 2 mg plant-Se/kg BW was 68.907 µg-h/L, followed by the 4 mg plant-Se/kg BW group at 136.92 µg-h/L and then
the 6 mg plant-Se/kg BW group at 184.303 µg-h/L. The selenite AUC fell between the 4 and 6 mg plant-Se/kg BW groups at 147.664 µg-h/L. The 2 and 6 mg plant-Se/kg BW groups were the only groups significantly different from each other.

Whole Blood

As with serum, curve stripping could not be performed on the following animals due to early death: both animals in the Se-Met group (died between 16-18 h), all six animals in the 8 mg plant-Se/kg BW group (died between 12-21 h) and three animals in the 6 mg plant-Se/kg BW group (died between 13-30 h). These animals were eliminated from the statistical analysis. A two-term model was applied to one animal in the 4 mg plant-Se/kg BW group (dead by 29 h); therefore the elimination phase data for that animal was lost. There were enough data points from the final animal that died early from the 6 mg plant-Se/kg BW group (56 h) to perform curve stripping using a three-term model.

Absorption

Treatment did not have a significant effect on the absorption rate constant \( (P = 0.1725) \) or half-life \( (P = 0.0586) \) (Tables 4.2 and 4.3). The mean absorption half-life in the selenite group was 7.2 h, in the 2 mg plant-Se/kg BW group was 4.6 h, in the 4 mg plant-Se/kg BW was 3.7 h and in the 6 mg plant-Se/kg BW was 3.9 h.

Distribution

Treatment had a highly significant effect on the distribution rate constant \( (P = 0.0007) \) (Table 4.2). The 6 mg plant-Se/kg BW group was significantly greater than all
other groups. The selenite and 2 mg plant-Se/kg BW groups were not significantly different and the 2 and 4 mg plant-Se/kg BW groups were not significantly different. Treatment had a significant effect on the distribution half-life ($P = 0.0196$) (Table 4.3). The mean half-lives of the 2 and 4 mg plant-Se/kg BW groups were not significantly different with values of 11.6 h and 28.2 h, respectively. The selenite group had a mean half-life of 6.7 h and the 6 mg plant-Se/kg BW group had a mean half-life of 4.2 h. The selenite, 2 and 6 mg plant-Se/kg BW group half-lives were not significantly different from each other.

Elimination

Treatment had a significant effect on the elimination rate constant ($P = 0.0068$) (Table 4.2). The 2 mg plant-Se/kg BW group was significantly lower than all other groups. The selenite, 6 and 4 mg plant-Se/kg BW groups were not significantly different from each other. Treatment also had a significant effect on the elimination half-life ($P = 0.0100$) (Table 4.3). The 2 mg plant-Se/kg BW group was significantly greater than all other groups with a mean of 430.1 h. The selenite, 6 and 4 mg plant-Se/kg BW groups were not significantly different. The mean half-life of the 6 mg plant-Se/kg BW group was 117.6 h, was 117.1 h in the 4 mg plant-Se/kg BW group and 86.5 h in the selenite group.

Other Kinetic Parameters

Treatment had a significant effect on $t_{\text{max}}$ ($P = 0.0037$) (Table 4.4). The 2 mg plant-Se/kg BW and selenite groups were not significantly different from each other; however both groups were significantly different from both the 6 and 4 mg plant-Se/kg
BW groups. The 2 mg plant-Se/kg BW group had the longest $t_{\text{max}}$ at 30.4 h, followed closely by the selenite group at 27.8 h, next was the 6 and 4 mg plant-Se/kg BW groups at 18.7 and 17.2 h, respectively.

Treatment had a highly significant effect on $C_{\text{max}}$ ($P < 0.0001$) (Table 4.4). $C_{\text{max}}$ increased with each increase in dose as seen with serum. The 2 mg plant-Se/kg BW group was significantly different from all other groups with a $C_{\text{max}}$ of 0.582 ppm. The selenite group was also significantly different from all other groups with a $C_{\text{max}}$ of 1.059 ppm. The 4 and 6 mg plant-Se/kg BW groups were not significantly different with $C_{\text{max}}$ values of 1.399 ppm and 1.653 ppm, respectively.

Treatment also had a significant effect on AUC ($P = 0.0068$) (Table 4.4). AUC increased with increasing Se dose. The 2 mg plant-Se/kg BW group was significantly less than the 4 and 6 mg plant-Se/kg BW groups with an AUC of 67.896 µg-h/L. The AUC for the 4 mg plant-Se/kg BW group was 130.711 µg-h/L followed by the 6 mg plant-Se/kg BW group at 155.917 µg-h/L. The AUC of the selenite group was between 2 and 4 mg plant-Se/kg BW groups at 118.331 µg-h/L.

Discussion

The study was performed to evaluate the toxicokinetics of Se in whole blood and serum following an oral dosing of Se-containing plant material. The results are somewhat limited due to the lethal effect of the high dose plant group and the Se-Met group. Many of these animals died prior to reaching a peak Se concentration in the whole blood.
The $t_{\text{max}}$ is the time at which the absorption and elimination rates are equal. Prior to reaching $t_{\text{max}}$ the absorption rate is greater than the elimination rate. After $t_{\text{max}}$ the elimination rate is greater than the absorption rate. Lower dose animals of the selenite and 2 mg plant-Se/kg BW groups had longer $t_{\text{max}}$. Those in the higher dose groups of 4 and 6 mg plant-Se/kg BW group had shorter $t_{\text{max}}$. The mean $t_{\text{max}}$ in whole blood ranged from 17-30 h across all groups. The mean $t_{\text{max}}$ ranged from 14-26 h across all groups in serum. Although not significant, a slight increase in the length of time to peak concentration as dose decreased in both whole blood and serum is not surprising since the absorption rates of both whole blood and serum increased slightly with each increase in dose. Tiwary\textsuperscript{17} observed $t_{\text{max}}$ in the range of 12-24 h and 3-18 h in whole blood of sheep dosed with selenite and Se-Met, respectively. The $t_{\text{max}}$ ranged from 12-24 h and 6-12 h in the serum of selenite and Se-Met treated sheep, respectively. This plant material generally showed longer $t_{\text{max}}$ than both selenite and Se-Met in the Tiwary study,\textsuperscript{17} but was most similar to the selenite treated animals in both whole blood and serum. However, a comparison by Wang \textit{et al}.\textsuperscript{20} of the effect of monensin on Se bioavailability in sheep given 0.4 mg Se/kg as selenite showed different $t_{\text{max}}$ ranges. The $t_{\text{max}}$ ranged from 4-12 h in whole blood and 6-12 h in plasma of both selenite and selenite/monensin treated animals. This difference could be due to much higher doses used in this toxicokinetic study.

The $C_{\text{max}}$ for each plant-Se treatment increased with increasing dose. This was also seen in studies of macaques given increasing does of Se-Met.\textsuperscript{10,23} A similar increase in $C_{\text{max}}$ values was seen by Tiwary\textsuperscript{17} who dosed sheep with increasing doses of sodium selenite and Se-Met. Tiwary drew the conclusion that Se-Met is more bioavailable than
selenite due to animals in the Se-Met group exhibiting higher $C_{\text{max}}$ values. Interestingly, the average peak concentration of the selenite group (4 mg Se/kg BW) in this study fell between the 2 and 4 mg plant-Se/kg BW groups in both whole blood and serum. This could possibly mean that the chemical form of Se in this plant was slightly more bioavailable than sodium selenite. However this conclusion cannot be made because this data did not compare plant Se with selenite over several doses.

The AUC parameter is related to the amount of drug absorbed in the body. Not surprisingly in this study, the calculated AUC increased with each increase in plant treatment dose in both whole blood and serum. Two separate studies in macaques also showed an increase in plasma AUC as dose increased after administering varying doses of Se-Met through nasogastric intubation.\textsuperscript{10,23}

AUC has been shown to be affected by organic versus inorganic Se.\textsuperscript{24} They compared intravenous and oral dosing of selenate and Se-Met in hamsters. Se-Met dosed hamsters had much larger plasma AUC regardless of route of administration compared to hamsters receiving selenate. At equivalent doses, intravenous administration of Se-Met resulted in an AUC value 9.9 times greater than when given as selenate. Similarly at equivalent doses, oral administration of Se-Met resulted in an AUC value almost 3.8 times greater than when given as selenate. Tiwary\textsuperscript{17} also saw higher AUC values for Se-Met when compared with selenite at equal Se doses in both whole blood and serum of sheep, however the increases observed were not as drastic as seen by Willhite \textit{et al.}\textsuperscript{24} In this study, the selenite AUC was similar to the 4 mg plant-Se/kg BW group in both serum and whole blood. However, the selenite group mean was lower than the 4 mg plant-Se/kg BW group in the whole blood and higher than the 4 mg plant-Se/kg BW group in
serum. Unfortunately, the untimely deaths of the animals in the Se-Met and 8 mg plant-Se/kg BW groups prevented comparison of those AUC values. More research needs to be done to compare this plant material with varying doses of purified Se compounds such as selenocysteine, selenate, and other organic Se forms to determine which chemical form this particular plant most closely resembles.

The absorption rate was observed to increase as dose increased in serum. This is in contrast to what Tiwary\textsuperscript{17} observed. Tiwary\textsuperscript{17} found that as dose of Se-Met increased, the absorption rate decreased, presumably due to a saturation of methionine uptake pathways shared by Se-Met. This would suggest that the form of Se in the plant material used in this study is absorbed differently than Se-Met. Tiwary\textsuperscript{17} observed that Se-Met had a higher absorption rate than sodium selenite. In the current study, selenite had a lower absorption rate than the plant groups. This supports the idea that organic Se species are absorbed faster than inorganic Se species.\textsuperscript{12,19}

Davis \textit{et al.}\textsuperscript{5} and Cristaldi \textit{et al.}\textsuperscript{4} observed an increase in serum and whole blood Se as dietary Se increased when dosing sheep with selenite. Echevarria \textit{et al.},\textsuperscript{6} observed an increase in serum Se as dietary Se increased when dosing with selenite. Goehring \textit{et al.}\textsuperscript{9} also saw blood Se in pigs increase with an increase in dietary Se when dosing with either selenite or seleniferous wheat and oats. Similarly in this study, Se concentrations in whole blood and serum were greater in the 4 and 6 mg plant-Se/kg BW groups compared with the 2 mg plant-Se/kg BW group. This shows that increasing the dose of the chemical form of Se in this plant correspondingly increases the amount of Se absorbed into the body such that large doses can and will be toxic and should generally
be avoided, though there have been reports suggesting that livestock can become accustomed to high Se concentrations.\textsuperscript{18}

According to Raisbeck,\textsuperscript{15} serum Se concentrations increase rapidly after a toxic dose and then quickly drop off, whereas whole blood Se concentrations are not as affected by “short-term fluctuations” and remain elevated longer after dosing than serum Se concentrations. A possible explanation for the whole blood Se concentration staying elevated longer than the serum Se concentration after a single dose could be because Se can become bound to proteins in the blood.\textsuperscript{1} In this study, the absorption rate was at least slightly higher for the 2, 4, and 6 mg plant-Se/kg BW groups in the serum than in the same treatment group for whole blood. The absorption rates for the 2 and 4 mg plant-Se/kg BW groups were only slightly different. The absorption rate constant of serum was about 1.3 times greater than the absorption rate constant in whole blood in the 2 mg plant-Se/kg BW group and almost 1.1 times greater in the 4 mg plant-Se/kg BW. However, the serum absorption rate was over 2.6 times greater than the whole blood in the 6 mg plant-Se/kg BW group. This tends to support the statement by Raisbeck\textsuperscript{15} that serum Se concentrations increased quicker following toxic doses than do whole blood Se concentrations.

Of the plant groups, the elimination half-life was found to be greatest in the 2 mg plant-Se/kg BW group in both whole blood and serum. It was 258 h compared to about 60-70 h in the other groups in serum (Table 4.3). In whole blood, the half-life was 430 h compared to 85-120 h in the other treatment groups (Table 4.3). Elimination was much slower at the lowest dose, indicating that higher concentrations of Se in the body are eliminated faster. Burk \textit{et al}.\textsuperscript{2} saw a large increase in urinary Se excretion over 10 days
with increasing doses of selenite in rats. The elimination rate constants for Se in serum increased with increasing dose. Tiwary$^{17}$ also reported an increase in elimination rates with an increase in dose when lambs were given either sodium selenite or Se-Met. The selenite group in this study had a higher elimination rate constant and correspondingly lower half life, in serum and whole blood than the plant groups, indicating that it was eliminated faster than the Se from the plant material. This could be because the plant Se may exist in multiple chemical forms, possibly both organic and inorganic forms, leading to longer elimination rates. It has been noted that organic versus inorganic Se can have an effect on the elimination rates though some research shows contradictions. Willhite et al.$^{24}$ saw higher elimination rates in hamsters given selenate compared to those given Se-Met both orally and intravenously, indicating faster elimination when given an inorganic form of Se. However, Ryszka et al.$^{16}$ saw the opposite effect in rats. They observed a higher elimination rate constant in rats when dosed with Se yeast compared with rats given selenite.

Of those animals that died early, the elimination pathways were overloaded allowing Se to build up to high concentrations causing severe tissue damage leading to death, as seen in the heart. The histopathology results of the hearts of animals that died early showed severe myocardial degeneration and necrosis. The tissue Se concentrations in the hearts of animals in the 8 mg plant-Se/kg BW group were over 41 and 44 times greater than the tissue concentrations seen in the negative control animals. As seen with the serum/whole blood data, those in the higher dose groups that survived beyond 3 days post dosing had eliminated much of the dosed Se and were therefore able to survive until the end of the study period of 7 days.
Se elimination occurs mainly through fecal and urinary elimination. At toxic doses, Se is known to be eliminated through respiration. Absorbed Se is generally metabolized to selenide in the mammalian body and then selenide is methylated to form excretion products. It is first methylated to methyl selenide then to dimethylselenide and finally to trimethylselenonium ion. Dimethylselenide is the major product found in expired air following toxic doses. Trimethylselenonium ion is the major metabolite found in urine and the amount found is dose dependent as seen in the study by Burk et al. who fed rats Se and observed that the amount eliminated in urine was found to be dose dependent, whereas fecal elimination was consistent throughout the study. Ruminants eliminate more Se than monogastrics through fecal material because rumen microbes convert Se to insoluble forms that the ruminant cannot absorb. Although urinary and fecal elimination were not measured in this study, it is assumed that much of the ingested Se was eliminated in urine because a large concentration was absorbed as seen in the whole blood and serum. It is also assumed that the unabsorbed Se was eliminated in the feces.

Though the erythrocyte Se content was not analyzed separately from whole blood in this study, contrasting the whole blood data with the serum data can predict the Se content in the erythrocytes. It would be expected that the erythrocyte concentrations would be approximately equal to the serum concentrations subtracted from the whole blood concentrations. The elimination half-life of Se in the erythrocytes would be expected to be greater than that observed for serum, but not greater than that observed in the whole blood. This would be expected because the elimination half-life was greater in the whole blood than in the serum and serum makes up only a part of whole blood. The
remainder of whole blood, including the erythrocytes, would likely have a longer elimination half-life than serum, leading to a longer half-life in whole blood.

Though limited, the data reported here provides more insight into the oral-dose kinetics of Se in the lamb. The oral-dose kinetic work done by Tiwary\textsuperscript{17} using purified organic and inorganic forms of Se in lambs provided a base for the current research using plant material. This study is important to see how the chemical form(s) of Se in plants, specifically Se-accumulator plants, affect livestock and how these affects are similar or different from purified compounds commonly given as supplements. As seen from this study, Se from accumulator plants can cause rapid toxic responses in sheep. Fessler \textit{et al.}\textsuperscript{7} also described a large number of sheep deaths in an area of Idaho with high soil and plant Se content. Knowledge of these plants and the Se stored within these plants is important for livestock producers who graze animals in highly seleniferous areas, as they will need to exercise careful management of grazing.

Conclusions

The Se from this plant caused a dose dependent response on the $C_{\text{max}}$ and AUC as has been observed with purified selenite and Se-Met.\textsuperscript{17} There was also a dose dependent response on elimination rates in both serum and whole blood. Thus as the concentration of Se ingested increased, Se elimination occurred at a faster rate.

Unfortunately, the specific chemical form of Se that predominates in this particular plant is unknown. However, Se-accumulators are known to accumulate Se in variety of forms.\textsuperscript{21} The most common forms found in accumulators are thought to be Se-methylselenocysteine and selenocystathionine. Other forms can include selenate,
selenite, Se-methylselenomethionine, γ-glutamyl-Se-methylselenocysteine, γ-glutamyl-selenocystathionine and selenohomocysteine. Identification of the forms of Se in this plant will be useful to future study comparisons. There is also potential for future research on other Se-accumulator plants as well as studies in other species of mammals.

References


Table 4.1. Mean selenium (Se) concentration in parts per million (ppm) ± 1 standard deviation (SD) in negative control receiving water and plant negative control groups receiving low Se Aster in serum and whole blood.

<table>
<thead>
<tr>
<th>Time-point</th>
<th>Serum*</th>
<th>Whole Blood*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative Control (n=2)</td>
<td>Plant Negative Control (n=4)</td>
</tr>
<tr>
<td>Pre-dose</td>
<td>0.075 ± 0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.075 ± 0.008&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>12 h</td>
<td>0.063 ± 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.076 ± 0.006&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>168 h</td>
<td>0.065 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.078 ± 0.008&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Means with different letters indicate significant differences within serum or whole blood (<i>P</i> < 0.05).

Table 4.2. Mean absorption, distribution, & elimination rate constants (h<sup>-1</sup>) ± 1 SD in serum and whole blood of sheep dosed with differing amounts of selenium.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Absorption Rate Constant (mean ± 1 SD)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Distribution Rate Constant (mean ± 1 SD)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Elimination Rate Constant (mean ± 1 SD)&lt;sup&gt;*&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenite</td>
<td>0.090 ± 0.028&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.070 ± 0.013&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.013 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 mg plant-Se/kg</td>
<td>0.199 ± 0.120&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.048 ± 0.013&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.003 ± 0.002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 mg plant-Se/kg</td>
<td>0.241 ± 0.060&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.028 ± 0.012&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.009 ± 0.002&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 mg plant-Se/kg</td>
<td>0.574 ± 0.648&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.682 ± 1.373&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.012 ± 0.003&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Whole Blood</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenite</td>
<td>0.101 ± 0.028&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.105 ± 0.020&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.008 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 mg plant-Se/kg</td>
<td>0.151 ± 0.013&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.067 ± 0.028&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.002 ± 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 mg plant-Se/kg</td>
<td>0.223 ± 0.098&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.034 ± 0.020&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.006 ± 0.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 mg plant-Se/kg</td>
<td>0.216 ± 0.107&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.198 ± 0.091&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.007 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Means with different letters indicate significant differences within each treatment group in serum or whole blood (<i>P</i> < 0.05).
Table 4.3. Mean absorption, distribution, & elimination half-lives (h) ± 1 SD in serum and whole blood of sheep dosed with differing amounts of selenium.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Absorption Half-life (mean ± 1 SD)*</th>
<th>Distribution Half-life (mean ± 1 SD)*</th>
<th>Elimination Half-life (mean ± 1 SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenite</td>
<td>8.112 ± 2.477&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.164 ± 2.032&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.568 ± 3.306&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 mg plant-Se/kg</td>
<td>4.265 ± 1.664&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.775 ± 6.262&lt;sup&gt;a&lt;/sup&gt;</td>
<td>258.732 ± 83.340&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 mg plant-Se/kg</td>
<td>3.055 ± 0.896&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.457 ± 15.207&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.923 ± 10.904&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 mg plant-Se/kg</td>
<td>2.650 ± 2.047&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.313 ± 26.849&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.904 ± 15.229&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Means with different letters indicate significant differences within each treatment group in serum or whole blood (P < 0.05).

Table 4.4. Other kinetic parameters in serum and whole blood of sheep dosed with differing amounts of selenium, mean ± 1 SD: time to peak Se concentration (t max) (h), peak Se concentration (C max) (ppm), area under the curve (AUC) (µg-h/L).

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>t max (mean ± 1 SD)*</th>
<th>C max (mean ± 1 SD)*</th>
<th>AUC (0-168 h) (mean ± 1 SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenite</td>
<td>26.1 ± 4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.527 ± 0.246&lt;sup&gt;a&lt;/sup&gt;</td>
<td>147.664 ± 36.807&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 mg plant-Se/kg</td>
<td>25.7 ± 9.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.802 ± 0.197&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.907 ± 13.378&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 mg plant-Se/kg</td>
<td>14.4 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.828 ± 0.421&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>136.920 ± 35.853&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 mg plant-Se/kg</td>
<td>15.3 ± 7.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.360 ± 0.487&lt;sup&gt;c&lt;/sup&gt;</td>
<td>184.303 ± 105.137&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Means with different letters indicate significant differences within each group in serum or whole blood (P < 0.05).
Figure 4.1. Mean serum selenium (Se) concentrations of negative control sheep and ones dosed with selenite at 4 mg Se/kg body weight (BW) and selenomethionine (Se-Met) at 8 mg Se/kg BW.
Figure 4.2. Mean serum Se concentrations of negative control sheep and ones dosed with plant Se at 2, 4, 6, or 8 mg Se/kg BW.
Figure 4.3. Mean whole blood Se concentrations of negative control sheep and ones dosed with selenite at 4 mg Se/kg BW and Se-Met at 8 mg Se/kg BW.
Figure 4.4. Mean whole blood Se concentrations of negative control sheep and ones dosed with plant Se at 2, 4, 6, or 8 mg Se/kg BW.
CHAPTER 5

RESPIRATORY ELIMINATION OF SELENIUM IN SHEEP GIVEN THE ACCUMULATOR PLANT SYMPHYOTRICUM SPATHULATUM (WESTERN MOUNTAIN ASTER)¹

Abstract

Selenium (Se) is a necessary mineral required by mammals and poultry. If toxic amounts are ingested, expired air becomes a potentially important, but poorly investigated, route of elimination. A study was performed to evaluate respiratory toxicokinetics of Se in sheep. Sheep were gavaged with the accumulator plant Symphyotrichum spathulatum at Se equivalent doses of 0, 2, 4, 6, or 8 mg/kg BW. As positive controls an additional two sheep were gavaged with purified sodium selenite at 4 mg Se/kg BW and two sheep were gavaged with purified selenomethionine (Se-Met) at 8 mg Se/kg BW. Expired air samples were collected prior to dosing and at 1, 2, 4, and 8 h post dosing. Samples were collected from both sheep in the control, selenite and Se-Met groups and from 4 sheep in each of the plant-Se treatment groups. The post-dose air Se concentrations of the Se-Met group were statistically higher (P < 0.05) than all other groups at 1, 2, and 4 h. The selenite, 2 and 4 mg plant-Se/kg BW groups all had peak concentrations at the 2 h collection time. The 8 mg plant-Se/kg BW group showed a linear increase in respiratory Se concentration through 8 h. The 6 mg plant-Se/kg BW group peaked at 1 h, then dropped and peaked again at 4 h and finally dropped between 4

¹ This data was presented as a poster at the Western Section American Society of Animal Science meeting in Moscow, ID in June 2007.
and 8 h. At 8 h, the 8 mg plant-Se/kg BW group was significantly higher than the pre-dose collections of all groups and all collections of the control group \((P < 0.05)\). The elimination profile for Se-Met was dissimilar to any of the other treatments, with concentrations ranging from about 3.7 to 30 times more Se than other treatments at the highest Se dose at all time points. The 4 mg selenite and 4 mg plant-Se groups had similar elimination profiles. The total dose of the plant-Se appreciably altered the elimination profile. These findings indicate that both dose and chemical form of Se affect respiratory elimination kinetics.

**Introduction**

The trace mineral selenium (Se) is essential to mammals and poultry. However there is a narrow margin of safety between deficiency and toxicosis when supplementing Se. Generally 0.3 parts per million (ppm) Se is recommended in the diets of domestic livestock.\(^{10}\) Several cases of accidental Se overdose have been documented by Gabbedy and Dickson, \(^{6}\) Lambourne and Mason, \(^{13}\) Janke, \(^{8}\) and Kyle and Allen.\(^{12}\) Acute poisoning with Se results in vomiting (in monogastrics), dyspnea and death.\(^{11}\) Lesions are often seen affecting the liver, lungs and myocardium.

Certain species of plants can accumulate large amounts of Se and are referred to as Se-accumulator plants.\(^{18}\) Accumulator plants such as *Astragalus*, *Aster*, *Symphyotrichum*, and others can contain several hundred to several thousand ppm Se. If ingested, these plants cause Se poisoning in livestock. Over 500 sheep deaths were documented over a 4 year period due to ingestion of plants and water containing large amounts of Se in southeastern Idaho on reclaimed phosphate mining sites.\(^{3}\)
After entering the body, Se compounds are converted to hydrogen selenide, which is either utilized by the body or methylated to form excretion products.\(^4\) The first methylation step gives rise to methylselenol that is again methylated to form dimethyl selenide. This can be further methylated to trimethylselenonium ion.

Trimethylselenonium ion is the most common metabolite excreted in the urine.\(^2\) After large amounts of Se have been ingested, the methylation reaction of dimethyl selenide to trimethylselenonium ion becomes overloaded because this is the rate-limiting step.\(^{17}\) Dimethyl selenide is then excreted through respiration. Jiang et al.\(^9\) observed dimethyl selenide to be the major compound excreted in expired air following selenite exposure and dimethyl diselenide to be the major compound excreted in expired air following selenomethionine (Se-Met) exposure. The objective of this study was to evaluate the respiratory elimination kinetics of Se from the accumulator plant, *Symphyotrichum spathulatum* (Western Mountain Aster), in sheep and compare it to that of Se-Met and selenite.

Materials and Methods

Animals

Twenty-two sheep ranging in weight from 23.6 to 29.0 kg were group housed with *ad lib* access to alfalfa hay and water. Prior testing of the hay indicated background Se content of between 0.11 and 0.13 ppm. The sheep were allowed to acclimate for 2 weeks prior to the study. This study was approved by the Institutional Animal Care and Use Committee of Utah State University.
Dosing Material

Western Mountain Aster (*Symphyotrichum spathulatum*) was collected from an area of southeastern Idaho. A plant specimen is found in the Intermountain Herbarium at Utah State University in Logan, Utah under the accession number 246175. Purified sodium selenite compound was purchased from United States Biochemical Corporation, Cleveland, Ohio. Purified Se-Met compound was purchased from Sigma-Aldrich, St. Louis, Missouri.

Treatment

The sheep were randomly assigned to the following study groups: control, sodium selenite, Se-Met and 2, 4, 6, or 8 mg plant-Se/kg BW. Two animals were assigned to each of the control, selenite and Se-Met groups. Four animals were assigned to each level of plant treatment. The selenite group received 4 mg Se/kg BW as purified sodium selenite. The Se-Met group received 8 mg Se/kg BW as purified Se-Met. These two groups served as positive controls in the study, as they allowed comparison back to previous work performed in this laboratory. The treatments were prepared for each individual animal and administered through an intraruminal gavage tube, followed by a flush of water to make certain each animal received the full assigned dose.

Air samples were collected as described previously by Tiwary *et al.*\(^{17}\) and as shown in Figure 5.1. Air was collected into 3 L tedlar bags (SKC Inc., Eighty Four, PA). Following collection, air was pumped across a labeled charcoal filter (SKC Inc., Eighty Four, PA) using a Gilian pump (Sensidyne Inc., Clearwater, FL) set at 1 L/min. The pump was allowed to run for 2 min to allow 2 L of air to cross the filter. Respired air was
collected prior to dosing and at 1, 2, 4, and 8 h post dosing from all animals. All charcoal filters were capped tightly and stored at room temperature prior to extraction for analysis.

Tiwary et al.\textsuperscript{17} found a 50:50 ratio of ethanol to water to be the best extraction solvent for the charcoal trapped Se. They found the highest concentration of Se (99\%) was extracted when using 3 ml of solvent in each of two sequential extraction procedures. We performed two extractions using 3 ml of 50:50 ethanol to water on the primary charcoal collection chambers for all samples.

Each charcoal filter contains two compartments of charcoal and each compartment was analyzed separately. The end of the charcoal tube was broken and the charcoal from each compartment was placed into separate, labeled 15 ml polypropylene, metal-free tubes (CPI International, Santa Rosa, CA). Three milliliters of the ethanol:water solvent was added to each tube and the tubes were capped tightly. The tubes were rotated on a shaker for 2 h at 200 rpm. Following rotation, the tubes were centrifuged for 10 min at 500 x g. One milliliter of supernatant was removed to another labeled 15 ml metal-free tube containing 8.5 ml of ultra-pure water and 0.5 ml of trace mineral grade nitric acid and served as the analytical matrix. The charcoal from the primary compartment was then blotted dry and returned to the original tube. A second extraction was performed identically to the first extraction on the primary compartment of all samples. Only one extraction was performed on the secondary compartment because it is used as a breakthrough compartment. Initial extractions from the secondary compartment for all samples contained no detectable Se. Standards were prepared in the same matrix. Samples and standards were analyzed by Inductively Coupled Plasma-Mass Spectrometry on an ELAN 6000 (PerkinElmer, Shelton, CT).
Statistical Methods

Means and Mixed procedures of SAS (SAS Inst., Inc., Cary, NC) were used to evaluate data as a repeated measures experiment. A setting of $P < 0.05$ was used to establish significance. The Means function was used to separate significance versus insignificance between the treatment groups.

Results and Discussion

Prior to dosing, no group means for the quantitative results were significantly different from each other. All samples were below the detection limit of 0.010 μg/L prior to dosing.

The mean Se-Met respiratory elimination at 1, 2, and 4 h post-dosing was significantly different from all other groups at all time points post-dosing ($P < 0.05$). At 1, 2, and 4 h post dosing, the Se concentration of the Se-Met group ranged from 13 to 30 times more Se than the 6 mg plant-Se/kg BW group, which had the next highest concentration of Se at those time points (Table 5.1). At 8 h, the Se concentration of the Se-Met group was about 3.7 times greater than the 8 mg plant-Se/kg BW treatment group, which had the next highest concentration of Se at that time point. Following statistical evaluation comparing all treatment groups, statistical evaluation was performed again without including the Se-Met group because the Se-Met group was so much larger than all other treatments it skewed the data and prevented an accurate statistical comparison of the Se concentrations expired by the other treatment groups. The following data reflect the statistical results of the evaluation without Se-Met. The 8 mg plant-Se/kg BW group at 8 h was significantly greater than the pre-dose collections in all
groups ($P < 0.05$). Also at 8 h, the 8 mg plant-Se/kg BW group was significantly greater than the 2 mg plant-Se/kg BW group at 8 h ($P = 0.0283$) and significantly greater than the control group at all collection time points ($P < 0.05$). The pre-dose collections from the plant-treated groups were all significantly lower than the 4 mg plant-Se/kg BW group at 2 h ($P < 0.05$), the 6 mg plant-Se/kg BW group at 1 h ($P < 0.05$) and the 6 mg plant-Se/kg BW group at 4 h ($P < 0.01$).

The Se-Met group elimination peaked at 1 h and gradually decreased through 8 h (Figure 5.2). This agrees with Hirooka and Galambos$^7$ and McConnell and Roth$^{14}$ who found the majority of Se exhaled in 24 h following administration occurred during the first 6 h.

The selenite group peaked at 2 h and decreased drastically between 2 and 4 h (Figure 5.3). The elimination curve then stayed consistent from 4 to 8 h. The mean of the 2 and 4 mg plant-Se/kg BW groups peaked at 2 h and then gradually decreased (Figure 5.3). The peak of the 4 mg plant-Se/kg BW group (0.178 ug Se/L air) was slightly higher than the peak of the 2 mg plant-Se/kg BW group (0.092 ug Se/L air). Hirooka and Galambos$^7$ observed an increase in exhaled Se with each increase in dose administered as selenate.

The 6 mg plant-Se/kg BW group peaked at 1 h, then dropped and peaked again at 4 h before a final decrease through 8 h (Figure 5.3). The four animals in the 6 mg plant-Se/kg BW group eliminated Se differently, which led to the two peaks in the mean of the group. One animal peaked at 1 h with 0.405 µg Se/L air. Two other animals reached peak respiratory elimination at 4 h. The final animal peaked at 1 h, dropped slightly at 2 h and peaked again at 4 h. The 1 h peak of this animal was 0.173 µg Se/L air, the 4 h
peak was 0.206 μg Se/L air with a drop at 2 h to 0.111 μg Se/L air. The differences in each of the individual animals explain the two peaks in the curve of the 6 mg plant-Se/kg BW group. The first animal that peaked at 1 h had a much higher concentration than all other animals at that time point causing the mean curve to peak at 1 h. The other animals peaked at 4 h causing the mean curve to peak again at that time. As the chemical forms of Se were not identified in the plant material, kinetic differences in differing chemical forms within the plant may have contributed to this as well, but one would have expected other dosing groups to exhibit similarities.

The 8 mg plant-Se/kg BW group increased linearly through 8 h (Figure 5.3). One of the animals in the 8 mg plant-Se/kg BW group reached a plateau at 4 h but the respiratory elimination did not drop prior to the 8 h collection. Two of the animals increased most rapidly through 2 h then only gradually increased through 8 h. The final animal saw a drastic increase in respiratory Se elimination from 0.084 μg Se/L air at 4 h to 0.309 μg Se/L air at 8 h. This animal caused the mean curve to appear to increase linearly even though one animal reached plateau at 4 h and two of the animals only increased slightly through 8 h.

The animals receiving Se-Met were given the same dose of Se as the highest plant Se group of 8 mg Se/kg BW. Until recently, Se-Met is believed to be the most common chemical form of Se found in many plants and is still believed to be a predominant form found in non-accumulator plants. Recent research shows that a predominant chemical form in accumulator plants is Se-methylselenocysteine. The vast difference in the amount eliminated between the plant and Se-Met groups indicates this particular plant
contained Se in a different chemical form. Another possible explanation is that the Se-Met in plants is not as readily bioavailable to sheep as the purified compound of Se-Met.

A similar study performed by Tiwary et al.\textsuperscript{17} observed the effects of varying amounts of purified sodium selenite and Se-Met on respiratory elimination in sheep. Tiwary et al.\textsuperscript{17} reported the concentration of Se per 2 L expired air whereas this data is listed in 1 L expired air. The data from this study was doubled to make the following comparisons to that of the previous study. At the same dose (4 mg Se/kg BW) and times (4 and 8 h), the selenite group reported here had only about 17% and 19% of the respired Se as that reported previously. The Se-Met group at the same dose (8 mg Se/kg BW) had about 62% of the content found by Tiwary et al.\textsuperscript{17} at 4 h and about 23% at 8 h. This could be due to differences in the amount of Se in these sheep prior to dosing. It has been reported that animals can tolerate high doses of Se better if the Se status in the body prior to dosing satisfies the bodies need rather than is deficient.\textsuperscript{18} The prior Se status of our sheep was on the deficient side.

The respiratory elimination of Se in sheep from the plant, \textit{Symphyotrichum spathulatum,} is dependent upon dose. The highest dose of 8 mg plant-Se/kg BW caused Se to be increasingly eliminated up through 8 h and likely beyond. The lower doses caused a major elimination of Se in expired air at the early collection times, followed by a decrease in the amount eliminated.

Se has a distinct odor when eliminated in expired air.\textsuperscript{18} Faint odor was observed in all plant treatment groups, but occurred most often in the 6 and 8 mg plant-Se groups between 2 and 4 h. The strongest odor observed occurred in the Se-Met group. Tiwary et al.\textsuperscript{17} recorded ‘Strong’ odor in the 4 mg Se-Met group at all time points. Surprisingly,
in the present study, the odor of the 8 mg Se-Met group was recorded as ‘Obvious,’ but not ‘Very Strong.’

Overall Se eliminated in expired air was a very small amount of the total administered dose. A typical 30 kg sheep would be expected to expire approximately 180 L air/hour at 25 breaths/minute and a tidal volume of 4 ml/kg BW.\textsuperscript{1} The largest animal (29 kg) was treated with 6 mg plant-Se/kg BW. The total dose this animal received was 174.0 mg Se. This animal peaked at 4 h with 0.206 \( \mu \text{g} \) Se eliminated at that point in 1 L of expired air. Thus, even if one used the peak elimination, this sheep would have only eliminated 0.15 mg in the first 4 hours. This is a very small percentage of the total dose. Respiratory Se elimination does occur following ingestion of this plant; however, only a small amount of the dosed Se is eliminated in expired air through the observed 8 h. Thus, respiratory kinetics is a very minor elimination pathway. This was also observed by Ohta \textit{et al.}\textsuperscript{15} who measured selenium elimination in both urine and expired air from rats. The rats were given an oral mixture of three labeled compounds of selenium: Se-Met, methylselenocysteine and methylselenic acid. The percentage of selenium eliminated as dimethylselenide was less than 0.01\% for all three selenium compounds.

Plants high in Se content can also produce and release volatile Se compounds into the environment.\textsuperscript{16,20} As was noted on the breath of the sheep in our study, there was also a unique garlic-like odor in the area where this plant was collected. If this odor is present in particular areas, these areas should be avoided for grazing of livestock. The presence of this odor both in the environment and on the breath of affected animals can help in the diagnosis of Se poisoning. Clinically this odor could be important because the
recognition of it could lead to faster treatment and removal of animals from affected areas which could reduce mortality.

Conclusions

All groups appear to peak and decrease during the observed 8 h except the 8 mg plant-Se/kg BW group. More research should be done to evaluate the respiratory elimination of 8 mg plant-Se/kg BW beyond 8 h to see when respiratory elimination peaks and when elimination drops following administration of this particular plant.

From this study, we know the elimination of Se through expired air does occur following ingestion of Se in sheep. We also know that the amount of Se ingested has an effect on the amount eliminated through expired air, as the amount eliminated generally increased with the amount dosed. However, although it does occur, we observed that only a small amount of the total dose was eliminated through expired air indicating that this is a minor Se elimination pathway.

References


Table 5.1. Mean selenium (Se) concentration (μg) ± 1 standard deviation (SD) from 1 L of expired air at each collection time point.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>0 hours (mean ± 1 SD)*</th>
<th>1 hour (mean ± 1 SD)*</th>
<th>2 hours (mean ± 1 SD)*</th>
<th>4 hours (mean ± 1 SD)*</th>
<th>8 hours (mean ± 1 SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.000 ± 0.000&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.001 ± 0.001&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.001 ± 0.001&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.000 ± 0.000&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.001 ± 0.001&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Selenite</td>
<td>0.001 ± 0.001&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.107 ± 0.031&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.142 ± 0.050&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.019 ± 0.010&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.016 ± 0.003&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Se-Met†</td>
<td>0.000 ± 0.000&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.844 ± 0.407&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.496 ± 0.982&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.694 ± 1.510&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.809 ± 0.417&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 mg plant-Se/kg</td>
<td>0.000 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.048 ± 0.022&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.092 ± 0.033&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.066 ± 0.019&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.033 ± 0.010&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 mg plant-Se/kg</td>
<td>0.000 ± 0.000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.098 ± 0.143&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.178 ± 0.122&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.081 ± 0.035&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.065 ± 0.013&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 mg plant-Se/kg</td>
<td>0.000 ± 0.000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.194 ± 0.148&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.158 ± 0.044&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.203 ± 0.099&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.067 ± 0.056&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>8 mg plant-Se/kg</td>
<td>0.000 ± 0.000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.056 ± 0.032&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.092 ± 0.046&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.139 ± 0.073&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.215 ± 0.082&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Means with different letters indicate statistically significant differences (P < 0.05).
†Se-Met, selenomethionine.
Figure 5.1. Air collection apparatus showing funnel around sheep muzzle attached to nonrebreathing tee, which is attached to 3 L tedlar bag.
Figure 5.2. Mean respiratory elimination of selenium (Se) in 1 L expired air of negative control sheep and ones dosed with selenite at 4 mg Se/kg body weight (BW), selenomethionine (Se-Met) at 8 mg Se/kg BW and plant material at 2, 4, 6, or 8 mg Se/kg BW.
Figure 5.3. Mean respiratory elimination of Se in 1 L expired air of negative control sheep and ones dosed with selenite at 4 mg Se/kg BW and plant material at 2, 4, 6, or 8 mg Se/kg BW.
The data collected from this study showed surprising and unexpected results. Based on previously reported data, we expected to see more pulmonary lesions such as pulmonary edema. Although this was observed, the majority of the gross and histopathologic lesions were myocardial. Rather than observing many cases of tachypnea and dyspnea, a majority of animals appeared relatively healthy until just prior to death. Several died after minor stress or movement, likely due to the observed myocardial damage caused by the selenium found in this plant, which led to reduced pumping capacity of the heart such that it could no longer function at its full potential.

We found that expired air played a fairly small role in the elimination of selenium from the body of these sheep. We saw that the elimination of selenium from both whole blood and serum occurred faster in the higher dose groups, likely due to the body’s effort to rid itself of selenium at toxic concentrations, thus avoiding some of the damage and even death seen in the highest dosed sheep.

This study brought about new questions that need answering with future research. First, we do not know what chemical form or forms of selenium are found in this particular accumulator plant. Once the form(s) have been determined, this data could be better compared to data of the most similar purified chemical form of selenium. Another question that arises is how other species of mammals respond to this accumulator plant in similar dosing studies.

The plant studied is only one of a variety of selenium accumulating plants. Other accumulator plants could have different combinations of selenium chemical forms. Each
of these plants could possibly elicit different responses in sheep at the same selenium doses given in this study.

Another question arising, specifically from the respiratory portion of the data collected here, is how the respiratory elimination curve would have looked with collections beyond 8 hours. All treatments showed a decrease in respiratory selenium elimination by 8 hours except the highest plant dose group, which showed a continual increase through that time. In future research of respiratory selenium elimination, it would be wise to include expired air collections beyond 8 hours.

Other potential for future research could include dosing sheep with this plant for long periods of time rather than just a single oral dose. Since the response to an acute dose of this plant in sheep was different to that seen in other studies with purified chemicals, it is likely the results of a chronic feeding study would also be different from what is already documented for purified selenium compounds.