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RESEARCH PAPER

Detection of rare species of volatile organic selenium metabolites in male golden hamster urine

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Abstract Selenium has been considered as an essential trace element in mammals and its intake comes mainly from food. Mammals can metabolize both inorganic and organic species, and urinary excretion is the primary elimination route of selenium. Selenosugars and trimethylselenonium ion have been identified as major urinary metabolites. Other metabolites have been reported, but they were detected in some studies and not in others. Still, a large portion of the ingested selenium eliminated from the body is unknown. Volatile selenium species may account for a certain portion of the unknown species since they can easily be lost during sample analyses. While we analyzed male golden hamster urine in search of potential volatile pheromone(s), four volatile selenium compounds were detected. They were dimethyl selenenylsulfide, dimethyl diselenide, dimethyl bis(thio)selenide, and dimethyl selenodisulfide. When the urine samples were aged and dried for 48 h, dimethyl selenodisulfide tended to increase, while others decreased. The increase might be due to the formation of dimethyl selenodisulfide via reaction of dimethyl diselenide and dimethyl trisulfide whose concentration increased as urine aged. To our knowledge, dimethyl bis(thio)selenide and dimethyl selenodisulfide have never been demonstrated in urine. It remains to be determined whether these species are common metabolites in other animals or hamster-specific.

Keywords Volatile organic selenium species · Golden hamster · Urine · Gas chromatography–mass spectrometry · Solid-phase microextraction

Introduction

Selenium has been considered as an essential trace element for mammals, and its intake comes mostly from food. Selenium can function as an antioxidant and an anticarcinogenic agent [1]. Selenium deficiency has been linked to heart diseases, arthritis, cancer, AIDS, etc. [1, 2]. Selenomethionine (SeMet) has been used for dietary supplements in animals and humans [3].

Selenium has different oxidation states: selenide (Se^{2-}), selenite (SeO_3^{2-}), and selenate (SeO_4^{2-}) [4], and a variety of inorganic and organic species have been identified. Mammals can metabolize both inorganic and organic selenium species to hydrogen selenide (H_2Se) or closely related compounds [1, 5]. Then, the selenide is utilized for selenoproteins such as glutathione peroxidase (GPx) [1, 6, 7] or excreted as methylated metabolites such as dimethyl selenide (CH_3SeCH_3), trimethylselenonium ion ($(CH_3)_3Se^+$), and selenosugars [8–11].

Urinary excretion is the primary elimination route of selenium [5], although dimethyl selenide can be exhaled [7, 11–13]. While trimethylselenonium ion had previously been considered as a major urinary metabolite [14], recent studies revealed that selenosugars such as methyl-2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside, methyl-2-acetamido-2-deoxy-1-seleno-β-D-glucosopyranoside, and methyl-2-

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amino-2-deoxy-1-seleno-β-D-galactopyranoside are the major selenium metabolites in urine [6, 15–21]. However, the major metabolites of selenium excreted in urine vary considerably from person to person. For example, the selenosugars were the major metabolites in 80 % of the German study participants, whereas trimethylselenonium ion was the major one in the remaining subjects [22]. Furthermore, the relative proportion of the urinary metabolites was altered by the source of selenium ingested [23]. Other urinary selenium metabolites including selenite, selenate, selenocystine, methylselenocysteine, SeMet, selenoadenosylmethionine, selenocystamine and *Se*-methylselenoneine, and selenourea have been reported, but they were detected in some studies and not in others [22, 24–30]. Still, a large portion of the ingested selenium eliminated from the body is not known [23]. Volatile organic selenium species may account for a certain portion of the unknown species since they can easily be lost due to evaporation during sample preparations and analyses of the non-volatile metabolites listed above. Several volatile species such as dimethyl selenide, dimethyl diselenide ($CH_3SeSeCH_3$), and dimethyl selenenylsulfide (CH_3SeSCH_3) have been demonstrated in human urine [31, 32].

While we analyzed male golden hamster urine in search of potential volatile pheromone(s), we unexpectedly detected four volatile organic selenium compounds two of which have never been reported in urine. As identification of urinary metabolites would help us understand better the selenium metabolism in mammals, their identities are documented here.

Materials and methods

Chemicals

Dimethyl disulfide and zinc (powder) were obtained from Merck (Vienna, Austria). Dimethyl trisulfide, dimethyl diselenide, sodium selenite pentahydrate, sodium borohydride, sodium hydroxide, hydrochloric acid, and sulfuric acid were purchased from Sigma-Aldrich (Vienna, Austria).

Synthesis of dimethyl selenenylsulfide, dimethyl selenodisulfide, and dimethyl bis(thio)selenide

Dimethyl selenenylsulfide (CH_3SeSCH_3) was synthesized based on the procedure described by Chasteen [33]. Five hundred milligrams of zinc powder was placed in a 20-mL headspace vial and acidified with 2 mL 5 M hydrochloric acid, and the acid was then removed. To the vial containing the acidified zinc powder, 25 μL each of dimethyl disulfide and dimethyl diselenide was added. The vial was capped and left for several hours at room temperature for reaction. Five microliters of the headspace was taken from the vial with a 10-μL gas-tight syringe and analyzed by a Shimadzu gas chromatograph–mass spectrometer (GC–MS) QP2010 Plus (Duisburg, Germany). In this reaction, methanethiol, dimethyl sulfide, dimethyl selenide, dimethyl selenenylsulfide, and dimethyl trisulfide were formed.

Dimethyl selenodisulfide ($CH_3SeSSCH_3$) was synthesized based on the procedure described by Swearingen et al. [34]. Sixty milligrams of zinc powder was placed in a 20-mL headspace vial and acidified with 1 mL 1 M sulfuric acid. To the vial, 25 μL each of dimethyl trisulfide and dimethyl diselenide was added. The vial was capped and left for several hours at room temperature for reaction. Five microliters of the headspace was taken from the vial with a 10-μL gas-tight syringe and analyzed by the Shimadzu GC–MS. In this reaction, methanethiol, methaneselenol (trace), dimethyl selenide, dimethyl disulfide, dimethyl selenenylsulfide, dimethyl selenodisulfide, and dimethyl diselenenylsulfide ($CH_3SSeSeCH_3$) were generated.

Dimethyl bis(thio)selenide ($CH_3SSeSCH_3$) was synthesized based on the procedure described by Swearingen et al. [34]. To a 20-mL headspace vial, 24 mg sodium borohydride, 1 mg sodium hydroxide, 526 mg sodium selenite pentahydrate, 1 mL deionized water, and 1 mL sulfuric acid were placed. Reaction started upon addition of 50 μL dimethyl trisulfide to the vial. The vial was capped and left for several hours at room temperature. Five microliters of the headspace was taken from the vial with a 10-μL gas-tight syringe and analyzed by the Shimadzu GC–MS. In this reaction, methanethiol, dimethyl disulfide, and dimethyl bis(thio)selenide were produced.

Animals and urine collection

Urine samples were collected from a golden hamster (*Mesocricetus auratus*) colony raised at the Research Institute of Wildlife Ecology. The founder population consisting of eight individuals was obtained originally from Charles River (Bad Sulzfeld, Germany). Only male hamsters were used in this study and housed individually in a polycarbonate cage (Eurostandard Type IV, Tecniplast, Italy) containing sawdust (Lignocel FS 14, JRS, Rosenberg, Germany). Hamster diet (Ssniff, Germany) and water were provided ad libitum and temperature was maintained at 22 ± 2 °C. The selenium content was 0.3 μg per gram of diet. The daily selenium intake was estimated to be 1.8 to 2.4 μg as the animals consumed 6 to 8 g diet per day. The hamsters were kept on a 16:8 h light–dark cycle. Twelve male hamsters were used as urine donors and their age ranged from 75 to 387 days. Each hamster was transferred to an empty cage

for urine collection, and then urine was taken with a pipette as soon as each individual urinated in the cage. The urine samples collected were stored at −20 °C until needed.

Collection of volatile organic selenium compounds released from hamster urine

One hundred fifty microliters of intact or aged urine was placed in a 4-mL glass vial, and a 2-cm, three-component solid-phase microextraction (SPME) fiber (30 μm carboxen, 50 μm divinylbenzene, polydimethylsiloxane; Supelco Corp., Bellefonte, PA, USA) was used for collection of the headspace volatile organic selenium compounds released from urine in the vial for 30 min at 37 °C. The analytical procedure has been described elsewhere in detail [35].

Aging procedure of urine

One hundred fifty microliters of urine was transferred to a 4-mL glass vial, placed into a fume hood, and aged for 48 h at room temperature. The 48-h-aged sample was nearly dried. The aged urine sample was then capped and extracted with SPME followed by GC–MS analysis.

Analysis of volatile organic selenium compounds by gas chromatography–mass spectrometry

A Shimadzu GC–MS was used for separation and analysis of the selenium compounds. A Supelcowax® 10 column (30 m × 0.25 mm with 0.50 μm film thickness; Sigma-Aldrich, Vienna, Austria) was used. The analytical procedure has been described elsewhere in detail [35].

Results and discussion

A total of 12 male hamster urine samples collected from each animal were analyzed in both intact and aged conditions. Four volatile organic selenium compounds were detected in the samples. They were dimethyl selenenylsulfide (CH_3SeSCH_3), dimethyl diselenide ($CH_3SeSeCH_3$), dimethyl bis(thio)selenide ($CH_3SSeSCH_3$), and dimethyl selenodisulfide ($CH_3SeSSCH_3$), and three of them were sulfur-containing compounds. Representative, total, and selected ion chromatograms are illustrated in Fig. 1. The retention times and mass spectra of the selenium compounds detected in urine matched those of synthetic standards. The syntheses of CH_3SeSCH_3 and $CH_3SeSSCH_3$ were based on sulfur/selenium exchange reactions [33, 34]. In the synthesis of $CH_3SSeSCH_3$, selenite was reduced to selenide (Se^{2-}) by $NaBH_4$, and then selenide reacted with dimethyl trisulfide to form dimethyl bis(thio)selenide [34]. The mass spectra of the compounds are shown in Fig. 2. The spectra of dimethyl bis(thio)selenide and dimethyl selenodisulfide obtained from urine contained many background ions since they were detected at trace levels. Dimethyl diselenide was demonstrated in human urine [32]. It was identified as a degradation product of methyl-2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside, a major urinary selenium metabolite [31]. Dimethyl selenenylsulfide has also been demonstrated in human urine [31, 32], and suggested to be formed by the reaction between dimethyl diselenide and dimethyl disulfide or dimethyl trisulfide [33, 34]. Dimethyl selenide (CH_3SeCH_3) has been demonstrated in human urine [31, 32]. However, it was not detected in our urine

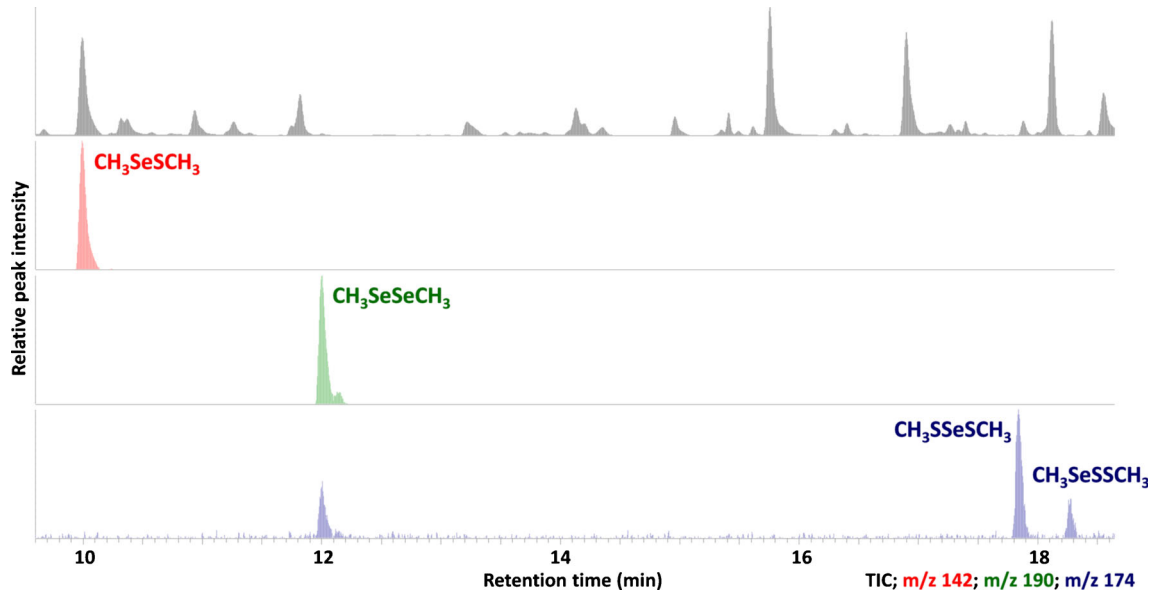

Fig. 1 Representative, total, and selected ion chromatograms containing volatile organic selenium compounds detected in male golden hamster urine

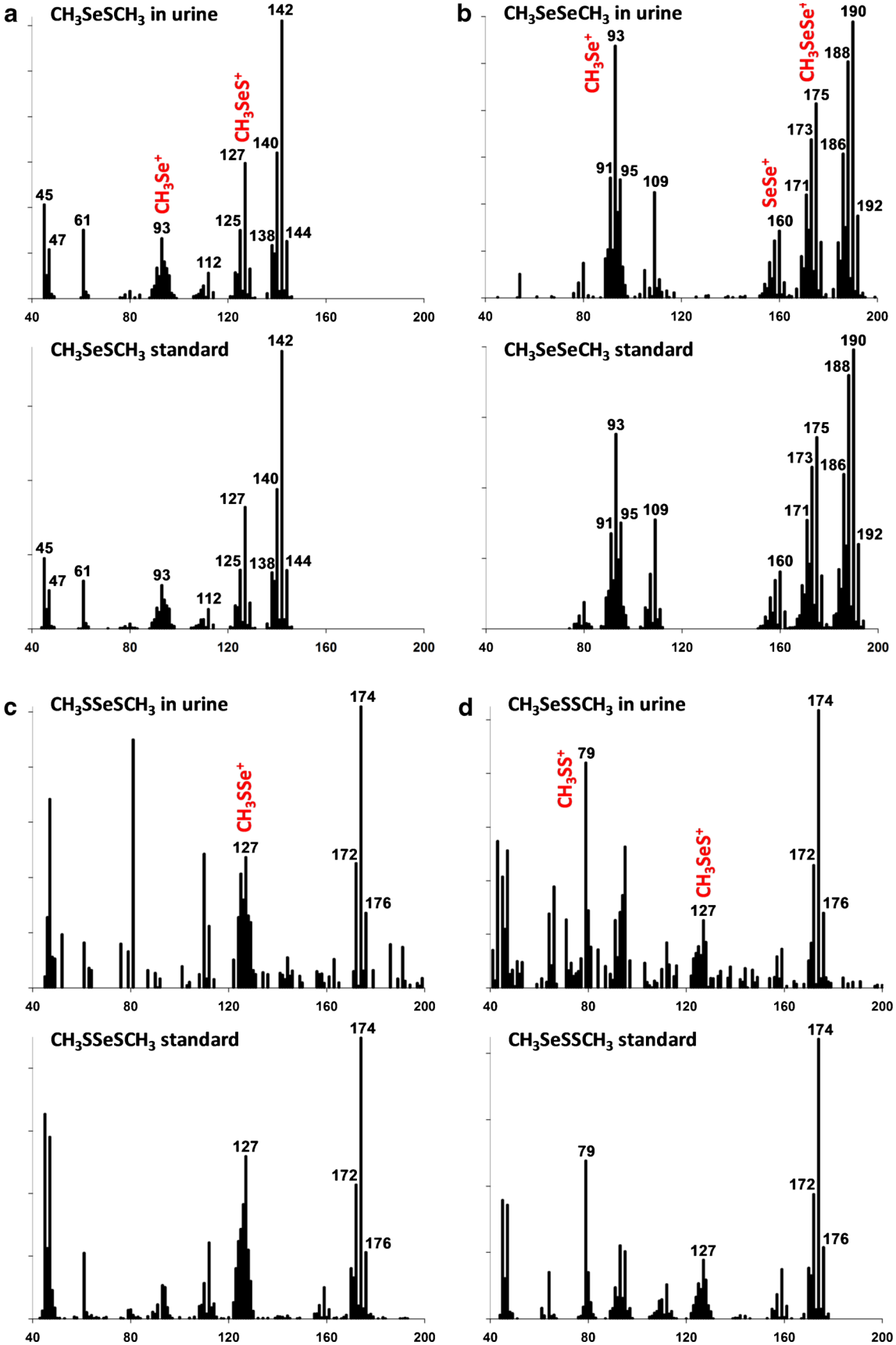

Fig. 2 The mass spectra of volatile organic selenium compounds detected in male golden hamster urine and those of the synthesized compounds

samples analyzed. To our best knowledge, dimethyl bis(thio)selenide and dimethyl selenodisulfide have never been demonstrated in urine.

As the hamster urine samples were aged and dried for 48 h, dimethyl selenenylsulfide and dimethyl bis(thio)selenide significantly decreased (Fig. 3A, C; Mann–Whitney rank sum test $p < 0.001$), indicating that these compounds are degraded or lost in the air due to evaporation. Dimethyl diselenide tended to decrease, but the change was not significant (Fig. 3B; Mann–Whitney rank sum test $p = 0.089$), suggesting that it could be formed from other urinary selenium compounds (e.g., methyl-2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside [31]) while some of dimethyl diselenide is lost. In contrast, dimethyl selenodisulfide tended to increase (Fig. 3D; Mann–Whitney rank sum test $p = 0.069$). The increase might be due to its formation via reaction of dimethyl diselenide and dimethyl trisulfide whose concentration increased while urine aged (Fig. 4D), as we used these two chemicals for the synthesis of dimethyl selenodisulfide, and as dimethyl selenenylsulfide has been suggested to be formed by the reaction between dimethyl diselenide and sulfides [33, 34]. The changes in the headspace concentrations of other volatile sulfides detected in the urine samples during the aging process are also illustrated in Fig. 4. Dimethyl selenide was not detected in the aged urine samples as it was not in the intact samples.

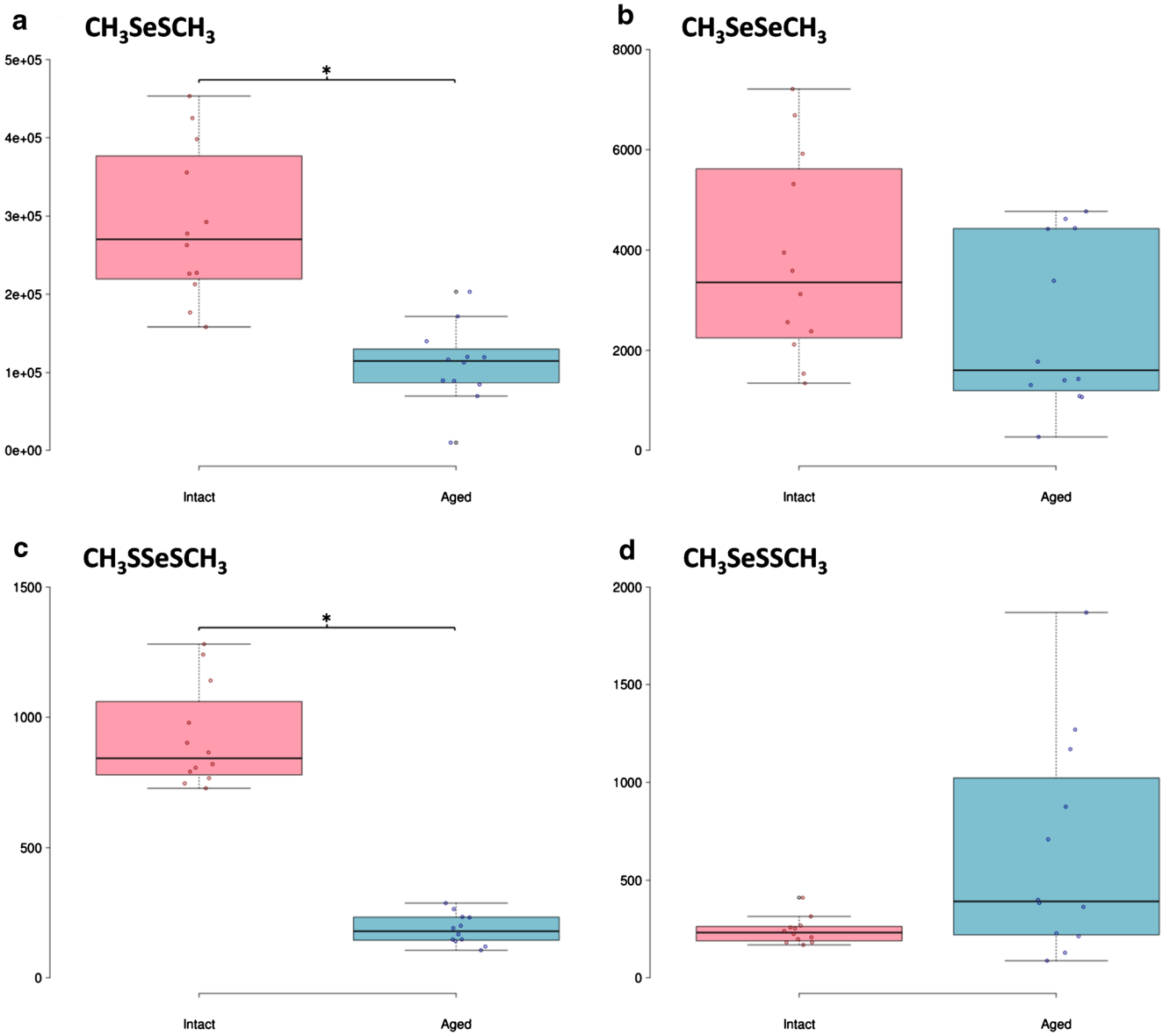

Fig. 3 The headspace concentrations of volatile organic selenium compounds detected in intact vs. aged male golden hamster urine. The box plots were created online using the BoxPlotR application [36] (http://boxplot.tyerslab.com/). *Asterisk* $P < 0.001$ (Mann–Whitney rank sum test)

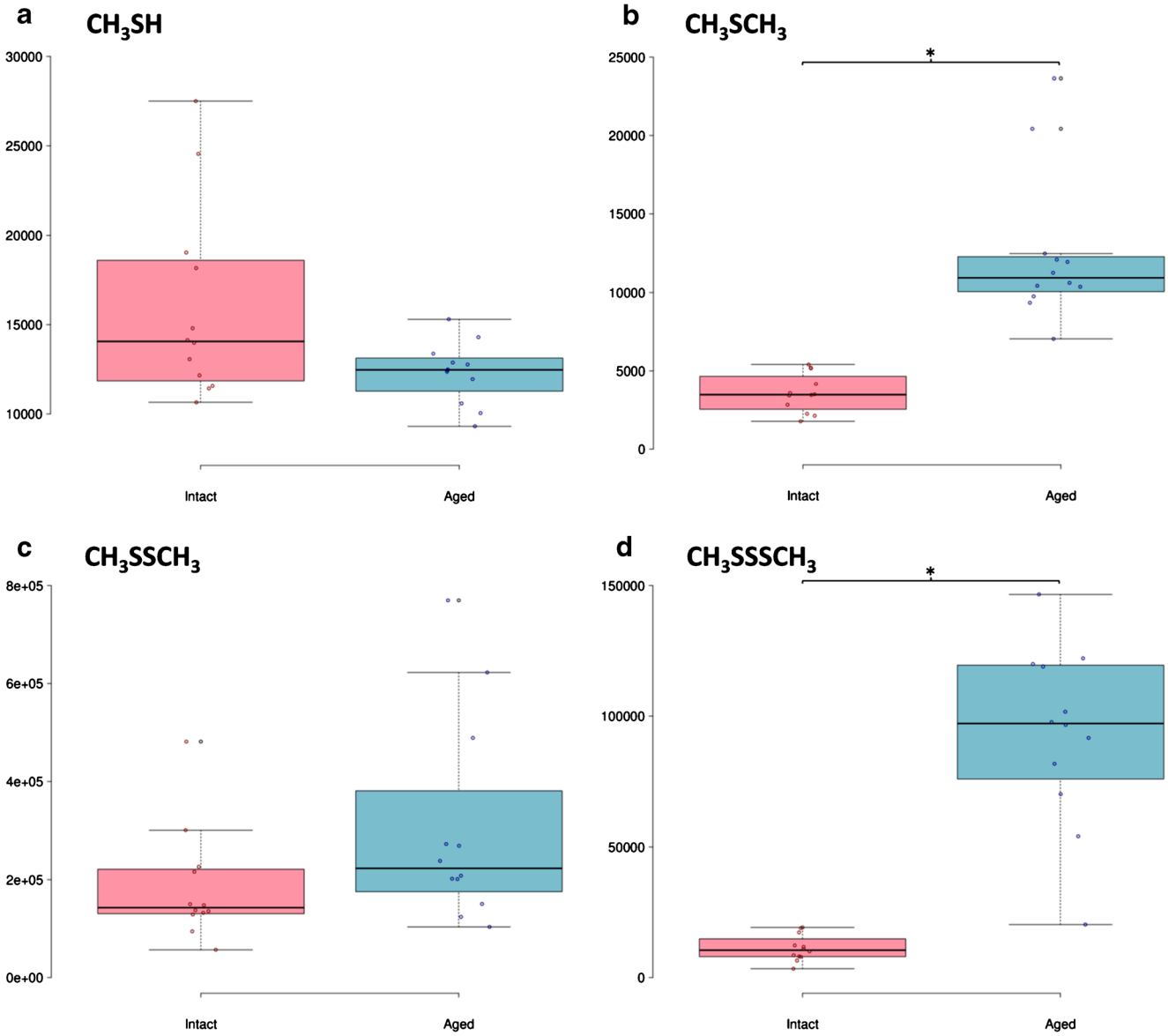

Fig. 4 The headspace concentrations of volatile sulfides detected in intact vs. aged male golden hamster urine. The box plots were created online using the BoxPlotR application [36] (http://boxplot.tyerslab.com/). *Asterisk* $P < 0.001$ (Mann–Whitney rank sum test)

Although it remains to be determined whether these volatile organic selenium species detected in the urine samples are formed by hamsters and/or the intestinal microorganisms, the latter is mostly likely to be involved in the formation of the volatile species possibly by degrading heavier selenium-containing molecules. Inorganic and organic selenium compounds can be transformed to a variety of volatile selenium compounds by many different microorganisms [37, 38]. The degradation of methyl-2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside that was spiked into human urine and stored at 4 °C for 17 weeks was minimized when an antimicrobial agent (NaN_3) was added, although tested in vitro [31]. These findings support the involvement of microorganisms in formation of volatile selenium species in urine. It remains to be determined whether these volatile selenium species are common metabolites in other animals or hamster-specific.

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Compliance with ethical standards The experimental procedures were in accordance with ethical standards and guidelines on both care and use of experimental animals of the Ethical and Animal Welfare Commission of the University of Veterinary Medicine Vienna (Permit No. ETK-13/07/2015).

Conflict of interest The authors declare that they have no conflict of interest.

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