Evaluation of the respiratory elimination kinetics of selenium after oral administration in sheep

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Objective—To evaluate the respiratory excretion and elimination kinetics of organic and inorganic selenium after oral administration in sheep.

Animals—38 crossbred sheep.

Procedures—Selenium was administered PO to sheep as a single dose of 0, 1, 2, 3, or 4 mg/kg as sodium selenite or selenomethionine. Expired air was collected and analyzed from all sheep at 4, 8, and 16 hours after administration.

Results—Clinical signs consistent with selenium intoxication were seen in treatment groups given sodium selenite but not in treatment groups given the equivalent amount of selenium as selenomethionine. However, a distinct garlic-like odor was evident in the breath of all sheep receiving 2 to 4 mg of selenium/kg. The intensity of odor in the breath did not correlate with clinical signs in affected animals receiving sodium selenite treatment.

Conclusions and Clinical Relevance—The concentration of selenium in expired air was greater in sheep receiving selenium as selenomethionine than sodium selenite. The concentration of selenium in expired air from sheep receiving high doses of selenium (3 and 4 mg of selenium/kg) was larger and selenium was expired for a longer duration than the concentration of selenium in expired air from sheep receiving low doses of selenium (1 and 2 mg of selenium/kg). (Am J Vet Res 2005;66:2142–2148)
Se 75 radioisotopes. Such studies are expensive and impractical in field conditions. We focused on developing a simpler method for collection, analysis, and quantitation of selenium in expired air and correlating results of this method with clinical signs of toxicosis. Although not reported in animals, the intensity of odor has been proposed as a qualitative indicator of selenium content in plants. The purpose of the study reported here was to evaluate the respiratory excretion and elimination kinetics of organic and inorganic selenium after oral administration in sheep.

Reviews regarding the metabolism and elimination of selenium in expired air have been published. After exposure, sodium selenite is converted to hydrogen selenide (H₂Se) in the liver and RBCs, in which reduced glutathione is readily available. In the liver, H₂Se is methylated to monomethyl selenide or methyl selenol and then to dimethyl selenide (DMSe) by S-adenosyl methionine-dependent methylation reactions, involving thiol-S-methyl transferase. Dimethyl selenide is a precursor, which is further methylated to form TMSe⁺, and requires thioether-S-methyl transferase that is present in the liver and lungs. The conversion from DMSe to TMSe⁺ is considered the ratelimiting step; therefore, selenium in urine, primarily as TMSe⁺, remains constant, whereas DMSe in expired air increases with dose. Formation of TMSe⁺ in the liver of an animal given SeMet occurs earlier than that in animals given selenite or selenocysteine (SeCys). This may be because SeMet can be directly catabolized to produce methyl selenol that is methylated to DMSe and then to TMSe⁺. Selenomethionine can also undergo conversion to SeCys by the trans-sulfuration pathway. Following this, SeCys-γ-lase in the liver can produce H₂Se from the SeCys, which can follow the subsequent methylation steps.

Information concerning excretion of the forms of volatile selenium that are responsible for the characteristic odor is not known. Dimethyl selenide and dimethyl diselenide (DMDSe) are the primary volatile selenium compounds in expired air of rats, although some other unidentified compounds may also be involved. Activated charcoal has been successfully used to trap volatile selenium compounds from soil and plants. This adsorptive property of activated charcoal was used as the basic framework of trapping the expired selenium in the study reported here.

### Materials and Methods

Sheep and administration of selenium—Thirty-eight 8- to 12-week-old crossbred sheep (19 males and 19 females) were acclimated to the local conditions for 1 month prior to the study. During the entire acclimatization period and the study phase, sheep had access to water and long-stem alfalfa-grass hay ad libitum. All sheep appeared healthy and had access to a trace mineral block that did not contain selenium. This study was approved by the Institutional Animal Care and Use Committee, Utah State University.

One day prior to the beginning of the study, sheep were randomly allocated to one of 10 treatment groups; each treatment group contained 4 sheep, except for group 5, which contained 2 sheep. Each group was placed into a separate pen. Sheep in each treatment group received 0, 1, 2, 3 or 4 mg of selenium/kg as either sodium selenite or SeMet (Table 1). The 2 control groups were treated either with 10 mL of water (group 1) or an amount of methionine equivalent to the highest selenomethionine treatment (group 6). Bacteriologic-grade sodium selenite and seleno-DL-methionine were used to prepare all doses of selenium. All sheep were weighed the evening prior to treatment. Individual doses were prepared, dissolved in approximately 10 mL of water, and administered intraruminally via an intragastric tube. The tube was flushed with water to ensure no residue remained in the tube. Neither sodium selenite nor SeMet had a detectable garlic-like odor when administered to sheep.

#### Qualitative expired air assessment

Four, 8, and 16 hours after treatment, expired air samples were collected from all sheep. In addition, the breath of each sheep was evaluated for a garlic-like odor and scored as 0, 1, 2, or 3 for no odor, faint odor, readily detectable odor, and strong odor, respectively. Evaluations were performed by 3 individuals who were unaware of treatment group allocations. A rebreathing apparatus was made by use of a plastic funnel, polyvinyl tubes of various sizes, a rubber inner tube, and a nonrebreathing tee having two 1-way valves in opposing directions (Figure 1). The joints were sealed with parafilm tape. This apparatus was used to collect expired air into the air sampling bags. Rubber tubing was lined with polyethylene because DMSe is known to adsorb to rubber and to avoid contamination of the sample. For air sampling, 3-L capacity bags that had a metal eyetlet, a polypropylene hose with valve, and a septum fitting were used. For sampling, the mask was gently placed over the muzzle of the sheep and secured on the sides to avoid leakage. After bags were filled, the valves were closed and kept for further processing.

Activated charcoal tubes (8-mm outer diameter and 110 mm long) having 400 and 200 mg of sorbent in compartments I and II, respectively, were used. After collection of expired air in the air sampling bags, the hoses of the bags were attached to the compartment I end of the charcoal tube by use of polyvinyl tubing. After opening the valves on the air bags, vacuum was applied from the compartment II end of the charcoal tube by use of a pump. This permitted uniform flow of collected air samples from the air sampling bags through compartments I and II of the activated charcoal tubes. The flow rate of the pump was set at a constant rate of 1 L/min. The pump was switched off after exactly 2 minutes; therefore, 2 L was the total volume of expired air passed through the charcoal tube. The charcoal tubes were capped thereafter and stored at 22°C for 6 weeks before being analyzed.

### Table 1—Dose and type of selenium administered to sheep in various treatment groups.

<table>
<thead>
<tr>
<th>Treatment group*</th>
<th>Dose of selenium (mg/kg)</th>
<th>Amount and type of dosing compound†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>0</td>
<td>10 mL of water (vehicle only)</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>2.19 mg of NaSe/kg</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>4.38 mg of NaSe/kg</td>
</tr>
<tr>
<td>4</td>
<td>3.0</td>
<td>6.57 mg of NaSe/kg</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>8.76 mg of NaSe/kg</td>
</tr>
<tr>
<td>6 (control)</td>
<td>0</td>
<td>8.93 mg of Met/kg</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>2.48 mg of SeMet/kg</td>
</tr>
<tr>
<td>8</td>
<td>2.0</td>
<td>4.97 mg of SeMet/kg</td>
</tr>
<tr>
<td>9</td>
<td>3.0</td>
<td>7.45 mg of SeMet/kg</td>
</tr>
<tr>
<td>10</td>
<td>4.0</td>
<td>9.93 mg of SeMet/kg</td>
</tr>
</tbody>
</table>

* n = 4 for each group except group 5 for which n = 2. All doses were suspended in 10 mL of water just prior to intraruminal administration via intragastric intubation.

NaSe = Sodium selenite. SeMet = Selenomethionine. Met = Methionine.
Method development for extraction and analysis—To detect the absorption, breakthrough, and percentage extraction of selenium from the charcoal, tests were performed in triplicate, by use of extra air samples obtained from sheep in the highest selenium treatment groups (groups 3 and 10) and with a breath score of 3 (strong odor). The activated charcoal tubes were broken at the ends, and charcoal from compartments I and III were extracted and analyzed separately. Absolute ethanol, a 50:50 ratio of ethanol and water, and 5% nitric acid were tested as solvents for extraction of selenium from the activated charcoal. Test solvents (2, 3, or 4 mL) were added to polypropylene, metal-free tubes containing activated charcoal. Tubes were capped and placed on a rotary shaker (200 rpm) for 2 hours. Tubes were then centrifuged at 500 g for 10 minutes. One milliliter of supernatant was added to another tube containing 8.5 mL of 18.3 mega ohm water and 0.5 mL of trace metal–grade distilled water.

Samples were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) at the atomic mass of 78 and 82. Selenium standards were prepared in the same matrix, with standard curves and quality-control samples tested after every fifth sample. A second then third extract was similarly prepared after complete separation of the charcoal from the remaining first or second extract and blotting it dry. Sensitivity of the ICP-MS analysis was 1 ng/mL or 10 ng/extract.

Purified DMSe and DMDSe were obtained. Various concentrations of DMSe and DMDSe were prepared in a solution containing 5% nitric acid and 5% ethanol, the analyte matrix for ICP-MS, and were tested for loss of selenium attributable to volatilization. No loss of selenium was detected after tubes were kept open for 4 hours, which represented the amount of time required for batch analysis for the analytical runs. The maximum lag time between preparation of a sample and analysis was 20 hours. Results of quality-control testing indicated that leaving the capped tubes standing for 20 hours did not affect the selenium content.

Dimethyl selenide and DMDSe in 50% ethanol were added to activated charcoal to determine the adherance of these volatile selenium species to activated charcoal. Although most all of the DMSe was subsequently extracted, DMDSe was not totally extracted, indicating a stronger binding affinity of DMDSe to activated charcoal than DMSe. However, 2 extractions resulted in recovery of >99% of DMSe and >80% of DMDSe. The DMSes and DMDSe were also used to prepare standards for detection of these compounds by gas chromatography with a flame ionization detector. However, the low concentration of total selenium in the samples negated our attempts to speciate the volatile forms of selenium in the breath of sheep. Efforts by use of solid phase extraction of C-18 columns were not successful.

Statistical analysis—Statistical analyses were performed by use of computer software. Post hoc analysis was performed by means of the least significant difference (LSD test). The Student t test (α = 0.05) was applied to evaluate significant differences among the mean selenium concentrations at 4, 8, and 16 hours after treatment in sheep given various doses and forms of selenium.

Results

Sheep receiving 2, 3, and 4 mg of sodium selenite/kg had signs of depression, anorexia, tachypnea, and labored breathing. When forced to walk a few steps, sheep stood with their heads down and necks extended, taking short, rapid, shallow breaths. The onset of clinical signs was observed as early as 10 to 12 hours after administration of selenium. Severity of clinical signs and time to recovery varied but were dose dependent. None of the affected sheep became recu-
bent, and all sheep recovered within 1 to 2 days without necessitating treatment. Sheep receiving SeMet did not have noticeable clinical signs.

Breath of sheep had a distinct garlic-like odor within hours after oral administration of selenium compounds, especially in sheep receiving 3 and 4 mg of selenium/kg. The characteristic smell was detected before 4 hours, but sampling was not performed earlier than 4 hours after selenium administration. In general, the odor was strong at 4 and 8 hours but mild by 16 hours after administration of selenium (Table 2).

Total selenium content from the 2 L of expired air was calculated for each sheep at 4, 8, and 16 hours after selenium administration. The amount of selenium in expired air was highest in air samples obtained at 4 hours, followed by air samples obtained at 8 and 16 hours, and was also dose dependent. For the same amount of selenium administered, administration of SeMet resulted in approximately 1.5- to 3-fold higher selenium content in expired air at 4 hours, compared with sodium selenite. Mean total selenium in 2 L of expired air from sheep in the control groups was less than the instrument’s limit of detection.

Administration of sodium selenite resulted in significant increases in total selenium concentration in 2 L of expired air at each of the measured times. At 4 hours, mean selenium content from 2 L of expired air from sheep in treatment groups given 2, 3, and 4 mg of selenium/kg as sodium selenite (0.152 ± 0.098 µg, 0.153 ± 0.098 µg, and 0.226 ± 0.090 µg, respectively) was significantly higher than that in the control group in which no selenium was detected at a method detection limit of 0.01 µg (Figure 2). At 8 hours, mean selenium content from 2 L of expired air from sheep in treatment groups given 3 and 4 µg of selenium/kg as sodium selenite (0.083 ± 0.048 mg and 0.167 ± 0.068 µg, respectively) was significantly higher than that in the control group.

At 16 hours, mean selenium content from 2 L of expired air from sheep in the treatment group given 4 mg of selenium/kg as sodium selenite (0.126 ± 0.075 µg) was significantly higher than that in the control group. The selenium content in expired air samples reflected a dose-dependent increase. Similar effects were observed in sheep receiving SeMet. At 4 hours, mean selenium content from 2 L of expired air from sheep in treatment groups given 2, 3, and 4 µg of selenium/kg as SeMet (0.267 ± 0.139 µg, 0.556 ± 0.227 µg, and 0.465 ± 0.313 µg, respectively) was significantly higher than that in the control group (Figure 3). At 8 hours, mean selenium content from 2 L of expired air from sheep in treatment groups given 3 and 4 mg of selenium/kg as SeMet (0.204 ± 0.091 µg and 0.148 ± 0.151 µg) was significantly higher than that in the control group. At 16 hours, mean selenium content from 2 L of expired air from sheep in treatment groups given 2, 3, and 4 µg of sele-

![Figure 2](image1.png)

**Figure 2**—Mean ± SD total Se content in 2 L of expired air collected from sheep receiving Se (0 to 4 mg/kg, PO) as sodium selenite. The Se content in expired air from all sheep in the control group (Con) was < 0.01 µg (limit of detection). For each sampling time, different letters indicate a significant (P < 0.05) difference among treatment groups.

![Figure 3](image2.png)

**Figure 3**—Mean ± SD total Se content in 2 L of expired air collected from sheep receiving Se (0 to 4 mg/kg, PO) as selenomethionine. The Se content in expired air from all sheep in the control group was < 0.01 µg (limit of detection). See Figure 2 for key.

Table 3—Mathematical equations corresponding to nonlinear regression and correlation coefficients (R²) for selenium elimination in sheep given selenium (1 to 4 mg/kg, PO) as NaSe or SeMet.

<table>
<thead>
<tr>
<th>Dose of selenium (mg/kg)</th>
<th>NaSe Elimination equation</th>
<th>R²</th>
<th>SeMet Elimination equation</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>y = 0.06x⁻¹²</td>
<td>0.8695</td>
<td>y = 0.07x⁻¹³</td>
<td>0.9941</td>
</tr>
<tr>
<td>2</td>
<td>y = 0.15x⁻¹³</td>
<td>0.9953</td>
<td>y = 0.28x⁻¹⁴</td>
<td>0.9917</td>
</tr>
<tr>
<td>3</td>
<td>y = 0.17x⁻¹⁴</td>
<td>0.9118</td>
<td>y = 0.45x⁻¹⁵</td>
<td>0.9915</td>
</tr>
<tr>
<td>4</td>
<td>y = 0.23x⁻¹⁵</td>
<td>0.9633</td>
<td>y = 0.57x⁻¹⁶</td>
<td>0.9963</td>
</tr>
</tbody>
</table>

NA = Not applicable.
nium/kg as SeMet (0.056 ± 0.024 µg, 0.098 ± 0.050 µg, and 0.094 ± 0.062 µg, respectively) was significantly higher than that in the control group. The selenium content of expired air samples reflected a dose-dependent increase with the exception that the mean content for the group receiving 3 mg of selenium/kg as SeMet was higher than that for the group receiving 4 mg of selenium/kg as SeMet; however, this difference was not significant. The means of the total selenium concentration from the air samples were plotted against time for each of the treatment groups. The pattern obtained indicated a nonlinear regression in the selenium content versus time. The curve was best described by the mathematical equation, $y = ax^b$, with high coefficient of correlation values (Table 3).

Discussion
Ethanol has been used to extract selenium from activated charcoal.30,37 However, among the 3 solvents tested in the study reported here, a 50:50 ratio of ethanol and water was found to be the best solvent, with the highest concentration of selenium recovered after 2 extractions. Nitric acid had no desorptive property, and a significant amount of selenium was not detected in any of the extracts from either compartment of the activated charcoal tubes. Of the 3 volumes of solvents used to obtain information concerning the best recovery, the use of 3 mL of solvent resulted in a higher total concentration of selenium being obtained from activated charcoal than 2 mL of solvent. Use of 3 or 4 mL of solvent resulted in a similar total concentration of selenium being extracted; thus, 3 mL of solvent was used for all extraction procedures.

There was an absolute absence of selenium in all of the extracts of compartment II (breakthrough or secondary compartment) of the charcoal tubes. Selenium was not detected in any of the extracts by use of any of the 3 solvent types or volumes tested, indicating that there was no breakthrough and that all of the selenium, if any, was contained in compartment I, the primary compartment. Selenium contents of the third extracts were generally less than the limit of detection or <1% of the cumulative total amounts obtained from extracts 1 and 2, indicating that the combination of total selenium concentrations obtained from 2 extracts of the activated charcoal represented >99% recovery.

A garlic-like odor in the breath of sheep was detected as early as 1 hour after oral administration of selenium and persisted for as long as 16 hours, depending on the dose and type of selenium compound administered. The intensity of the odor was greater in sheep receiving SeMet than sodium selenite. Clinical signs of selenium toxicosis in sheep were apparent 10 to 12 hours after treatment. This observation corroborates results from other studies25,38 that further suggest that, unlike urine, selenium in expired air may not correlate with clinical signs or selenium concentrations in the blood or serum. The selenium concentration in expired air was associated with the metabolism that occurred in the liver after absorption and prior to being incorporated into the carrier selenoproteins.25,30

The total selenium concentration in expired air increased with dose. The peak concentration of selenium in expired air was detected 4 hours after treatment, with lower concentrations detected at 8 and 16 hours, respectively. Although this finding was consistent with the scoring of odor intensity in the breath of sheep receiving SeMet, sheep receiving sodium selenite appeared to have a higher score at the 8-hour sampling time, which may have been possible if the form of volatile selenium eliminated through respiration was different (less odorous) in the treatment groups given sodium selenite than in groups given SeMet. Dimethyl selenide was the predominant respiratory form in selenite or SeCys when administered to mice, whereas an unidentified compound and DMDSe were the main forms detected when SeMet was administered to mice.39 Results of a study in rats indicate a linear association between selenite exposure and the concentration of selenium in exhaled air after administration of low doses of selenite but a logarithmic pattern after administration of high doses of selenite, which appears to be similar to our results obtained in sheep in which a nonlinear power ($y = ax^b$) relationship was the best fit for the elimination curves. The variability may have been attributed to the low number of sheep in each treatment group (n = 4) and variations in rumen microflora that reduce and convert bioavailable selenium such as selenites, selenates, SeMet, and SeCys to unavailable forms such as selenides or elemental selenium. The effect of rate of respiration may also be an important factor because selenium toxicosis is known to alter the breathing pattern, therefore resulting in variations.

Among sheep receiving supplemental sodium selenite, those that expired 0.05 to 0.3 µg/2 L of expired air 4 hours after administration of selenium developed clinical signs. Interestingly, sheep receiving similar amounts of selenium as SeMet expired higher concentrations of selenium (0.15 to 0.8 µg/2 L of expired air) 4 hours after treatment than sheep receiving sodium selenite; however, clinical signs of toxicosis were not observed. Although the selenium concentration was dose dependent and highest 4 hours after treatment, the strength of the odor in expired air peaked at 8 hours in sheep receiving sodium selenite. At 16 hours, the total selenium concentration further decreased; however, the odor remained stronger than that detected 4 hours after treatment. In sheep receiving SeMet, the odor remained somewhat consistent at high doses. These findings support the potential for detecting multiple forms of selenium in expired air and, thus, the possibility of differing respiratory toxicosis with each form.

At doses higher than the minimum lethal dose, rats given sodium selenite exhaled 40% to 60% of the dose within a day.28 During the 24-hour respiratory elimination period, 70% to 90% of the total selenium administered was recovered within 6 hours.29,31 As the concentration of selenium, administered SC, was decreased, the percentage of selenium exhaled decreased rapidly.28 However, in rats in which selenium was administered orally as selenite, SeCys, or SeMet, SeMet caused the highest selenium concentrations in expired air.39 Results of that study appear to be in agreement with our observation, which suggests that
the excretion of volatile selenium is overall 1.5- to 3-fold higher in sheep receiving SeMet, compared with sheep receiving equal doses of selenite. The amount and route of administration, species (ruminant or non-ruminant), type of diet, and variations in methyltransferase activities among species are other important factors that should be considered.

Fractions of fecal and urinary selenium remained unchanged in rats in which the dose of selenium (as sodium selenate) was increased from 0.08 to 1.4 mg of selenium/kg. However, the selenium fraction in expired air was lower, whereas DMSe in expired air remained constant, whereas DMSe in expired air.38 Thiol-S-methyl transferases are inhibited by arsenite compounds and high amounts of SeCys; however, the thioether-S-methyl transferases remain unaffected.39 Because the primary clinical signs and lesions of selenium poisoning in sheep with selenium poisoning are associated with the respiratory system, it would be expected that the measured concentration of selenium in expired air would correlate with the severity of clinical signs and lesions. However, results of the study reported here disproved this hypothesis. In fact, the selenium content in expired air of sheep receiving SeMet was significantly greater than that in sheep receiving sodium selenite, but sheep receiving SeMet did not have clinical signs.

Because the primary clinical signs and lesions of sheep with selenium poisoning are associated with the respiratory system, it would be expected that the measured concentration of selenium in expired air would correlate with the severity of clinical signs and lesions. However, results of the study reported here disproved this hypothesis. In fact, the selenium content in expired air of sheep receiving SeMet was significantly greater than that in sheep receiving sodium selenite, but sheep receiving SeMet did not have clinical signs. The potential for multiple selenium metabolites in the excretion of volatile selenium is overall 1.5- to 3-fold higher in sheep receiving SeMet, compared with sheep receiving equal doses of selenite. The amount and route of administration, species (ruminant or non-ruminant), type of diet, and variations in methyltransferase activities among species are other important factors that should be considered.

References
Correction: Functional adaptation through changes in regional biochemical characteristics during maturation of equine superficial digital flexor tendons

In the report, “Functional adaptation through changes in regional biochemical characteristics during maturation of equine superficial digital flexor tendons” (AJVR, September 2005, pp 1623–1629), Figure 8 found on page 1626 should appear as follows:

Figure 8—Mean ± SD concentration of total collagen on a dry-weight basis, as a component of the collagen network, versus age in normal equine SDFT tissues from 2 differently loaded sites. See Figure 1 for remainder of key.