

# Antioxidant and Anticancer Properties and Mechanisms of Inorganic Selenium, Oxo-Sulfur, and Oxo-Selenium Compounds

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**Abstract** Inorganic selenium and oxo-sulfur compounds are widely available in dietary supplements and have been extensively studied for their antioxidant and anticancer properties. Although many in vivo and clinical trials have been conducted using these compounds, their biochemical and chemical mechanisms of efficacy are the focus of much current research. This review discusses the ability of inorganic selenium compounds, such as selenite, and selenate, to prevent damage from reactive oxygen species as well as their ability to promote cell death by reactive oxygen species generation. Oxo-sulfur and selenium compounds, such as alliin, dimethyl sulfone, methionine sulfoxide, and methylselenenic acid also have similar abilities to act as both antioxidants and pro-oxidants, but the mechanisms for these behaviors are distinctly different from those of the inorganic selenium compounds. The antioxidant and pro-oxidant properties of these small-molecule sulfur and selenium compounds are extremely complex and often greatly depend on experimental conditions, which may explain contradictory literature reports of their efficacy.

**Keywords** Antioxidant mechanisms · Anticancer mechanisms · Inorganic selenium compounds · Oxo-sulfur compounds · Oxo-selenium compounds · Reactive oxygen species · Selenium speciation

## Abbreviations

ACSO                      Alliin, *S*-allyl-L-cysteine sulfoxide  
AGE                        Aged garlic extracts

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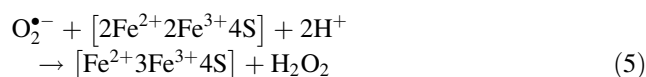
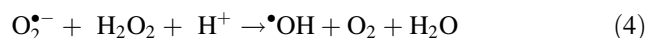
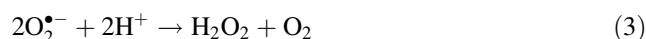
AIDS	Acquired immune deficiency syndrome
ARC	AIDS-related complex
[Cu(bipy) <sub>2</sub> ] <sup>+</sup>	Copper (I) bipyridine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
[Fe(EDTA)] <sup>2-</sup>	Iron(II) ethylenediaminetetraacetic acid
FMO	Flavin-containing monooxygenase
GPx	Glutathione peroxidase
GSH	Glutathione
GSSeSG	Selenoglutathione
GSSe <sup>-</sup>	Selenopersulfide anion
GSSH	Glutathione disulfide
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
H <sub>2</sub> Se	Hydrogen selenide
HDL	High density lipoprotein
HIV	Human immunodeficiency virus
HSC-3	Human oral squamous carcinoma cells
IC <sub>50</sub>	Concentration required for 50% inhibition of biological activity
LDL	Low-density lipoprotein
MeCysSO; MCSO	Methylcysteine sulfoxide, <i>S</i> -methyl-L-cysteine sulfoxide, methiin
Me <sub>2</sub> SO <sub>2</sub>	Dimethyl sulfone; methylsulfonyl methane
Met	Methionine
MePhSO	Methyl phenyl sulfoxide
MetSO	Methionine sulfoxide
MetSeO	Methionine selenoxide
MMTS	Methyl methane thiosulfonate
MSeA	Methylselenenic acid
Msr	Methionine sulfoxide reductase
MsrA	Methionine sulfoxide reductase A

MsrB	Methionine sulfoxide reductase B
NADH	Reduced nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
Na <sub>2</sub> Se	Sodium selenide
Na <sub>2</sub> SeO <sub>3</sub>	Sodium selenite
Na <sub>2</sub> SeO <sub>4</sub>	Sodium selenate
<sup>3</sup> O <sub>2</sub>	Triplet oxygen
O <sub>2</sub> <sup>•-</sup>	Superoxide anion radical
•OH	Hydroxyl radical
ONOOH	Peroxynitrous acid
PCSO	Propiin, S-propyl-L-cysteine sulfoxide
PhSO	Phenyl sulfoxide
RDA	Recommended daily allowance
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RSSeSR	Selenotrisulfide
SAR	Seasonal allergy rhinitis
Se <sup>0</sup>	Selenium (elemental)
SeCys	Selenocysteine
SeMet	Selenomethionine
SELECT	Selenium and Vitamin E Cancer Prevention Trial
SeO <sub>2</sub>	Selenium dioxide
SOD	Superoxide dismutase
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
TPCSO	Isoalliin, S-trans-1-propenyl-L-cysteine sulfoxide

## Introduction

The generation of reactive oxygen species (ROS) has dual functionality in biological systems, with both beneficial and detrimental effects in cells [1, 2]. ROS generation at low or moderate concentrations aids in the defense against infectious agents and functions in several cell signaling pathways [1, 2]. The damaging effects of ROS such as the superoxide anion radical (O<sub>2</sub><sup>•-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radical (•OH) caused by the overproduction of these species results in oxidative stress, an unavoidable consequence of aerobic cellular respiration [1–6]. ROS damage to lipids, proteins, and DNA [1, 2, 6, 7] is a result of this oxidative stress and leads to several health conditions including aging [1], cancer [1, 8], neurodegenerative diseases such as Parkinson's and Alzheimer's [9–12], and cardiovascular diseases such as arteriosclerosis [13–16].

Reactive oxygen species are generated during the reduction of molecular oxygen (O<sub>2</sub>) to produce water (H<sub>2</sub>O) via metabolic processes catalyzed by cytochrome oxidase in biological systems [5, 17]. The primary ROS formed as a byproduct of this respiratory process is the superoxide anion radical (O<sub>2</sub><sup>•-</sup>), generated when molecular oxygen gains an electron from either the mitochondrial electron transport chain (Reaction 1) or as a result of UV-irradiation (Reaction 2) [1, 4, 5, 17]. Further reduction of O<sub>2</sub><sup>•-</sup>, either directly or through enzyme- or metal-catalyzed



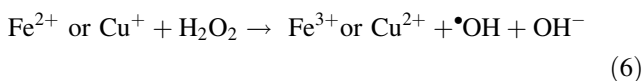
reactions, results in the formation of secondary ROS such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>; Reaction 3) and the hydroxyl radical (•OH; Reaction 4) [1, 4, 5, 17]. Hydrogen peroxide is also produced directly by protonation of the superoxide radical anion in solution (Reaction 3), and indirectly upon oxidation of iron-sulfur clusters (Reaction 5) [18, 19].

Compared to other ROS, hydrogen peroxide is a non-radical species with relatively low reactivity [20]. It is one of the more commonly studied ROS, and is produced endogenously by various physiological processes including respiratory burst and oxidative phosphorylation [21]. Calculations to determine the steady-state intracellular concentrations of hydrogen peroxide in unstressed *E. coli* cells determined a value of ~20 nM, with a high rate of H<sub>2</sub>O<sub>2</sub> production ranging from 9 to 22 μM/s [22]. Thus, any imbalance between the rate of H<sub>2</sub>O<sub>2</sub> generation and the decomposition may result in significantly increased H<sub>2</sub>O<sub>2</sub> concentrations and resultant oxidative stress [22–24].

Imlay and Linn [24] reported bimodal cell killing when *E. coli* is exposed to H<sub>2</sub>O<sub>2</sub>. Mode I cell killing occurs at low concentrations of H<sub>2</sub>O<sub>2</sub> (1–5 mM) and is faster than Mode II, which occurs at H<sub>2</sub>O<sub>2</sub> concentrations greater than 10 mM. Mammalian cells also show the same bimodal killing rates as *E. coli* upon hydrogen peroxide challenge [25]. Bimodal kinetics are observed for iron-mediated oxidative DNA damage in vitro, where maximal damage occurs at 50 μM H<sub>2</sub>O<sub>2</sub> for Mode I conditions, and at H<sub>2</sub>O<sub>2</sub> concentrations >10 mM for Mode II conditions [24, 26, 27]. H<sub>2</sub>O<sub>2</sub> reacts with redox-active metal ions to generate hydroxyl radical [5, 17, 18, 20, 28]. Hydroxyl radical has an extremely short half-life (~10<sup>-9</sup> s) [29, 30] and reacts quickly with biomolecules near its site of generation, resulting in DNA damage, lipid peroxidation, thiol depletion, and changes in calcium homeostasis [1, 21, 31].

Iron and copper are the most commonly studied redox-active metal ions found in biological systems and are essential in many proteins and enzymes, including ferritin, transferrin, ceruloplasmin, and superoxide dismutase [32]. In *E. coli*, normal intracellular non-protein-bound (labile) iron concentrations are  $\sim 20 \mu\text{M}$ . However, this concentration increases significantly to 80–320  $\mu\text{M}$  upon disruption of iron homeostasis and oxidative stress [20, 31, 33, 34]. Although the intracellular concentration of non-protein-bound copper was calculated to be approximately  $10^{-18} \text{ M}$  in yeast, significant amounts of labile copper are observed in mouse Golgi and mitochondria [35–37]. Studies have also reported extracellular copper concentrations in blood serum and cerebrospinal fluid between 10–25  $\mu\text{M}$  and 0.5–2.5  $\mu\text{M}$ , respectively, whereas copper concentrations in the synaptic cleft are approximately 30  $\mu\text{M}$  [38, 39]. Neural copper concentrations are significantly higher in the locus coeruleus (stress and panic response center) and substantia nigra (dopamine production region) with concentrations of 1.3 and 0.4 mM, respectively [38, 40].

In the reduced state,  $\text{Fe}^{2+}$  and  $\text{Cu}^+$  are oxidized by  $\text{H}_2\text{O}_2$  to  $\text{Fe}^{3+}$  and  $\text{Cu}^{2+}$ , generating hydroxyl radical in the Fenton or Fenton-like reaction (Reaction 6) [1, 21, 28, 41–43].



This production of  $\bullet\text{OH}$  becomes catalytic in vivo due to the presence of cellular reductants, such as NADH, which reduce  $\text{Fe}^{3+}$  and  $\text{Cu}^{2+}$  back to their reduced forms. This iron-mediated generation of  $\bullet\text{OH}$  is the main cause of oxidative DNA damage and cell death in prokaryotes and eukaryotes, including human cells, under oxidative stress conditions [24, 25, 43], and is a root cause of many health conditions such as cancer, aging, and cardiovascular and neurodegenerative diseases [9, 14, 43, 44].

Cellular defenses against the harmful effects of oxidative stress involve both enzymatic and nonenzymatic antioxidant activities [1, 4]. Enzymes such as glutathione peroxidases (GPx), catalases, and superoxide dismutases (SOD) act by directly scavenging ROS or by producing nonenzymatic antioxidants such as glutathione (GSH), thioredoxin, ubiquinone, and menaquinone [1, 4]. Nonenzymatic defenses involve small-molecule antioxidants such as carotenoids, lipoic acid, and vitamins C and E to protect against the damaging effects of oxidative stress [1, 45]. Both vitamins C and E reduce oxidative stress and malformations in the offspring of rats with diabetes [46–48]. Studies have also focused on the ability of various selenium, sulfur, and polyphenol compounds to act as antioxidants by preventing ROS-mediated DNA damage [23, 49–54].

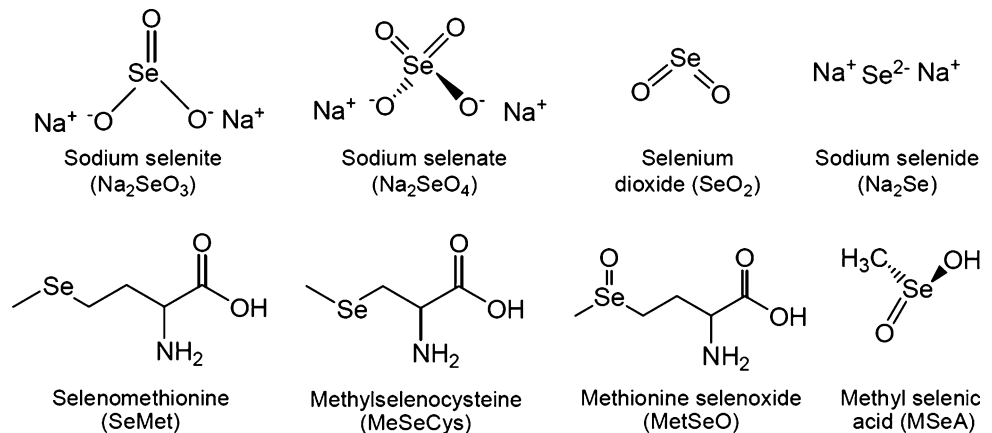
Selenium has been extensively studied for its antioxidant and cancer preventative properties and is an essential

trace element in human and animal metabolism [55–58]. It is found in many dietary supplements and multivitamins in forms such as selenite ( $\text{Na}_2\text{SeO}_3$ ), selenate ( $\text{Na}_2\text{SeO}_4$ ), or selenomethionine (SeMet) [56, 59]. Selenite and selenate are also found in fertilizers, animal feed, infant formulas, and protein shakes [56, 60]. Selenium is incorporated as selenocysteine (SeCys) in selenoproteins P, W, and R, as well as in the active sites of enzymes such as glutathione peroxidases (GPx) and thioredoxin reductases [23, 52, 57, 61–63]. In cells, these selenoproteins have important antioxidant activities and protect the mitochondria, plasma membrane, and DNA from oxidative damage by ROS [60, 64]. For example, GPx is found in the cytosol of cells and exerts its antioxidant activity by reducing intracellular hydrogen peroxide to water, preventing the generation of ROS [62, 63, 65–68]. Although selenoproteins are a significant part of the antioxidant properties of selenium, they have been extensively discussed [62, 69, 70] and are not the focus of this review.

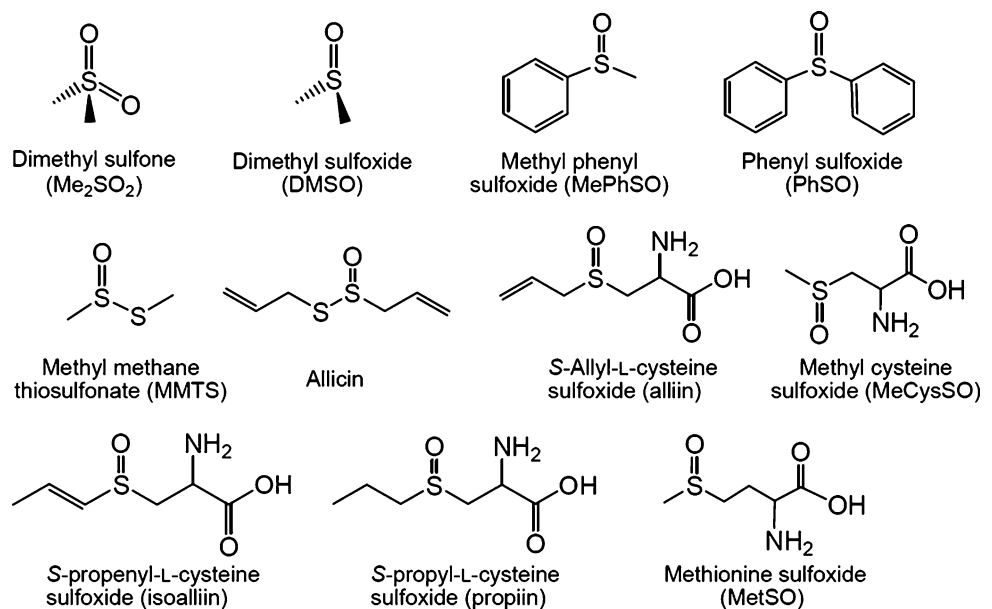
Studies to determine the antioxidant activity of small-molecule selenium- and sulfur-containing compounds have focused mainly on organoselenium and organosulfur compounds since they are more bioavailable and are more readily incorporated into amino acids and proteins when compared to the inorganic forms [62, 71]. Consumption of food products high in selenomethionine (SeMet) results in incorporation of this amino acid into proteins by replacing its sulfur analog, methionine (Met) [72]. SeMet is also more efficiently absorbed and retained than the inorganic sodium selenite and selenate [73]. While the organoselenium compounds have received a significant amount of attention for their role as antioxidants [49, 74–76], several studies indicate that inorganic selenium compounds such as selenite, selenate, selenium dioxide ( $\text{SeO}_2$ ), and sodium selenide ( $\text{Na}_2\text{Se}$ ; Fig. 1) also exhibit similar antioxidative properties [23, 51, 55, 57, 58, 77–79]. Fruits, vegetables, and dietary supplements also contain oxo-sulfur compounds (Fig. 2) such as allicin, methylcysteine sulfoxide (MeCysSO), methyl methane thiosulfonate (MMTS), and dimethyl sulfone or methylsulfonyl methane ( $\text{Me}_2\text{SO}_2$ ), which are also effective in preventing oxidative damage to cellular components [53, 54, 80–85]. Understanding the effects of oxidation on the antioxidant properties of organosulfur compounds is also important because amino acids such as methionine are susceptible to oxidation by ROS [86–88]. To prevent the disruption of protein function upon methionine oxidation to methionine sulfoxide (MetSO), cells have dedicated methionine reductase enzymes (Msr) to reduce MetSO back to Met [87, 89–92].

Research has focused primarily on the ability of organoselenium and organosulfur compounds in their reduced forms to prevent oxidative DNA damage and to treat or prevent diseases caused by oxidative stress. However, it is

**Fig. 1** Structures of inorganic and oxo-selenium compounds discussed in this review



**Fig. 2** Structures of oxo-sulfur compounds discussed in this review



important to acknowledge the fact that inorganic selenium and oxo-sulfur compounds are abundant in many food products such as dietary supplements, protein shakes, infant formulas, fruits, and vegetables. This review will therefore discuss the role and biochemical mechanisms of inorganic selenium, oxo-selenium, and oxo-sulfur compounds to act as antioxidants and pro-oxidants, both in vivo and in vitro, for the treatment or prevention of ROS-mediated diseases.

### Selenium Bioavailability, Related Pathologies, and Biological Effects

Selenium is an important micronutrient for both humans and animals and is obtained through the diet from several sources including cereals, grains, nuts, vegetables, meat, and seafood [62, 93, 94]. The recommended daily allowance (RDA) for selenium ranges from 55  $\mu\text{g}/\text{day}$  to an

upper limit of 350–400  $\mu\text{g}/\text{day}$ , and daily intake comes from dietary supplementation and foods rich in this mineral [56, 95]. Although selenium toxicity has been observed for supplementation greater than 400  $\mu\text{g}/\text{day}$  [60, 96], it is important to note that some studies conducted with a selenium intake ranging from 750 to 850  $\mu\text{g}/\text{day}$  ( $\sim 0.01$  mg/kg) reported no signs of selenium toxicity in humans [97, 98]. Animal studies reported selenium toxicity within 12 h upon supplementation of 2 mg/kg selenium [99, 100]. These seemingly contradictory observations of selenium toxicity in humans highlight the need for additional studies to establish accurate upper level RDA values for selenium supplementation.

The selenium content of plant and animal products in the diet is important to maintain adequate selenium status and is highly dependent on regionally variable selenium concentrations in soil [48, 56, 62, 63, 66, 68, 98]. In the United States, for example, soil in northern Nebraska and the Dakotas has high selenium levels, but areas such as the

Keshan province of China and some parts of Finland, New Zealand, Australia, and North America (northeast, northwest, Midwest, and southeast regions) have low soil selenium levels [66, 68, 98, 101–104]. To increase the selenium content in soils, and therefore increase animal and human consumption of selenium, these countries have implemented the use of fertilizers enriched with selenite or selenate for agricultural crops [105–108]. In the United States, selenite supplementation in animal feed has been shown to improve animal performance and increase the selenium dietary intake for Americans consuming meat products [60].

**Pathologies Associated with Selenium Deficiency and Toxicity**

Selenium deficiency occurs in regions where the selenium content in soil is low and can result in diseases such as hypothyroidism, weakened immune defenses, and cardiovascular diseases [63, 109, 110]. Keshan disease is a cardiomyopathy endemic to the Keshan province of China. This disease affects young children and women of child-bearing age as a result of low iodine and selenium contents in food products, leading to low blood plasma selenium levels [111–115]. The average intake of selenium for the development of symptoms due to deficiency was 10 µg/day with symptoms such as congestive heart failure, stroke, or sudden death (Fig. 3) [62, 115].

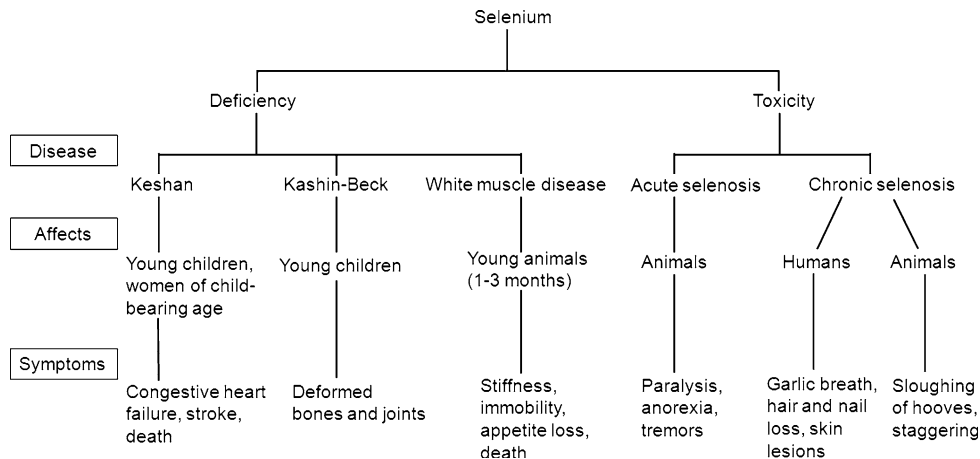
Also resulting from low selenium and iodine intake is the endemic osteoarthropathy known as Kashin–Beck disease found in several areas of China [113, 114]. Bone and joint deformations in growing children are characteristic of this disease (Fig. 3) [116]. The average serum selenium levels of patients with Kashin–Beck disease is significantly lower (11 ng/mL) than those without these mineral deficiencies (60–105 ng/ml) [117]. Serum thyroxine levels are also much lower in patients with Kashin–Beck disease,

resulting in higher incidences of goiter than those unaffected by the disease [118]. In farm animals, selenium deficiency causes a muscular dystrophy known as white muscle disease (Fig. 3) [68, 119]. This disease usually affects growing animals such as lambs and calves between 1 and 3 months old with symptoms including stiffness, inability to move, weakness, tiredness, accelerated breathing, elevated temperatures, difficulty in feeding, and death [68, 119, 120].

Intake of selenium higher than the upper limit range 350–400 µg/day [56, 95] of the RDA is also of major concern to humans and animals since it can result in selenium toxicity or selenosis (Fig. 3) [60, 96]. Acute selenosis is caused by consumption of high levels of selenium in a short period of time. Upon ingestion of 17.2 µg/ml selenium due to incorrect dosage in animal feed, pigs showed signs of acute selenium toxicity, including paralysis, hyperesthesia, anorexia, and tremors [96, 121]. Signs of acute selenosis in buffalo include anorexia, alopecia, mild convulsions, and lowered body temperature [96, 122]. Symptoms of chronic selenosis include hair loss, deformation or cracks on the skin, horns, and hooves of animals, resulting in the sloughing of hooves and staggering [60, 96]. In humans, signs of selenosis include garlic breath, hair and nail loss, thickened and brittle nails, teeth deformation, skin lesions, and lowered hemoglobin levels upon dietary selenium intake of 5 mg/day (Fig. 3) [97].

Although countries have implemented the use of fertilizers containing selenite and selenate to supplement foods grown in selenium-deficient soil, the effects of selenium supplementation vary for each of these inorganic selenium compounds. In an attempt to prevent or reduce the prevalence of selenium deficiency diseases in China, both selenite and selenate were introduced into the soils of rice crops [115]. In unsupplemented soil, selenium content is extremely low, less than 0.06 µg/g. Rice crops grown in selenite- or selenate-enriched soils had significantly increased

**Fig. 3** Pathologies associated with selenium deficiency and toxicity



selenium levels of 0.47 and 0.64  $\mu\text{g/g}$ , respectively, with no adverse effects on the plants [115]. In Chile, ryegrass supplemented with 0.1  $\mu\text{g/g}$  selenite or selenate increases selenium content from 0.07  $\mu\text{g/g}$  for grass grown in untreated soil to 0.28 and 5.72  $\mu\text{g/g}$ , respectively [66]. Soil enrichment at higher levels of selenate (4–10  $\mu\text{g/g}$ ), resulted in stunted growth of ryegrass, with selenium content ranging from 150 to 247  $\mu\text{g/g}$  [66]. Surprisingly, selenite-enriched soils (6–10  $\mu\text{g/g}$ ) decreased lipid peroxidation in the plants, whereas selenate supplementation of soil at the same levels had the opposite effect. Higher lipid peroxidation levels for plants grown in selenate supplemented soil may account for the observed stunting of plant growth [66].

Because inorganic forms of selenium effectively increase selenium levels in plant crops and prevent selenium deficiency diseases in people that consume them, it is important to understand the effect of selenite and selenate supplementation in crops. Plants more efficiently absorb selenate, as indicated by the higher concentrations of selenium in plants supplemented with selenate as compared to selenite supplementation, but selenite may be safer to use in fertilizers, since there are fewer adverse effects with supplementation at high concentrations [66].

To better treat selenium deficiency and to prevent selenium toxicity, an accurate evaluation of the effects of inorganic selenium compounds in fertilizers is required. Selenite- and selenate-enriched pastures and salt licks are also used to increase selenium concentrations in livestock [68]. It is therefore also important to understand the effects of this supplementation on animals, and further studies are necessary to determine the appropriate levels and forms of inorganic selenium supplementation that are most effective.

In humans, selenium deficiency causes poor immune response by reducing T-cell counts and impairing lymphocyte proliferation and response [63, 123]. Studies have shown that human supplementation of 200  $\mu\text{g/day}$  of sodium selenite over an 8-week period resulted in enhanced T-lymphocyte response [124]. In HIV and AIDS patients, selenium deficiency is associated with decreased immune cell count, higher rates of disease progression, and increased risk of death [125, 126]. Additionally, selenium was found to protect cells from oxidative stress, resulting in slower progression of this disease [127].

A study performed over a period of 5 years on HIV-positive children found that those with low selenium levels died at a younger age than patients with higher selenium status [128]. These experiments were corroborated by another study involving HIV-positive men and women that linked increased death rates with selenium deficiency [129]. Clinical studies performed on male patients with AIDS and AIDS-related complex (ARC) showed that blood selenium levels increased upon supplementation of 400  $\mu\text{g/day}$  Se-enriched yeast from 0.142 to 0.240  $\mu\text{g/ml}$

over a period of 70 days [130, 131]. Similar results were also observed in AIDS patients supplemented with sodium selenite (80  $\mu\text{g/day}$ ) [130, 132]. These investigations indicate that both organic and inorganic selenium supplementation is effective for the treatment of patients with immune deficiencies [129].

Numerous studies indicate that selenium also plays an important role in cancer prevention and treatment. Patients with uterine cervical carcinoma were found to have low glutathione peroxidases and selenium levels [133], and, in a random, double-blind cancer prevention trial, the incidence of prostate cancer was reduced by  $\sim 50\%$  compared to the placebo group upon selenium supplementation of 200  $\mu\text{g/day}$  as selenium-enriched yeast. Total cancer mortality also decreased by a statistically significant 40% [134]. In a separate trial, a reduction of only 25% in cancer mortality was observed with 200  $\mu\text{g/day}$  selenium supplementation but not with 400  $\mu\text{g/day}$  [135]. In a larger study of selenium and vitamin E supplementation on prostate cancer (SELECT), selenium supplementation was not uniformly protective against the disease [136]. The SELECT trial indicated that selenium, in the form of L-selenomethionine (200  $\mu\text{g/day}$ ), was not effective in preventing prostate cancer [137, 138]. This study contradicts similar studies such as the Nutritional Prevention of Cancer (NPC) trial, which showed a reduction in prostate (63%), lung (48%), and colorectal (58%) cancers upon selenium supplementation [134, 137, 139, 140]. Disparities in both the studies have been attributed to the form of selenium used for supplementation, plasma selenium levels of patients, and the type of cancer to be treated [134, 137, 139, 140]. While SELECT trials focused on selenomethionine and prostate cancer, the NPC trials used selenite and selenium-enriched yeast as the selenium source to prevent skin cancer. In addition, the selenium status of patients in SELECT studies was higher (135  $\text{ng/ml}$ ) than those in the NPC trials (113  $\text{ng/ml}$ ) [137–139]. The results of these two studies show the importance of understanding the role and mechanism of selenium speciation in preventing or treating cancer. Mechanisms proposed for the observed anticancer properties of selenium include antioxidant activity, regulation of gene expression, cell proliferation control, and angiogenesis inhibition [141, 142].

### **Antioxidant and Anticancer Activities of Inorganic Selenium Compounds**

ROS generation is directly linked to cellular and DNA damage and is the primary cause of many diseases [9, 14, 18, 44, 143]. Antioxidants have been used to prevent or reduce the effects of ROS-mediated DNA and other cellular damage, and selenium has been extensively studied for its antioxidant properties [55, 57, 58]. Inorganic

selenium compounds can also act as pro-oxidants to produce DNA damage and cell death, an activity that plays an important role in the treatment of cancer [23, 51, 144–147]. Although sometimes confused in the literature, this distinction between antioxidant (cancer prevention) and pro-oxidant (cancer treatment) behavior is important to make, since the chemical and cellular mechanisms behind each type of activity are distinct. The behavior of these inorganic selenium compounds is complex, and in several studies, both antioxidant and pro-oxidant behavior have been observed for the same selenium compound depending on experimental conditions.

The main inorganic selenium compound used in most cancer treatment studies is sodium selenite; however, a few studies use other forms, such as sodium selenate and selenium dioxide [57, 62, 144–146, 148, 149]. Selenate and selenite (0.1  $\mu\text{g}/\text{ml}$ ) are effective dietary supplements for the inhibition of tumor cell growth in rodents [146, 148]. These two inorganic selenium compounds also strongly inhibit the growth of mammalian tumor cells at cell cycle phases specific for each compound (Table 1) [145]. Selenite treatment (10  $\mu\text{M}$ ) of human lymphocytes resulted in accumulation in the S-phase with irreversible growth inhibition, whereas selenate-treated (250  $\mu\text{M}$ ) lymphocytes accumulated in the G<sub>2</sub> phase with reversible inhibitory effects [145]. In a separate study, selenium dioxide (1.5  $\mu\text{M}$ ) effectively enhanced lymphocyte progression into the S-phase of the cell cycle in patients with stage IV cancer, restoring immune function and controlling cancer progression [144]. Takahashi et al. [57] showed that both selenite (10  $\mu\text{M}$ ) and selenium dioxide (100  $\mu\text{M}$ ) induced  $\sim 80\%$  apoptosis in human oral squamous carcinoma (HSC-3) cells after treatment for 72 h, whereas selenate had no effect on cell survival (Table 1).

Ramoutar and Brumaghim [23] have shown that inorganic selenium compounds exert both antioxidant and pro-oxidant activities against iron-mediated oxidative DNA damage (Table 1). Selenite progressively inhibited DNA damage at all concentrations tested (0–5000  $\mu\text{M}$ ), with 91% inhibition at the highest concentration under Mode I conditions (50  $\mu\text{M H}_2\text{O}_2$ ) [23]. Selenate and selenide had no effect on damage under similar conditions, whereas  $\text{SeO}_2$  was found to be both a pro-oxidant and an antioxidant, increasing DNA damage by 20% at 50  $\mu\text{M}$ , but inhibiting 100% DNA damage at 5000  $\mu\text{M}$  [23]. Similar studies performed with organoselenium compounds, SeMet (1–1000  $\mu\text{M}$ ) showed no antioxidant activity, whereas methyl selenocysteine prevented  $\sim 76\%$  iron-mediated DNA damage at very high concentrations (20,000  $\mu\text{M}$ ; Table 1) [150].

In contrast, under Mode II conditions (50 mM  $\text{H}_2\text{O}_2$ ),  $\text{Na}_2\text{SeO}_3$  showed pro-oxidant activity at all concentrations tested (0.5–5000  $\mu\text{M}$ ), damaging 90% DNA at the highest concentration in the absence of iron. However,  $\text{SeO}_2$  was an

efficient antioxidant under similar conditions, preventing 81% iron-mediated DNA damage, whereas  $\text{Na}_2\text{SeO}_4$  and  $\text{Na}_2\text{Se}$  had no effect on such damage (Table 1) [23]. Similar studies were also performed to determine the antioxidant activity of inorganic selenium compounds to prevent copper-mediated DNA damage [151]. In these experiments, both  $\text{Na}_2\text{SeO}_3$  and  $\text{SeO}_2$  were effective antioxidants, preventing 95 and 63% of copper-mediated DNA damage at the highest concentration tested (5000  $\mu\text{M}$ ), whereas  $\text{Na}_2\text{SeO}_4$  and  $\text{Na}_2\text{Se}$  had no effect on such damage. Little activity was observed at more physiologically relevant concentrations (Table 1) [151]. The antioxidant behavior of these inorganic selenium compounds has been attributed to the oxidation state of the selenium atom, rather than the overall charge of the selenium compound [23]. Inorganic selenium compounds in the +4 oxidation state ( $\text{Na}_2\text{SeO}_3$  and  $\text{SeO}_2$ ) were more effective antioxidants than  $\text{Na}_2\text{SeO}_4$  and  $\text{Na}_2\text{Se}$ , with selenium oxidation states of +6 and –2, respectively [23]. In a separate study, Hamilton and Wilker [77] found that  $\text{Na}_2\text{SeO}_4$  (6.2 mM) was effective at inhibiting DNA damage caused by alkylating agents.

High levels of selenite (1  $\mu\text{g}/\text{ml}$ ) were also shown to increase thioredoxin reductase activity 2-fold in rat kidney, liver, and lung tissues as compared to rats with normal selenite intake (0.1  $\mu\text{g}/\text{ml}$ ) (Table 1) [152]. In human colon cancer cells supplemented with various dosages of selenite (0.1, 1, and 10  $\mu\text{M}$ ), thioredoxin reductase activity increased with increasing selenium concentration, resulting in a 65-fold increase at the highest concentration tested (Table 1) [153].

Although these studies make a strong case for selenium supplementation for the prevention or treatment of cancer, additional studies are required to better compare and elucidate the structural and chemical properties of inorganic selenium compounds that contribute to antioxidant or pro-oxidant behavior. For example, while selenite has been shown to be a more effective antioxidant when compared to selenate in many studies, selenite can also oxidatively damage DNA under conditions of oxidative stress. This pro-oxidant effect is not observed with selenate, suggesting that selenate may be safer for use in human or animal supplementation.

### Selenium Speciation and Anticancer Activity

Selenium bioavailability differs for organic and inorganic compounds, and studies have shown that the formulation of the selenium compound, and not the presence of the element itself, is essential for chemopreventative activity [154–156]. It is therefore critical to elucidate the specific selenium compounds that are required for such activity. For example, sodium selenite (5–10  $\mu\text{M}$ ) introduced into cell culture media induced DNA single-strand breaks and cell death via necrosis [154, 155, 157, 158]. Organoselenium

**Table 1** The antioxidant or pro-oxidant behavior of inorganic and organic selenium compounds

Compound	Antioxidant or pro-oxidant behavior	References
Na <sub>2</sub> SeO <sub>4</sub>	Fe <sup>2+</sup> Mode I: No antioxidant or pro-oxidant behavior	[23, 151]
	Fe <sup>2+</sup> Mode II: No antioxidant or pro-oxidant behavior	
	Cu <sup>+</sup> : No antioxidant or pro-oxidant behavior	
	Antioxidant against alkylating agents (6.2 mM)	[77]
	Inhibits tumor cell growth in rodents (0.1 µg/ml)	[146, 148]
Na <sub>2</sub> SeO <sub>3</sub>	Reversibly inhibits mammalian tumor cell growth in G <sub>2</sub> phase (250 µM)	[145]
	Fe <sup>2+</sup> Mode I: Antioxidant at all concentrations (0.5–5000 µM)	[23, 151]
	Fe <sup>2+</sup> Mode II: Pro-oxidant behavior with or without Fe <sup>2+</sup> at all concentrations (0.5–5000 µM)	
	Cu <sup>+</sup> : Antioxidant at high concentrations (500–5000 µM)	
	Inhibits tumor cell growth in rodents (0.1 µg/ml)	[146, 148]
SeO <sub>2</sub>	Irreversibly inhibits mammalian tumor cell growth in S phase (250 µM)	[145]
	Induced apoptosis (80%) in HSC-3 cells (10 µM)	[57]
	Increases thioredoxin reductase activity	[152, 153]
	Fe <sup>2+</sup> Mode I: Pro-oxidant at low concentrations (0.5–250 µM), antioxidant at higher concentrations (250–5000 µM)	[23, 151]
	Fe <sup>2+</sup> Mode II: Antioxidant at all concentrations	
Na <sub>2</sub> Se	Cu <sup>+</sup> : Antioxidant at higher concentrations (5000 µM)	
	Enhances lymphocyte progression into S-phase (1.5 µM)	[144]
	Induced apoptosis (80%) in HSC-3 cells (100 µM)	[57]
	Fe <sup>2+</sup> Mode I: No antioxidant or pro-oxidant behavior	[23, 151]
SeMet	Fe <sup>2+</sup> Mode I: No antioxidant or pro-oxidant behavior	[150]
	MeSeCys	Fe <sup>2+</sup> Mode I: Antioxidant behavior at high concentrations (100–20,000 µM)

compounds (10–50 µM), however, caused cell death by apoptosis with no DNA single-strand breaks (Table 2) [154, 155]. Similar results were obtained in a separate study by Lu and co-workers [159] to determine the effect of selenium form on mouse mammary carcinoma cells (Table 2). Although all selenium compounds tested inhibited cell proliferation and induced cell death, selenite and selenide induced both DNA single (51–59%) and double-strand breaks (4.8–14.6%) in a concentration-dependent manner (1–5 µM); no DNA damage was observed for the organic forms, methylselenocyanate (2–7 µM) and methylselenocysteine (20–100 µM) [159].

In another study, selenite was found to be more potent than either selenocystamine or selenomethionine in inducing apoptosis in mouse keratinocyte (BALB/cMK2) cells [143]. In this experiment, selenite (10 µg/ml) resulted in 100% apoptosis, whereas selenocystamine resulted in 2.8% apoptosis at the same concentration [143]. Selenocystamine (250 µg/ml) was capable of inducing 100% apoptosis, whereas selenomethionine (5–250 µg/ml) showed no effect on BALB/cMK2 cells (Table 2) [143]. A study to determine whether sodium selenite and methylselenic acid (MSeA) repressed interleukin-6-mediated (IL-6) androgen receptor action in prostate cancer

progression indicated that selenite significantly inhibited IL-6 activity in human prostate cancer (LNCaP) cells, but MSeA did not [160].

Although these investigations indicate that the inorganic forms of selenium may be more effective for the prevention or treatment of diseases when compared to the organic forms, further studies are necessary to evaluate the effects of selenium speciation for such purposes. While most studies have focused solely on selenite, the examination of other inorganic selenium compounds such as selenate, selenide, and selenium dioxide, in addition to organoselenium compounds, in antioxidant and anticancer experiments would aid in understanding the effects of selenium speciation within these inorganic and organo-selenium compounds on ROS-induced DNA damage and cell death.

### Mechanisms of Antioxidant and Anticancer Activity for Inorganic Selenium Compounds

While the precise mechanisms of cancer prevention or treatment has not been elucidated for inorganic selenium compounds, several reports indicate that the protection against oxidative damage may involve selenoproteins, such



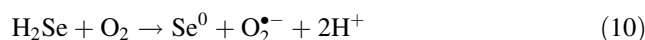
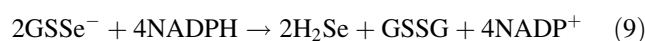
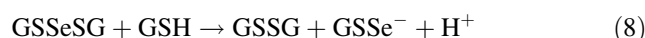
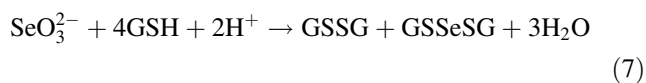
**Table 2** The effect of selenium speciation in anticancer studies

Se form	Se compound	Cell line	[Se]	Results	References
Inorganic	Selenite	Cell culture	5–10 $\mu\text{M}$	DNA single strand breaks; cell death (necrosis)	[154, 155, 157, 158]
		Mouse mammary carcinoma	1–5 $\mu\text{M}$	DNA single (51–59%) and double strand (4.8–14.6%) break	[159]
		Mouse keratinocyte	10 $\mu\text{g/ml}$	Apoptosis (100%)	[143]
	Selenide	Mouse mammary carcinoma	1–5 $\mu\text{M}$	DNA single (51–59%) and double stand (4.8–14.6%) break	[159]
		Cell culture	10–50 $\mu\text{M}$	No DNA single strand breaks; cell death (apoptosis)	[154, 155]
Organic	Organoselenium compounds	Cell culture	10–50 $\mu\text{M}$	No DNA single strand breaks; cell death (apoptosis)	[154, 155]
	Methyl selenocysteine	Mouse mammary carcinoma	2–7 $\mu\text{M}$	No DNA damage	[159]
	MeSeCys	Mouse mammary carcinoma	20–100 $\mu\text{M}$	No DNA damage	[159]
	SeCysta	Mouse keratinocyte	10 $\mu\text{g/ml}$ 250 $\mu\text{g/ml}$	Apoptosis (2.8%) Apoptosis (100%)	[143]
	SeMet	Mouse keratinocyte	5–250 $\mu\text{g/ml}$	No apoptotic effect	[143]

as GPx and thioredoxin reductase, and may require supranutritional levels of selenium [55, 152, 153, 161–170]. One proposed mechanism for the effects of cancer treatment by selenium compounds is the direct action of pro-oxidant selenometabolites to generate ROS, resulting in cellular toxicity [57, 149, 171].

This ROS-generation mechanism involves the metabolism of selenite and selenate to generate hydrogen selenide ( $\text{H}_2\text{Se}$ ), a by-product of selenium metabolic pathway [149, 172, 173]. High levels of selenide can then react with oxygen to produce ROS, resulting in oxidative damage to cells [149, 172, 173]. It has been suggested that the cytotoxicity of inorganic selenium compounds such as selenite and selenium dioxide is due to the formation of selenotrisulfides ( $\text{RSSeSR}$ ), such as selenogluthathione, upon reaction with disulfide peptides or proteins (Reaction 7) [149, 172, 173]. In more recent studies, this mechanism has also been attributed to the pro-oxidant effect of inorganic selenium compounds in different cell lines [57, 143, 174, 175].

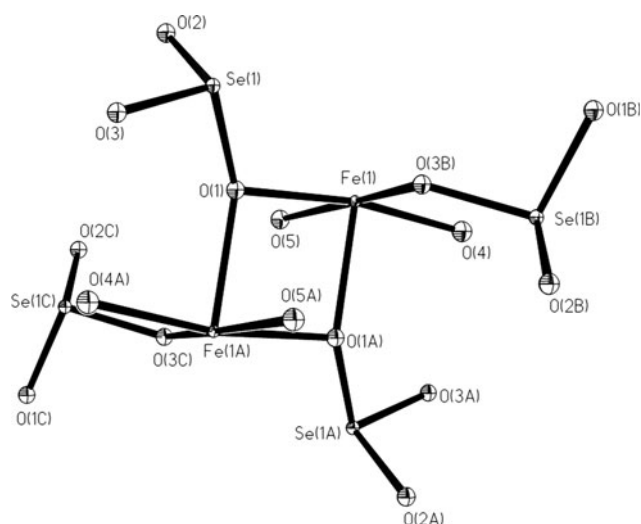
This proposed mechanism for selenite cytotoxicity has been further supported by generation of superoxide upon reduction of selenotrisulfide ( $\text{GSSeSG}$ ) to selenopersulfide anion ( $\text{GSSe}^-$ ) [149]. The selenopersulfide anion, in turn, is reduced by thiols to generate  $\text{H}_2\text{Se}$  (Reactions 7–9) [149]. Selenide then reacts with oxygen to form elemental selenium ( $\text{Se}^0$ ) and  $\text{O}_2^{\bullet-}$  (Reaction 10) [149, 172, 173]. Studies showing that selenite and selenium dioxide, but not selenate, are cytotoxic via this mechanism have been previously reviewed by Spallholz [149].



In contrast, the mechanism for the antioxidant ability of both inorganic and organoselenium compounds in preventing iron- and copper-mediated oxidative DNA damage is through coordination of the metal ion by the selenium compounds [23, 74, 150].  $\text{Na}_2\text{SeO}_3$  and  $\text{SeO}_2$  (0.5–5000  $\mu\text{M}$ ) showed no effect on DNA damage produced by completely coordinated  $[\text{Fe}(\text{EDTA})]^{2-}$  (400  $\mu\text{M}$ ) or  $[\text{Cu}(\text{bipy})_2]^+$  (50  $\mu\text{M}$ ), so coordination of  $\text{Fe}^{2+}$  or  $\text{Cu}^+$  to inorganic selenium compounds is a primary mechanism for both their antioxidant and/or pro-oxidant activities [23, 151]. Similar results were also observed for organoselenium compounds [49]. Although metal coordination to inorganic selenium compounds has not been directly observed in biological systems, iron, copper, mercury, and aluminum react with selenite, selenate or selenium dioxide to form complexes such as  $\text{Fe}_2(\text{H}_2\text{O})_4(\text{SeO}_3)_2$ ,  $\text{FeH}(\text{SeO}_3)_2$ ,  $\text{Fe}(\text{HSeO}_3)_3$ , and  $\text{Al}_2(\text{SeO}_3)_3 \cdot 3\text{H}_2\text{O}$  [176, 177], where iron is coordinated through the oxygen atoms of inorganic selenium compounds such as selenite (Fig. 4).

### Bioavailability and Activity of Oxo-Sulfur and Oxo-Selenium Compounds

Fruits, vegetables, cereal, nuts, and teas have been widely studied for their ability to ameliorate oxidative stress and their potential to prevent or treat cancer, aging, and



**Fig. 4** Coordination of iron to selenite in  $\text{Fe}_2(\text{H}_2\text{O})_4(\text{SeO}_3)_2$  reported by Xiao et al. [177]

cardiovascular diseases [54, 62, 93, 94, 178–180]. The antioxidant capabilities of many foods are attributed to their vitamin, polyphenolic, selenium, and sulfur content [53, 54, 62]. Although there are many members of the *Allium* genus, including onions, leeks, scallions, and chives, garlic has been widely studied for its antioxidant activity [53, 54, 81]. For many centuries, garlic (*Allium sativum* Liliaceae) has been cultivated and used in food preparation for its distinct flavor and aroma, as well as for its medicinal properties [53, 54, 81]. Throughout the years, this bulb has been used to treat the plague, animal bites, leprosy, and cancer, as well as bacterial, immune, and cardiovascular diseases [53, 54, 81, 181–188].

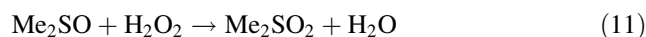
The characteristic flavor and aroma of garlic are attributed to the volatile organosulfur compounds produced upon tissue damage and enzymatic hydrolysis from non-volatile precursors [81, 189]. The vegetative parts of garlic are odorless and composed of non-volatile sulfur-storage compounds known as *S*-alk(en)yl-L-cysteine sulfoxides [81, 189]. These compounds (Fig. 2) are stored in the cytosol of undamaged *Allium* tissues protected from the enzyme alliinase, which is found in the vacuoles [81, 189]. Upon tissue damage, alliinase and *S*-alk(en)yl-L-cysteine sulfoxides react to generate sulfenic acid that then undergoes condensation to form the volatile thiosulfonate compounds [81, 189]. The *S*-alk(en)yl-L-cysteine sulfoxides detected in garlic and several other varieties of the *Allium* genus are *S*-allyl-L-cysteine sulfoxide (alliin, ACSO),

*S*-methyl-L-cysteine sulfoxide (methiin, MeCysSO, MCSO), *S*-propyl-L-cysteine sulfoxide (propiin, PCSO), and *S*-trans-1-propenyl-L-cysteine sulfoxide (isoalliin, TPCSO) [81, 89–192].

The medicinal properties of garlic are primarily attributed to the thiosulfonate compound, allicin produced from alliin by alliinase when garlic is crushed (Fig. 5) [193, 194]. While alliin is the main *S*-alk(en)yl-L-cysteine sulfoxide found in garlic, and is responsible for the volatile odor of cut or crushed garlic, MeCysSO is the most ubiquitous found in onions, chives, leeks, and scallions in various quantities [81].

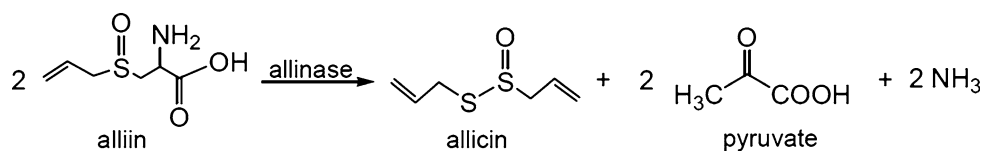
In folk medicine, cauliflower (*Brassica oleracea* Liliaceae var. *botrytis*) is also used for its medicinal purposes [80]. The juice extracts from raw cauliflower leaves are expectorants and are used in the treatment of gastric and duodenum ulcers, whereas the stewed leaves have been used as antipyretics or antirheumatics [80]. These medicinal properties have been attributed to *S*-methyl methane thiosulfonate (MMTS), an oxo-sulfur compound found in cauliflower, broccoli, and cabbage, as well as in the *Allium* vegetables [80, 195–197].

Dimethyl sulfone or methylsulfonylmethane ( $\text{Me}_2\text{SO}_2$ ) is another oxo-sulfur compound found in vegetables including broccoli, peppers, asparagus, and cabbage [82]. It can also be found in trace amounts in fish, meat, unpasteurized milk, beverages, and eggs and has more recently been used as a dietary supplement [83–85]. Currently,  $\text{Me}_2\text{SO}_2$  is sold in over 30 products in combination with other dietary supplements such as chondroitin sulfate and glucosamine, and in more than 50 different products as a single agent in tablets, capsules, creams, and lotions [198]. Although some physicians suggest a daily dose of only 300 mg, the recommended daily dose of  $\text{Me}_2\text{SO}_2$  is reported to be between 1000 and 6000 mg when taken as a dietary supplement [82].  $\text{Me}_2\text{SO}_2$  is a metabolite of dimethyl sulfoxide (DMSO), a byproduct of algae and phytoplankton decay, and is commercially synthesized by reacting hydrogen peroxide with DMSO to produce  $\text{Me}_2\text{SO}_2$  and water (Reaction 11) [198, 199]. Studies have shown that ~15% of orally ingested DMSO is recovered as  $\text{Me}_2\text{SO}_2$  in urine [198–201].



Amino acids are major targets for oxidation by reactive oxygen species, and this oxidation can disrupt protein structure and function [86]. Methionine can be oxidized to

**Fig. 5** Production of allicin from alliin by alliinase

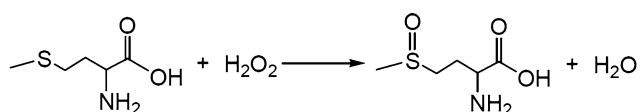


methionine sulfoxide (MetSO) by ROS and reactive nitrogen species (RNS) such as hydrogen peroxide, hydroxyl radical, and peroxyxynitrite anion [87, 88, 202]. ROS-mediated oxidation of Met results in mixtures of *R*- and *S*-isomers of MetSO [203]. Metal-catalyzed oxidation of methionine occurs through Fenton or Fenton-like reactions when metals such as iron and copper are reduced, subsequently producing hydroxyl radical upon reaction with hydrogen peroxide [87, 204–206].

MetSO can also be formed from methionine oxidized by H<sub>2</sub>O<sub>2</sub> alone in the absence of metal ions (Fig. 6) [207, 208], and both peroxyxynitrous acid (ONOOH) and peroxyxynitrite also react with methionine to produce MetSO [88, 202]. Methionine oxidation can result in changes in protein hydrophobicity, alterations in protein conformation, and disruption of biological function [91, 204, 209–214]. However, cells contain two enzymes that reduce MetSO to Met, repairing the oxidative damage [87, 89–91]. Methionine sulfoxide reductase A (MsrA) specifically reduces the *S*-isomer and methionine sulfoxide reductase B (MsrB) is specific for the reduction of the *R*-isomer [87, 89, 91]. Based on the reversibility of MetSO generation, MetSO formation is proposed to be important in regulating cell functions [87, 91, 204, 213, 215–217]. Interestingly, there have been reports on one biologically relevant oxo-selenium compound formed from the oxidation of selenomethionine (SeMet) by peroxyxynitrite to methionine selenoxide (MetSeO) [218, 219]. This oxidation is analogous to the peroxyxynitrite oxidation of methionine [218].

### Antioxidant and Pro-Oxidant Effects of Oxo-Sulfur Compounds in Disease Prevention

Many studies have reported antioxidant effects and disease amelioration by supplementation with aged garlic extracts (AGE) and garlic essential oils [220–222]. Although these products are generally considered to be safe and may be effective in preventing diseases such as cancer, it is difficult to determine the bioactive sulfur component or mixture of components that is responsible for the observed biological effects [220, 221]. Therefore, this review will focus on the ROS damage prevention (antioxidant) or ROS generation (pro-oxidant) abilities of individual oxo-sulfur compounds.



**Fig. 6** Oxidation of methionine to methionine sulfoxide by hydrogen peroxide

Several studies have shown that oxo-sulfur compounds play a significant role in preventing ROS-mediated cellular damage. For example, allicin, a major component in garlic, acts as a vasodilator, inhibits cholesterol biosynthesis, ameliorates serum lipid in hyperlipidemic rabbits, and lowers intraocular pressure in normal rabbits [193, 223–226]. In a separate study, both allicin and alliin had no effect in reducing lipid peroxidation induced by ferrous sulfate/ascorbic acid in microsomal membranes (Table 3) [227]. Another study to determine the effect of thiosulfonates on platelet aggregation showed that allicin inhibited 74% of such aggregation with an IC<sub>50</sub> of 0.27 mM [189]. Hirsh et al. [193] found that allicin (10–40 μM) inhibited cell proliferation in mammary, colon, and endometrial cancer cells with 50% inhibition at 10–25 μM. Alliin, the precursor to allicin, showed no inhibitory effect at all concentrations tested (0–64 μM) [193].

Another oxo-sulfur compound found in *Allium* vegetables is MeCysSO; however, only a few studies have been performed on this possible antioxidant sulfur derivative, despite the fact that it is a major oxo-sulfur compound in garlic (Table 3). MeCysSO is effective for the treatment of hyperglycemia and hyperlipidemia in diabetic rats [228–230]. In addition, daily oral administration of MeCysSO (200 mg/kg) for 45 days significantly controlled blood glucose and lipids in tissues and serum of diabetic rats [228]. The activities of HMG CoA reductases, liver hexokinase, and glucose-6-phosphate in these animals were close to normal upon MeCysSO treatment, effects similar to the anti-diabetic drugs glibenclamide and insulin [228]. Kumari and Augusti [229] also observed that MeCysSO was effective in lowering total cholesterol levels in rats.

Similar to allicin, MMTS also has chemopreventative properties [195]. Nakamura et al. [195–197] found that MMTS isolated from cauliflower homogenate showed strong antimutagenic activity against UV-induced mutation in wild-type *E. coli* (B/r WP2), but not in mutant cell strains lacking excision-repair activities. A separate study by the same group showed that MMTS (10 mg/kg) suppressed the frequency of aflatoxin B<sub>1</sub>-induced chromosome aberrations after 2 h [195]. Additionally, Kawamori et al. [231] reported that MMTS (20 and 100 ppm) inhibited 42 and 21% of intestinal neoplasm incidences induced by azoxymethane in rats, respectively.

MMTS also decreased the incidence of phenobarbital-promoted or diethylnitrosamine-initiated hepatocarcinogenesis in rats [232]. In *Drosophila melanogaster* and mouse micronuclei, MMTS was found to reduce mitomycin C-induced somatic mutation and recombination via oral administration [233]. Experiments performed with various antioxidants, including Kefir grain extracts, showed that both Kefir extracts (900–21,000 μg/ml) and MMTS (10 μg/ml) stimulated more than 50% thymine dimer repair

**Table 3** Dietary sources and application of various oxo-sulfur compounds

Oxo-Sulfur compound	Source	Treatment	References
Allicin	Garlic ( <i>Allium</i> vegetables)	Vasodilator, inhibits cholesterol biosynthesis, platelet aggregation and cancer cell proliferation, lowers serum lipids and intraocular pressure	[193, 223–226]
MeCysSO	Garlic, onions ( <i>Allium</i> vegetables)	Reduces hyperglycemia and hyperlipidemia, lowers cholesterol levels in rats	[228, 229]
MMTS	Cauliflower, broccoli, cabbage	Chemoprevention	[195–197, 231–233]
Me <sub>2</sub> SO <sub>2</sub>	Broccoli, peppers, asparagus, dietary supplements. Trace amounts in fish, meats and eggs, creams and lotions.	Inflammation, parasitic inflammation, arthritis, asthma, allergies, nourishes hair, nail and skin, maintenance of connective tissue	[198, 199, 235]

in UVC-irradiated HMV-1 cells, whereas other antioxidants tested (epigallocatechin and vitamins A, C, E, and K) showed little repair enhancement ( $\leq 10$ –30%) [234].

While the use of Me<sub>2</sub>SO<sub>2</sub> in commercially available products (Table 3) is generally considered safe, little data is available to assess the safety and toxicity of this oxo-sulfur compound. Unconfirmed side effects of Me<sub>2</sub>SO<sub>2</sub> consumption include headaches, hypertension, gastrointestinal symptoms, insomnia (if taken before bedtime), and increased hepatic enzyme levels [198]. Me<sub>2</sub>SO<sub>2</sub> has been used to treat inflammation, parasitic infections, allergies, asthma, cancer, arthritis, and rheumatic pain [198, 199]. Due to its sulfur content, it has also been used for nourishing hair, fingernails, and skin, and in the maintenance of normal connective tissues [198, 235]. In one study to determine the acute (2000 mg/kg/day) and chronic (1500 mg/kg/day) effects on rats, oral administration of Me<sub>2</sub>SO<sub>2</sub> for 90 days did not affect body weight, histopathological lesions in tissues and organs, hematological parameters, or mortality [82].

Early pharmacokinetic studies showed that  $\sim 64\%$  of [<sup>35</sup>S]Me<sub>2</sub>SO<sub>2</sub> (21 mg/kg) was excreted within 24 h upon intraperitoneal administration to rats [236]. In a more recent study, the distribution of Me<sub>2</sub>SO<sub>2</sub> administered orally for 7 days using a <sup>35</sup>S radioisotope tracer found that [<sup>35</sup>S]Me<sub>2</sub>SO<sub>2</sub> (470 mg/kg/day) was excreted in urine ( $\sim 70\%$ ) and feces ( $\sim 10\%$ ) [237]. The highest levels of radioactivity were found in hair, blood, and spleen [237]. Further investigations of Me<sub>2</sub>SO<sub>2</sub> pharmacokinetics in rats was performed by Magnuson et al. [238]. They also used radiolabeled [<sup>35</sup>S]Me<sub>2</sub>SO<sub>2</sub> to determine that Me<sub>2</sub>SO<sub>2</sub> is rapidly absorbed, well distributed, and efficiently eliminated [238]. Oral administration of 500 mg/kg in rats showed that the majority of Me<sub>2</sub>SO<sub>2</sub> was excreted in urine ( $\sim 57\%$ ), with only 1.6% excreted in feces [238]. After 48 h, Me<sub>2</sub>SO<sub>2</sub> was distributed evenly in several tissues, corresponding to blood concentrations of this compound [238].

Two separate studies performed by Cottler-Fox and co-workers [239] and Wever and co-workers [240] found that

Me<sub>2</sub>SO<sub>2</sub> was present in human plasma and cerebrospinal fluid ranging from 0 to 25  $\mu$ M. Me<sub>2</sub>SO<sub>2</sub> was also detected by magnetic resonance spectroscopy in the brains of patients with memory loss and in normal patients given doses of 1–3 g/day [241]. In this investigation, Me<sub>2</sub>SO<sub>2</sub> was found to be distributed equally between white and gray matter of the brain in all patients, ranging from 0.42 to 3.40 mmol/kg, with no adverse neurochemical or clinical effects [241]. Me<sub>2</sub>SO<sub>2</sub> was also detected in the brain at a concentration of 2.36 mM in a normal patient after taking Me<sub>2</sub>SO<sub>2</sub> at a dosage of 181 mg/kg/day [242].

While the suggested use of Me<sub>2</sub>SO<sub>2</sub> is as a dietary supplement for the treatment of arthritis and allergies, only a few studies have reported such activities [199, 243, 244]. A 12-week randomized and controlled study on individuals with knee osteoarthritis was conducted, with each of the 118 patients given Me<sub>2</sub>SO<sub>2</sub> (1.5 g/day). One-third reported decrease in pain; joint mobility and improved walking time was also observed [244]. In a second study, 21 patients with osteoarthritis given Me<sub>2</sub>SO<sub>2</sub> (3 g) twice daily for a 12-week period showed decreases in pain (25%), stiffness (20%), physical function (31%), and total symptoms (25%) [198]. Interestingly, in this study, patients in the placebo group also showed decreases in pain, stiffness, physical function, and total symptoms ( $\sim 13$ , 12, 17, and 14%, respectively) [198]. Although Me<sub>2</sub>SO<sub>2</sub> may ameliorate the effects of osteoarthritis, its full effects were not observed in such a short period of time, indicating the need for longer trials [198]. A study to evaluate the efficacy of Me<sub>2</sub>SO<sub>2</sub> for reduction of seasonal allergy rhinitis (SAR) symptoms such as headaches, sinus infections, breathing difficulties, and nasal congestion was performed by Barrager et al. [199]. The results of this study indicate that Me<sub>2</sub>SO<sub>2</sub> supplementation of 2600 mg/day over a period of 30 days was effective in reducing the symptoms of SAR [199]. Me<sub>2</sub>SO<sub>2</sub> was also found to have minimal side effects, suggesting that this oxo-sulfur compound may be therapeutic for the amelioration of SAR-associated symptoms [199].

## Antioxidant and Pro-Oxidant Mechanisms of Oxo-Sulfur Compounds

Although numerous studies show that oxo-sulfur compounds can be used in the treatment or prevention of several diseases including cancer and osteoarthritis, the mechanisms of such action have not been elucidated [193–195, 198, 212, 220, 230, 232]. Studies by Rabinkov and co-workers [194] indicate two possible mechanisms for the biological activity of allicin: radical scavenging or its ability to react with thiols. Spin trapping techniques and EPR measurements were used to show that allicin efficiently scavenged  $\cdot\text{OH}$  produced by the Fenton reaction [194]. The ability of allicin to inhibit the functions of thiol-containing proteins such as papain and alcohol dehydrogenase was also suggested as a possible mechanism [194].

The susceptibility of Met to oxidation results in antioxidant activity due to its radical scavenging capabilities, and the ability of Msr to reduce MetSO back to Met [212]. Evidence of such antioxidant activity is observed in studies performed in yeast and *Drosophila*, which showed that over-expression of the MsrA gene prevents oxidative stress induced by toxic levels of hydrogen peroxide and paraquat [245, 246]. Studies performed on various bacterial strains and yeast lacking the MsrA gene determined that these strains were more susceptible to paraquat- or  $\text{H}_2\text{O}_2$ -induced oxidative stress [245, 247, 248]. Under normal growth conditions, studies performed on MsrA knockout mice resulted in a 40% decrease of maximal life-span upon exposure to 20% oxygen [249]. In other studies, Met residues on the lipoprotein surface reduced low-density (LDL) and high-density (HDL) lipoprotein-generated peroxides and cholesterol peroxide to their respective hydroxyl derivatives [91, 250].

The oxidation of methionine to methionine sulfoxide has been implicated in aging, adult respiratory distress syndrome, and emphysema [213, 215]. A study by Costabel and co-workers [251] showed that methionine oxidation by neutrophil-generated ROS results in acute and chronic bronchitis, dependent on the Met/MetSO ratio in lavage fluid in the bronchialveolar. An investigation by Stadtman et al. [211] indicated that surface hydrophobicity of rat liver proteins increased with age over a period of 24 months. This study also indicated that increases in both hydrophobicity and MetSO levels were caused by protein oxidation from ROS [211].

Gradual decreases in the levels of MsrA activity in the liver and the kidney tissues of rats were found to be age-related, whereas in brain tissues, no age-related loss of enzyme activity was observed [252]. MsrA activity in rat kidney and liver tissues decreased from  $\sim 296$  and  $\sim 181$  pmol/min/mg, respectively, at 9 months of age to  $\sim 158$  and  $\sim 85$  pmol/min/mg, respectively, at 29 months

[252]. In addition, the proposed Met/MetSO antioxidant cycle may aid in the prevention of Alzheimer's disease [87, 253]. A study performed by Markesbery et al. showed that in various brain regions of Alzheimer's patients, the level of MetSO and protein carbonyls, another measure of oxidative damage, was significantly greater than in the brains of patients without this disease [253].

In an animal study, 67% of Met residues in rat brain calmodulin were oxidized to MetSO in aged rats [254]. Experiments by Wells-Knecht et al. [255] showed that in humans, the methionine sulfoxide content of skin collagen increases from approximately 4%, while young to approximately 12% at 80 years of age. Collectively, these studies indicate the oxidation and reduction processes of methionine, as well as the expression of MsrA, play an important role in the prevention of ROS-mediated diseases. Interestingly, methionine oxidation increases protein hydrophobicity, despite the fact that Met is more hydrophobic than MetSO [211, 256]. Further investigations are therefore needed to better understand effects of the Met/MetSO oxidation cycle at the molecular level, since local changes in protein folding may play a role in hydrophobicity alterations [215].

Similar to DNA inhibition experiments performed with inorganic selenium compounds, the ability of oxo-sulfur compounds to prevent metal-mediated DNA damage has also been investigated (Table 4). MetSO, MeCysSO, MMTS,  $\text{Me}_2\text{SO}_2$ , and methyl phenyl sulfoxide (MePhSO; Fig. 2) showed little inhibition of iron-mediated oxidative DNA damage. While MeCysSO and MMTS inhibited 17 and 20% DNA damage at 1000  $\mu\text{M}$ , respectively, the other oxo-sulfur compounds showed no effect on DNA damage [51]. Likewise,  $\text{Me}_2\text{SO}_2$  and MePhSO have no effect on copper-mediated DNA damage, whereas MMTS (1000  $\mu\text{M}$ ) is a pro-oxidant, producing 35% damaged DNA, and both MetSO and MeCysSO are efficient antioxidants with  $\text{IC}_{50}$  values of  $18 \pm 3$  and  $8.1 \pm 1$   $\mu\text{M}$ , respectively [51]. Interestingly, the reduced forms of MeCysSO and MetSO, MeCys and Met, were also effective antioxidants with  $\text{IC}_{50}$  values of  $8.9 \pm 0.02$   $\mu\text{M}$  and  $11.2 \pm 0.02$   $\mu\text{M}$ , respectively [49]. Thus, the ability of these sulfur-containing amino acids to prevent copper-mediated DNA damage does not significantly change upon oxidation [51].

Similar to the results obtained with the inorganic selenium compounds, metal coordination is also a mechanism for antioxidant activity of oxo-sulfur compounds [23, 49, 51, 74, 150]. Brumaghim et al. showed that sulfur compounds with the ability to prevent copper-mediated DNA damage have a Cu–S charge transfer band at  $\sim 240$  nm when combined with  $\text{Cu}^+$ , indicative of copper-sulfur coordination. Both MetSO and MeCysSO show similar UV bands with  $\text{Cu}^+$ , also indicating copper binding to these compounds [49, 51, 74]. Interestingly, in gel electrophoresis experiments

**Table 4** The antioxidant and pro-oxidant activity of sulfur and oxo-sulfur compounds

Compound	Antioxidant or pro-oxidant behavior	References
MetSO	Fe <sup>2+</sup> : No effect on DNA damage Cu <sup>+</sup> : Antioxidant (IC <sub>50</sub> = 18 ± 1.0 μM)	[51]
MeCysSO	Fe <sup>2+</sup> : Pro-oxidant behavior (17% ± 3.0 DNA damage at 1000 μM) Cu <sup>+</sup> : Antioxidant (IC <sub>50</sub> = 8.1 ± 3.0 μM)	[51]
MMTS	Fe <sup>2+</sup> : Pro-oxidant behavior (20% ± 4.0 DNA damage at 1000 μM) Cu <sup>+</sup> : Pro-oxidant behavior (35% ± 4.0 DNA damage at 1000 μM)	[51]
MePhSO	Fe <sup>2+</sup> : No effect on DNA damage Cu <sup>+</sup> : No effect on DNA damage Photoactivation: Pro-oxidant (83% DNA damage at 360 μM)	[51] [258]
Me <sub>2</sub> SO <sub>2</sub>	Fe <sup>2+</sup> : No effect on DNA damage Cu <sup>+</sup> : No effect on DNA damage	[51]
PhSO	Photoactivation: Pro-oxidant (83% DNA damage at 180 μM)	[258]
Met	Cu <sup>+</sup> : Antioxidant (IC <sub>50</sub> = 11.2 ± 0.02 μM)	[49]
MeCys	Cu <sup>+</sup> : Antioxidant (IC <sub>50</sub> = 8.9 ± 0.02 μM)	[49]

using 2,2'-bipyridine (bipy) to completely coordinate Cu<sup>+</sup>, MetSO and MeCysSO at 1000 μM, inhibited 43 and 88% of [Cu(bipy)<sub>2</sub>]<sup>+</sup>-mediated DNA damage, respectively, significantly less than the inhibition observed for uncoordinated Cu<sup>+</sup> [51]. The results of these studies demonstrate that metal coordination is a primary mechanism for the antioxidant activity of MetSO and MeCysSO, but a second mechanism, such as radical scavenging, may also be responsible for their DNA damage prevention at high concentrations [51]. Under similar conditions, MMTS (5000 μM) is a pro-oxidant, damaging 44% DNA in the presence of [Cu(bipy)<sub>2</sub>]<sup>+</sup>/H<sub>2</sub>O<sub>2</sub>, indicating that copper coordination is not required for its pro-oxidant activities [51]. Taken together, these experiments indicate that the antioxidant or pro-oxidant activities of sulfur compounds are quite complex, and highlight the importance of understanding the conditions and mechanisms for such behavior.

Recently, aryl sulfoxides such as phenyl sulfoxide (PhSO) and MePhSO (Fig. 2) have been found to generate ROS upon irradiation, with implicated use in cancer therapy [257–259]. Interestingly, few studies have reported on the effects of this ROS generation on DNA damage (Table 4). In one such study, Predecki and co-workers [258] showed that photoactivation at 240 nm of PhSO and MePhSO results in 83% DNA damage at 180 and 360 μM, respectively. In these experiments, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) scavenged carbon-based radicals, indicating that the possible mechanism for photoinduced-DNA damage by aryl sulfoxides is caused by hydrogen abstraction from the DNA backbone to produce a DNA radical [258]. It is assumed that the reaction between the DNA radical and the oxygen is responsible for DNA damage generated by PhSO and MePhSO [258, 259]. Oxygen, in its ground state (<sup>3</sup>O<sub>2</sub>), may also be necessary for the pro-oxidant effects of PhSO, since photo-induced DNA damage was inhibited in

the absence of oxygen [258]. These studies clearly indicate that the mechanistic action of oxo-sulfur compounds either as antioxidants or as pro-oxidants is quite complex, and further studies under biologically relevant conditions are essential to better understand the activity of these compounds in biological systems.

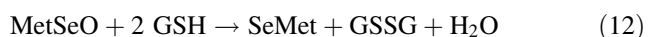
### Oxo-Selenium Compounds

Many studies have examined the antioxidant and anticancer effects of the oxo-selenium compound, methylselenic acid (MSeA), and this work has been recently reviewed [70, 260]. It is thought that MSeA exerts its observed anticancer properties both through oxidation of protein thiols, similar to selenite, and through formation of MeSe<sup>-</sup>, a species that can generate superoxide upon reacting with O<sub>2</sub> [260]. Thus, MSeA acts as a prooxidant to promote cell dysfunction and death. More recently, MSeA treatment (2.5–5 μM) was found to significantly increase thiol concentration and oxidative stress in the endoplasmic reticulum of human lung carcinoma cells [261]. In addition, MSeA has been found to decrease expression of several kinases involved in cell growth and apoptotic pathways [262], particularly protein kinase C [263], at low micromolar concentrations.

In prostate cancer cell lines (DU145 and LNCaP), Li et al. [264] reported that MSeA induced apoptosis without generating hydroxyl radical or superoxide. Oral administration of MSeA (4 mg Se/kg) significantly inhibited the growth of prostate cancer tumors in xenograft mice (DU145), comparable to effects observed for similar treatment with methylselenocysteine, and more effective than similar selenite treatment. Additionally, MSeA treatment resulted in significantly less genotoxicity and higher inhibition of tumor

angiogenesis compared to methylselenocysteine and selenite [265]. Clearly, the observed anticancer properties of MSeA may arise from a combination of several different mechanisms, and more work is required to develop a complete understanding of its biological activity.

The naturally occurring amino acid, selenomethionine (SeMet) is found in many proteins in place of its sulfur analog, methionine [266, 267]. Similar to methionine oxidation to its corresponding sulfoxide, SeMet is also oxidized by peroxytrioxide and enzymes such as flavin-containing monooxygenases (FMOs) to methionine selenoxide (MetSeO) [218, 219, 266]. A significant amount of research has investigated Met/MetSO interconversion, but little work has investigated the properties of MetSeO. Oxidation kinetics of SeMet are 10–1000 times faster than oxidation of methionine to produce MetSO [219]. While the effects of MetSeO in biological systems have not been reported, it is possible that this compound may lead to similar changes in hydrophobicity, conformation, and disruption of biological functions associated with its sulfur analog, MetSO [91, 204, 209–214]. In light of this, Sies and colleagues [218] investigated the reduction of MetSeO to SeMet using glutathione (GSH) as a reductant. This study showed that addition of MetSeO (0.4 mM) to increasing concentrations of GSH resulted in a loss of MetSeO with an increase in SeMet (Reaction 12), suggesting that GSH in low concentrations is effective in the protection against oxidants and that GSH may be responsible for redox cycling of selenoxides [218]. Similar reduction of MetSeO to SeMet was also reported to occur with cysteine, *N*-acetylcysteine, and ascorbic acid under conditions that did not reduce MetSO to Met [268].



Apart from its role in protein function, SeMet metabolism by the methionine transsulfuration pathway produces selenocysteine, an essential amino acid for the function of several antioxidant enzymes including glutathione peroxidase and thioredoxin reductase [266, 269]. Interestingly, FMOs also have been shown to oxidize other selenium compounds, including the selenium containing drug ebselen, to their corresponding selenoxides [270]. Thus, further investigations are necessary to determine the effects of oxidation on protein function and activity and the conditions required for antioxidant and pro-oxidant activity of oxidized sulfur and selenium compounds, including selenium-containing drugs.

## Conclusions

Investigating the antioxidant and anticancer properties of inorganic selenium and oxo-sulfur compounds in the

treatment of diseases such as cancer, aging, and neurodegenerative diseases generated from reactive oxygen species is an active and promising area of research. In the case of inorganic selenium compounds, sodium selenite is the compound of choice for both antioxidant and anticancer studies. While the mechanism for its antioxidant activity is unclear, it has been proposed that the ability of selenocysteine containing enzymes such as GPx and thioredoxin reductases to prevent radical formation is a possible mechanism [57]. The generation of hydrogen selenide from selenometabolites such as selenite to produce toxic ROS is also suggested as a possible mechanism for the pro-oxidant and anticancer properties of inorganic selenium compounds [55, 57].

The antioxidant activity of oxo-sulfur compounds is much less understood than that of the inorganic selenium compounds. Most studies have focused on allicin, an oxo-sulfur compound produced from crushed garlic; however, disparities concerning the antioxidant activity of allicin are attributed to other endogenous components remaining from the extraction process of crude garlic extracts [53, 54]. While MMTS and other oxo-sulfur compounds may be effective in the treatment or prevention of cancer, their mode of action has not been investigated. Similarly, little is known about the antioxidant activity of MeCysSO, MetSeO, and MePhSO; however, the ability of MetSO to prevent ROS-mediated diseases such as aging, emphysema, and Alzheimer's has been attributed to the cyclic interconversion of MetSO by methionine sulfoxide reductases [213, 215]. Although, MeCysSO and MetSeO are analogous to MetSO, little work has explored the effects of these compounds in biological systems. Since MeCysSO and MetSeO are formed from the oxidation of amino acids essential for protein function, investigating the ability of these compounds to act as antioxidants, as well as their role in disease prevention is of great interest. Therefore, further experiments including both in vitro, and cellular and animal studies are required to investigate such behaviors. In addition, studies are also needed to determine the effects of the sulfur compounds' structural and chemical properties on their antioxidant activity.

Investigations of the ability of inorganic selenium and oxo-sulfur compounds to inhibit metal-mediated oxidative DNA damage determined that coordination between iron or copper and the antioxidant compound is required for prevention of DNA damage [23, 51]. Additionally, the ability of oxo-sulfur compounds to scavenge radicals was also suggested as a possible mechanism for this activity, particularly at high concentrations [51]. Further investigations to determine the role of metal coordination and radical scavenging mechanisms of antioxidant behavior as well as the conditions required to observe pro-oxidant properties of oxo-sulfur compounds are necessary to fully understand the biological activities of these compounds.

In addition, the role of less studied compounds such as selenate, selenium dioxide, methylcysteine sulfoxide, and methionine selenoxide in the prevention of ROS-mediated disease is necessary to better understand and elucidate a mechanism for the antioxidant and pro-oxidant activities of both inorganic and oxo-sulfur compounds. Although the selenium speciation is an important factor in the biological activities of these compounds [154–156], additional studies using larger numbers of selenium compounds including selenide, selenate, and selenoxides are required to fully understand this effect. Most of the inorganic selenium and oxo-compounds discussed in this review are obtained from food products such as vegetables, fruits, nuts, and dietary supplements. However, these foods and supplements may also contain other antioxidant or bioactive ingredients [1, 46, 47]. It is therefore equally important to understand the effects of other bioactive compounds on the biological activity of inorganic and oxo-sulfur compounds. Additional studies exploring the antioxidant, pro-oxidant, and mechanistic actions of inorganic selenium, oxo-sulfur, and oxo-selenium compounds are essential to fully understand the biological implications of sulfur- and selenium-containing food products and supplements in both humans and animals.

## References

- Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telsler, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*, 39, 44–84.
- Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, 160, 1–40.
- Storz, G., & Imlay, J. A. (1999). Oxidative stress. *Current Opinion in Microbiology*, 2, 188–194.
- Farr, S. B., & Kogoma, T. (1991). Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*. *Microbiological Reviews*, 55, 561–585.
- Cadenas, E., & Davies, K. J. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radical Biology and Medicine*, 29, 222–230.
- Orrenius, S., Gogvadze, V., & Zhivotovsky, B. (2007). Mitochondrial oxidative stress: Implications for cell death. *Annual Review of Pharmacology and Toxicology*, 47, 143–183.
- Marnett, L. J. (2000). Oxyradicals and DNA damage. *Carcinogenesis*, 21, 361–370.
- Huang, X. (2003). Iron overload and its association with cancer risk in humans: Evidence for iron as a carcinogenic metal. *Mutation Research*, 533, 153–171.
- Halliwell, B. (2001). Role of free radicals in the neurodegenerative diseases: Therapeutic implications for antioxidant treatment. *Drugs and Aging*, 18, 685–716.
- Markesbery, W. R. (1997). Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biology and Medicine*, 23, 134–147.
- Markesbery, W. R., & Carney, J. M. (1999). Oxidative alterations in Alzheimer's disease. *Brain Pathology*, 9, 133–146.
- Markesbery, W. R., & Lovell, M. A. (2006). DNA oxidation in Alzheimer's disease. *Antioxidants and Redox Signaling*, 8, 2039–2205.
- Ide, T., Tsutsui, H., Hayashidani, S., Kang, D., Suematsu, N., Nakamura, K., et al. (2001). Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. *Circulation Research*, 88, 529–535.
- Steinberg, D. (1997). Low density lipoprotein oxidation and its pathobiological significance. *The Journal of Biological Chemistry*, 272, 20963–20966.
- Dhalla, N. S., Temsah, R. M., & Nettican, T. (2000). Role of oxidative stress in cardiovascular diseases. *Journal of Hypertension*, 18, 655–673.
- Kastan, M. B. (2008). DNA damage responses: Mechanisms and roles in human disease: 2007 G.H.A. Clowes Memorial Award Lecture. *Molecular Cancer Research*, 6, 517–524.
- McCormick, M. L., Buettner, G. R., & Britigan, B. E. (1998). Endogenous superoxide dismutase levels regulate iron-dependent hydroxyl radical formation in *Escherichia coli* exposed to hydrogen peroxide. *Journal of Bacteriology*, 180, 622–625.
- Henle, E. S., & Linn, S. (1997). Formation, prevention, and repair of DNA damage by iron/hydrogen peroxide. *The Journal of Biological Chemistry*, 272, 19095–19098.
- Flint, D. H., Tuminello, J. F., & Emptage, M. H. (1993). The inactivation of Fe-S cluster containing hydro-lyases by superoxide. *The Journal of Biological Chemistry*, 268, 22369–22376.
- Park, S., & Imlay, J. A. (2003). High levels of intracellular cysteine promote oxidative DNA damage by driving the fenton reaction. *Journal of Bacteriology*, 185, 1942–1950.
- Rodriguez, H., Holmquist, G. P., D'Agostino, R., Jr., Keller, J., & Akman, S. A. (1997). Metal ion-dependent hydrogen peroxide-induced DNA damage is more sequence specific than metal specific. *Cancer Research*, 57, 2394–2403.
- Seaver, L. C., & Imlay, J. A. (2004). Are respiratory enzymes the primary sources of intracellular hydrogen peroxide? *The Journal of Biological Chemistry*, 279, 48742–48750.
- Ramoutar, R. R., & Brumaghim, J. L. (2007). Effects of inorganic selenium compounds on oxidative DNA damage. *Journal of Inorganic Biochemistry*, 101, 101028–101035.
- Imlay, J. A., & Linn, S. (1988). DNA damage and oxygen radical toxicity. *Science*, 240, 1302–1309.
- Hoffmann, M. E., Mello-Filho, A. C., & Meneghini, R. (1984). Correlation between cytotoxic effect of hydrogen peroxide and the yield of DNA strand breaks in cells of different species. *Biochimica et Biophysica Acta*, 781, 234–238.
- Henle, E. S., Han, Z., Tang, N., Rai, P., Luo, Y., & Linn, S. (1999). Sequence-specific DNA cleavage by Fe<sup>2+</sup>-mediated Fenton reactions has possible biological implications. *The Journal of Biological Chemistry*, 274, 962–971.
- Imlay, J. A., Chin, S. M., & Linn, S. (1988). Toxic DNA damage by hydrogen peroxide through the Fenton reaction in vivo and in vitro. *Science*, 240, 640–642.
- Nunoshiba, T., Obata, F., Boss, A. C., Oikawa, S., Mori, T., Kawanishi, S., et al. (1999). Role of iron and superoxide for generation of hydroxyl radical, oxidative DNA lesions, and mutagenesis in *Escherichia coli*. *The Journal of Biological Chemistry*, 274, 34832–34837.
- Pastor, N., Weinstein, H., Jamison, E., & Brenowitz, M. (2000). A detailed interpretation of OH radical footprints in a TBP-DNA complex reveals the role of dynamics in the mechanism of sequence-specific binding. *Journal of Molecular Biology*, 304, 55–68.
- Emerit, J., Beaumont, C., & Trivin, F. (2001). Iron metabolism, free radicals, and oxidative injury. *Biomedicine and Pharmacotherapy*, 55, 333–339.



31. Stohs, S. J., & Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology and Medicine*, *18*, 321–336.
32. Lippard, S. J., & Berg, J. M. (1994). *Principles of bioinorganic chemistry*. Mills Valley: University Science Books.
33. Keyer, K., & Imlay, J. A. (1996). Superoxide accelerates DNA damage by elevating free-iron levels. *Proceedings of the National Academy of Sciences of the United States of America*, *93*, 13635–13640.
34. Woodmansee, A. N., & Imlay, J. A. (2002). Quantitation of intracellular free iron by electron paramagnetic resonance spectroscopy. *Methods in Enzymology*, *349*, 3–9.
35. Lippard, S. J. (1999). Free copper ions in the cell? *Science*, *284*, 748–749.
36. Rae, T. D., Schmidt, P. J., Pufahl, R. A., Culotta, V. C., & O'Halloran, T. V. (1999). Undetectable intracellular free copper: The requirement of a copper chaperone for superoxide dismutase. *Science*, *284*, 805–808.
37. Yang, L., McRae, R., Henary, M. M., Patel, R., Lai, B., Vogt, S., et al. (2005). Imaging of the intracellular topography of copper with a fluorescent sensor and by synchrotron x-ray fluorescence microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 11179–11184.
38. Que, E. L., Domaille, D. W., & Chang, C. J. (2008). Metals in neurobiology: Probing their chemistry and biology with molecular imaging. *Chemical Reviews*, *106*, 1517–1549.
39. Brown, D. R., Qin, K. F., Herms, J. W., Madlung, A., Manson, J., Strome, R., et al. (1997). The cellular prion protein binds copper in vivo. *Nature*, *390*, 684–687.
40. Stockel, J., Safar, J., Wallace, A. C., Cohen, F. E., & Prusiner, S. B. (1998). Prion protein selectively binds copper(II) ions. *Biochemistry*, *37*, 7185–7193.
41. Imlay, J. A., & Linn, S. (1986). Bimodal pattern of killing of DNA-repair-defective or anoxically grown *Escherichia coli* by hydrogen peroxide. *Journal of Bacteriology*, *166*, 519–527.
42. Imlay, J. A., & Linn, S. (1987). Mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. *Journal of Bacteriology*, *169*, 2967–2976.
43. Mello-Filho, A. C., & Meneghini, R. (1991). Iron is the intracellular metal involved in the production of DNA damage by oxygen radicals. *Mutation Research*, *251*, 109–113.
44. Singal, P. K., Khaper, N., Palace, V., & Kumar, D. (1998). The role of oxidative stress in the genesis of heart disease. *Cardiovascular Research*, *40*, 426–432.
45. Collins, A. R. (1999). Oxidative DNA damage, antioxidants, and cancer. *BioEssays*, *21*, 238–246.
46. Siman, C. M., & Eriksson, U. J. (1997). Vitamin E decreases the occurrence of malformations in the offspring of diabetic rats. *Diabetes*, *46*, 1054–1061.
47. Siman, C. M., & Eriksson, U. J. (1997). Vitamin C supplementation of the maternal diet reduces the rate of malformation in the offspring of diabetic rats. *Diabetologia*, *40*, 1416–1424.
48. Ames, B. N. (2001). DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. *Mutation Research*, *475*, 7–20.
49. Battin, E. E., & Brumaghim, J. L. (2008). Metal specificity in DNA damage prevention by sulfur antioxidants. *Journal of Inorganic Biochemistry*, *102*, 2036–2042.
50. Perron, N. R., Hodges, J. N., Jenkins, M., & Brumaghim, J. L. (2008). Predicting how polyphenol antioxidants prevent DNA damage by binding to iron. *Inorganic Chemistry*, *47*, 6153–6161.
51. Ramoutar, R. R., & Brumaghim, J. L. (2007). Investigating the antioxidant properties of oxo-sulfur compounds on metal-mediated DNA damage. *Main Group Chemistry*, *6*, 143–153.
52. Combs, G. F., & Gray, W. P. (1998). Chemopreventive agents: Selenium. *Pharmacology and Therapeutics*, *79*, 179–192.
53. Xiao, H., & Parkin, K. L. (2002). Antioxidant functions of selected allium thiosulfonates and S-alk(en)yl-L-cysteine sulfonides. *Journal of Agriculture and Food Chemistry*, *50*, 2488–2493.
54. Yin, M.-C., & Cheng, W.-S. (1998). Antioxidant activity of several *Allium* members. *Journal of Agriculture and Food Chemistry*, *46*, 4097–4101.
55. Holmgren, A. (2006). Selenite in cancer therapy: A commentary on “Selenite induces apoptosis in sarcomatoid malignant mesothelioma cells through oxidative stress”. *Free Radical Biology and Medicine*, *41*, 862–865.
56. Schrauzer, G. N. (2001). Nutritional selenium supplements: Product types, quality, and safety. *Journal of the American College of Nutrition*, *20*, 1–4.
57. Takahashi, M., Sato, T., Shinohara, F., Echigo, S., & Rikiishi, H. (2005). Possible role of glutathione in mitochondrial apoptosis of human oral squamous cell carcinoma caused by inorganic selenium compounds. *International Journal of Oncology*, *27*, 489–495.
58. Zhong, W., & Oberley, T. D. (2001). Redox-mediated effects of selenium on apoptosis and cell cycle in the LNCaP human prostate cancer cell line. *Cancer Research*, *61*, 7071–7078.
59. Finley, J. W., Ip, C., Lisk, D. J., Davis, C. D., Hintze, K. J., & Whanger, P. D. (2001). Cancer-protective properties of high-selenium broccoli. *Journal of Agriculture and Food Chemistry*, *49*, 2679–2683.
60. Spallholz, J. E., Mallory Boylan, L., & Rhaman, M. M. (2004). Environmental hypothesis: Is poor dietary selenium intake an underlying factor for arsenicosis and cancer in Bangladesh and West Bengal, India? *Science of the Total Environment*, *323*, 21–32.
61. Gladyshev, V. N., & Kryukov, G. V. (2001). Evolution of selenocysteine-containing proteins: Significance of identification and functional characterization of selenoproteins. *BioFactors*, *14*, 87–92.
62. Tapiero, H., Townsend, D. M., & Tew, K. D. (2003). The antioxidant role of selenium and seleno-compounds. *Biomedicine and Pharmacotherapy*, *57*, 134–144.
63. Brown, K. M., & Arthur, J. R. (2001). Selenium, selenoproteins and human health: A review. *Public Health Nutrition*, *4*, 593–599.
64. Stadtman, T. C. (2002). Discoveries of vitamin B<sub>12</sub> and selenium enzymes. *Annual Review of Biochemistry*, *71*, 1–16.
65. Brigelius-Flohe, R. (1999). Tissue-specific functions of individual glutathione peroxidases. *Free Radical Biology and Medicine*, *27*, 951–965.
66. Cartes, P., Gianfreda, L., & Mora, M. L. (2005). Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. *Plant and Soil*, *276*, 359–367.
67. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. G. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, *179*, 588–590.
68. Tinggi, U. (2003). Essentiality and toxicity of selenium and its status in Australia: A review. *Toxicology Letters*, *137*, 103–110.
69. Beilstein, M. A., & Whanger, P. D. (1986). Chemical forms of selenium in rat tissues after administration of selenite or selenomethionine. *Journal of Nutrition*, *116*, 1711–1719.
70. Abdulah, R., Miyazaki, K., Nakazawa, M., & Koyoma, H. (2005). Chemical forms of selenium for cancer prevention. *Journal of Trace Elements in Medicine and Biology*, *19*, 141–150.
71. Atmaca, G. (2004). Antioxidant effects of sulfur-containing amino acids. *Yonsei Medical Journal*, *45*, 776–788.
72. Waschulewski, I. H., & Sunde, R. A. (1988). Effect of dietary methionine on tissue selenium and glutathione peroxidase (EC

- 1.11.1.9) activity in rats given selenomethionine. *British Journal of Nutrition*, 60, 57–68.
73. Fairweather-Tait, S. J. (1997). Bioavailability of selenium. *European Journal of Clinical Nutrition*, 51, S20–S23.
  74. Battin, E. E., Perron, N. R., & Brumaghim, J. L. (2006). The central role of metal coordination in selenium antioxidant activity. *Inorganic Chemistry*, 45, 499–501.
  75. Davis, C. D., Feng, Y., Hein, D. W., & Finley, J. W. (1999). The chemical form of selenium influences 3,2'-dimethyl-4-aminobiphenyl-DNA adduct formation in rat colon. *Journal of Nutrition*, 129, 63–69.
  76. Ip, C., & Hayes, C. (1989). Tissue selenium levels in selenium-supplemented rats and their relevance in mammary cancer protection. *Carcinogenesis*, 10, 921–925.
  77. Hamilton, E. E., & Wilker, J. J. (2004). Inhibition of DNA alkylation damage with inorganic salts. *Journal of Biological Inorganic Chemistry*, 9, 894–902.
  78. Nilsson, G., Sun, X., Nystrom, C., Rundlof, A. K., Potamitou Fernandes, A., Bjornstedt, M., et al. (2006). Selenite induces apoptosis in sarcomatoid malignant mesothelioma cells through oxidative stress. *Free Radical Biology and Medicine*, 41, 874–885.
  79. Zhou, N., Xiao, H., Li, T. K., Nur, E. K. A., & Liu, L. F. (2003). DNA damage-mediated apoptosis induced by selenium compounds. *The Journal of Biological Chemistry*, 278, 29532–29537.
  80. Lima, E. A. C., Dire, G., Mattos, D. M. M., Freitas, R. S., Gomes, M. L., de Oliveira, M. B. N., et al. (2002). Effect of an extract of cauliflower (leaf) on the labeling of blood elements with technetium-99 m and on the survival of *Escherichia coli* AB1157 submitted to the treatment with stannous chloride. *Food and Chemical Toxicology*, 40, 919–923.
  81. Rose, P., Whiteman, M., Moore, P. K., & Zhu, Y. Z. (2005). Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus *Allium*: The chemistry of potential therapeutic agents. *Natural Product Reports*, 22, 351–368.
  82. Horvath, K., Noker, P. E., Somfai-Relle, S., Glavits, R., Financsek, I., & Schauss, A. G. (2002). Toxicity of methylsulfonylmethane in rats. *Food and Chemical Toxicology*, 40, 1459–1462.
  83. Magnuson, B. A., Appleton, J., Ryan, B., & Matulka, R. A. (2007). Oral developmental toxicity study of methylsulfonylmethane in rats. *Food and Chemical Toxicology*, 45, 977–984.
  84. Pearson, T. W., Dawson, H. J., & Lackey, H. B. (1981). Natural occurring levels of dimethyl sulfoxide in selected fruits, vegetables, grains, and beverages. *Journal of Agriculture and Food Chemistry*, 29, 1089–1091.
  85. Silva Ferreira, A. C., Rodrigues, P., Hogg, T., & Guedes, D. P. (2003). Influence of some technological parameters on the formation of dimethyl sulfide, 2-mercaptoethanol, methionol, and dimethyl sulfone in port wines. *Journal of Agriculture and Food Chemistry*, 51, 727–732.
  86. Berlett, B. S., & Stadtman, E. R. (1997). Protein oxidation in aging, disease, and oxidative stress. *The Journal of Biological Chemistry*, 272, 20313–20316.
  87. Hoshi, T., & Heinemann, S. (2001). Regulation of cell function by methionine oxidation and reduction. *Journal of Physiology*, 531, 1–11.
  88. Perrin, D., & Koppenol, W. H. (2000). The quantitative oxidation of methionine to methionine sulfoxide by peroxynitrite. *Archives of Biochemistry and Biophysics*, 377, 266–272.
  89. Levine, R. L., Berlett, B. S., Moskovitz, J., Mosoni, L., & Stadtman, E. R. (1999). Methionine residues may protect proteins from critical oxidative damage. *Mechanisms of Ageing and Development*, 107, 323–332.
  90. Schoneich, C. (2002). Redox processes of methionine relevant to beta-amyloid oxidation and Alzheimer's disease. *Archives of Biochemistry and Biophysics*, 397, 370–376.
  91. Stadtman, E. R., Moskovitz, J., & Levine, R. L. (2003). Oxidation of methionine residues of proteins: Biological consequences. *Antioxidants and Redox Signaling*, 5, 577–582.
  92. Kim, H. Y., & Gladyshev, V. N. (2007). Methionine sulfoxide reductases: Selenoprotein forms and roles in antioxidant protein repair in mammals. *Biochemical Journal*, 407, 321–329.
  93. Pennington, J. A., & Schoen, S. A. (1996). Total diet study: Estimated dietary intakes of nutritional elements, 1982–1991. *International Journal for Vitamin and Nutrition Research*, 66, 350–362.
  94. Pennington, J. A., & Schoen, S. A. (1996). Contributions of food groups to estimated intakes of nutritional elements: Results from the FDA total diet studies, 1982–1991. *International Journal for Vitamin and Nutrition Research*, 66, 342–349.
  95. Burk, R. F. (2002). Selenium, an antioxidant nutrient. *Nutrition in Clinical Care*, 5, 75–79.
  96. Deore, M. D., Srivastava, A. K., & Sharma, S. K. (2005). Effect of reduced glutathione treatment on selenosis, blood selenium concentration and glutathione peroxidase activity after repeated short-term selenium exposure in buffalo calves. *Toxicology*, 213, 169–174.
  97. Yang, G., Wang, S., Zhou, R., & Sun, S. (1983). Endemic selenium intoxication of humans in China. *American Journal of Clinical Nutrition*, 37, 872–881.
  98. Longnecker, M. P., Taylor, P. R., Levander, O. A., Howe, M., Veillon, C., McAdam, P. A., et al. (1991). Selenium in diet, blood, and toenails in relation to human health in a seleniferous area. *American Journal of Clinical Nutrition*, 53, 1288–1294.
  99. Koller, L. D., & Exon, J. H. (1986). The two faces of selenium - deficiency and toxicity - are similar in animals and man. *Canadian Journal of Veterinary Research*, 50, 297–306.
  100. MacDonald, D. W., Christian, R. G., Strausz, K. I., & Roff, J. (1981). Acute selenium toxicity in neonatal calves. *Canadian Veterinary Journal*, 22, 279–281.
  101. Gissel-Nielsen, G., Gupta, U. C., Lamand, M., & Westermarck, T. (1984). Selenium in soils and plants and its importance in livestock and human nutrition. *Advances in Agronomy*, 37, 397–460.
  102. Gupta, U. C., & Gupta, S. C. (2000). Selenium in soils and crops, its deficiencies in livestock and humans: Implications for management. *Communications in Soil Science and Plant Analysis*, 31, 1791–1807.
  103. Gupta, U. C., & Watkinson, J. H. (1985). Agricultural significance of selenium. *Outlook on Agriculture*, 14, 183–189.
  104. Kubota, J., Allaway, W. H., Carter, D. L., Cary, E. E., & Lazar, V. A. (1967). Selenium in crops in the United States in relation to selenium-responsive diseases of animals. *Journal of Agriculture and Food Chemistry*, 15, 448–453.
  105. Aro, A., Alfthan, G., & Varo, P. (1995). Effects of supplementation of fertilizers on human selenium in Finland. *Analyst*, 120, 841–843.
  106. Thomson, C. D., & Robinson, M. F. (1996). The changing selenium status of New Zealand residents. *European Journal of Clinical Nutrition*, 50, 107–114.
  107. Whelan, B. R., & Barrow, N. J. (1994). Slow-release selenium fertilizers to correct selenium deficiency in grazing sheep in Western Australia. *Fertilizer Research*, 38, 183–188.
  108. Whelan, B. R., Peter, D. W., & Barrow, N. J. (1994). Selenium fertilizers for pastures grazed by sheep. I. Selenium concentrations in whole blood and plasma. *Australian Journal of Agricultural Research*, 45, 863–875.
  109. Pedrero, Z., Madrid, Y., & Camara, C. (2006). Selenium species bioaccessibility in enriched radish (*Raphanus sativus*): A

- potential dietary source of selenium. *Journal of Agriculture and Food Chemistry*, 54, 2412–2417.
110. Taylor, E. W. (1995). Selenium and cellular immunity. Evidence that selenoproteins may be encoded in the +1 reading frame overlapping the human CD4, CD8, and HLA-DR genes. *Biological Trace Element Research*, 49, 85–95.
  111. Cheng, Y. Y., & Qian, P. C. (1990). The effect of selenium-fortified table salt in the prevention of Keshan disease on a population of 1.05 million. *Biomedical and Environmental Sciences*, 3, 422–428.
  112. Hartikainen, H. (2005). Biogeochemistry of selenium and its impact on food chain quality and human health. *Journal of Trace Elements in Medicine and Biology*, 18, 309–318.
  113. Moreno-Reye, R., Mathieu, F., Boelaert, M., Begaux, F., Suetens, C., Rivera, M. T., et al. (2003). Selenium and iodine supplementation of rural Tibetan children affected by Kashin-Beck osteoarthropathy. *The American Journal of Clinical Nutrition*, 78, 134–144.
  114. Vanderpas, J. B., Contempre, B., Duale, N. L., Goossens, W., Bebe, N., Thorpe, R., et al. (1990). Iodine and selenium deficiency associated with cretinism in northern Zaire. *American Journal of Clinical Nutrition*, 52, 1087–1093.
  115. Chen, L., Yang, F., Xu, J., Hu, Y., Hu, Q., Zhang, Y., et al. (2002). Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. *Journal of Agriculture and Food Chemistry*, 50, 5128–5130.
  116. Yang, C., Niu, C., Bodo, M., Gabriel, E., Notbohm, H., Wolf, E., et al. (1993). Fulvic acid supplementation and selenium deficiency disturb the structural integrity of mouse skeletal tissue. An animal model to study the molecular defects of Kashin-Beck disease. *Biochemical Journal*, 289, 829–835.
  117. Allander, E. (1994). Kashin-Beck disease. An analysis of research and public health activities based on a bibliography 1849–1992. *Scandinavian Journal of Rheumatology—Supplement*, 99, 1–36.
  118. Arthur, J. R., & Beckett, G. J. (1999). Thyroid function. *British Medical Bulletin*, 55, 658–668.
  119. Higuchi, T., Ichijo, S., Osame, S., & Ohishi, H. (1989). Studies on serum selenium and tocopherol in white muscle disease of foal. *Japanese Journal of Veterinary Science*, 51, 52–59.
  120. Schofield, F. W. (1953). The prevention and treatment of white muscle disease (muscular dystrophy). *Canadian Journal of Comparative Medicine*, 17, 422–424.
  121. Schroder, G., Weissenbock, H., Baumgartner, W. C., & Truschner, K. (1991). Selenium toxicosis in weaned pigs. *Wiener Tierärztliche Monatsschrift*, 80, 171–176.
  122. Prasad, T., & Arora, S. P. (1991). Influence of different sources of injected selenium on certain enzymes, glutathione and adenosylmethionine concentration in buffalo (*Bubalus bubalis*) calves. *British Journal of Nutrition*, 66, 261–267.
  123. Kiremidjian-Schumacher, L., Roy, M., Wishe, H. I., Cohen, M. W., & Stotzky, G. (1994). Supplementation with selenium and human immune cell functions. II. Effect on cytotoxic lymphocytes and natural killer cells. *Biological Trace Element Research*, 41, 115–127.
  124. Roy, M., Kiremidjian-Schumacher, L., Wishe, H. I., Cohen, M. W., & Stotzky, G. (1994). Supplementation with selenium and human immune cell functions. I. Effect on lymphocyte proliferation and interleukin 2 receptor expression. *Biological Trace Element Research*, 41, 103–114.
  125. Look, M. P., Rockstroh, J. K., Rao, G. S., Kreuzer, K. A., Spengler, U., & Sauerbruch, T. (1997). Serum selenium versus lymphocyte subsets and markers of disease progression and inflammatory response in human immunodeficiency virus-1 infection. *Biological Trace Element Research*, 56, 31–41.
  126. Singhal, N., & Austin, J. (2002). A clinical review of micronutrients in HIV infections. *Journal of the International Association of Physicians in AIDS Care*, 1, 63–75.
  127. Romero-Alvira, D., & Roche, E. (1998). The keys of oxidative stress in acquired immune deficiency syndrome apoptosis. *Medical Hypotheses*, 51, 169–173.
  128. Campa, A., Shor-Posner, G., Indacoche, F., Zhang, G., Lai, H., Asthana, D., et al. (1999). Mortality risk in selenium-deficient HIV-positive children. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology*, 15, 508–513.
  129. Baum, M. K., Shor-Posner, G., Lai, S., Zhang, G., Lai, H., Fletcher, M. A., et al. (1997). High risk of HIV-related mortality is associated with selenium deficiency. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology*, 15, 370–374.
  130. Chen, C., Zhou, J., Xu, H., Jiang, Y., & Zhu, G. (1997). Effect of selenium supplementation on mice infected with LP-BM5 MuLV, a murine AIDS model. *Biological Trace Element Research*, 59, 187–193.
  131. Olmsted, L., Schruazer, G. N., Flores-Acre, M., & Dowd, J. (1989). Selenium supplementation of symptomatic immunodeficiency virus infected patients. *Biological Trace Element Research*, 25, 89–96.
  132. Schruazer, G. N., & Sacher, J. (1994). Selenium in the maintenance and therapy of HIV-infected patients. *Chemico-Biological Interactions*, 91, 199–205.
  133. Bhuvaramurthy, V., Balasubramanian, N., & Govindasamy, S. (1996). Effect of radiotherapy and chemoradiotherapy on circulating antioxidant system of human uterine cervical carcinoma. *Molecular and Cellular Biochemistry*, 158, 17–23.
  134. Duffield-Lillico, A. J., Reid, M. E., Turnbull, B. W., Combs, G. F. H. S. E., Fischbach, L. A., Marshall, J. R., et al. (2002). Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: A summary report of the Nutritional Prevention of Cancer trial. *Cancer Epidemiology, Biomarkers and Prevention*, 11, 630–639.
  135. Reid, M. E., Duffield-Lillico, A. J., Slate, E., Natarajan, N., Turnbull, B., Jacobs, E., et al. (2008). The nutritional prevention of cancer: 400 Mcg per day selenium treatment. *Nutrition and Cancer*, 60, 155–163.
  136. Rayman, M. P., Combs, G. D., Jr., & Waters, D. J. (2009). Selenium and vitamin E supplementation for cancer prevention. *Journal of the American Medical Association*, 301, 1976.
  137. Hatfield, D. L., & Gladyshev, V. N. (2009). The outcome of Selenium and Vitamin E Cancer Prevention Trial (SELECT) reveals the need for better understanding of selenium biology. *Molecular Interventions*, 9, 18–21.
  138. Lippman, S. M., Klein, E. A., Goodman, P. J., Thompson, I. M., Ford, L. G., Parnes, H. L., et al. (2009). Effect of selenium and vitamin E on risk of prostate cancer and other cancers: The Selenium and Vitamin E Cancer Prevention Trial (SELECT). *Journal of the American Medical Association*, 301, 39–51.
  139. Clark, L. C., Combs, G. F., Jr., Turnbull, B. W., Slate, E. H., Chalker, D. K., Chow, J., et al. (1996). Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *Journal of the American Medical Association*, 276, 1957–1963.
  140. Duffield-Lillico, A. J., Dalkin, B. L., Reid, M. E., Turnbull, B. W., Slate, E. H., Jacobs, E. T., et al. (2003). Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: An analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial. *British Journal of Urology International*, 91, 608–612.

141. Jackson, M. I., & Combs, G. F., Jr. (2008). Selenium and anticarcinogenesis: Underlying mechanisms. *Current Opinion in Clinical Nutrition and Metabolic Care*, *11*, 718–726.
142. Zeng, H., & Combs, G. F., Jr. (2008). Selenium as an anticancer nutrient: Roles in cell proliferation and tumor cell invasion. *Journal of Nutritional Biochemistry*, *19*, 1–7.
143. Stewart, M. S., Spallholz, J. E., Neldner, K. H., & Pence, B. C. (1999). Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis. *Free Radical Biology and Medicine*, *26*, 42–48.
144. Mantovani, G., Maccio, A., Madeddu, C., Serpe, R., Massa, E., Gramignano, G., et al. (2004). Selenium is effective in inducing lymphocyte progression through cell cycle in cancer patients: Potential mechanisms for its activity. *Journal of Experimental Therapeutics and Oncology*, *4*, 69–78.
145. Spyrou, G., Bjornstedt, M., Skog, S., & Holmgren, A. (1996). Selenite and selenate inhibit human lymphocyte growth via different mechanisms. *Cancer Research*, *56*, 4407–4412.
146. Vadhanavikit, S., Ip, C., & Ganther, H. E. (1993). Metabolites of sodium selenite and methylated selenium compounds administered at cancer chemoprevention levels in the rat. *Xenobiotica*, *23*, 731–745.
147. Shen, C. L., Song, W., & Pence, B. C. (2001). Interactions of selenium compounds with other antioxidants in DNA damage and apoptosis in human normal keratinocytes. *Cancer Epidemiology, Biomarkers and Prevention*, *10*, 385–390.
148. Ip, C. (1986). The chemopreventive role of selenium in carcinogenesis. *Journal of the American College of Toxicology*, *5*, 7–20.
149. Spallholz, J. E. (1994). On the nature of selenium toxicity and carcinostatic activity. *Free Radical Biology and Medicine*, *17*, 45–64.
150. Battin, E. E., Zimmerman, M. T., Ramoutar, R. R., Quarles, C., & Brumaghim, J. L. Preventing metal-mediated oxidative DNA damage with selenium compounds (submitted).
151. Ramoutar, R. R. (2009). Understanding the antioxidant mechanism of inorganic selenium, oxo-sulfur, and polyphenol compounds, and the biological implications of functionalized nanoparticles, Ph.D. Dissertation, Clemson University.
152. Berggren, M. M., Mangin, J. F., Gasdaska, J. R., & Powis, G. (1999). Effect of selenium on rat thioredoxin reductase activity increase by supranutritional selenium and decrease by selenium deficiency. *Biochemical Pharmacology*, *57*, 187–193.
153. Berggren, M., Gallegos, A., Gasdaska, J., & Powis, G. (1997). Cellular thioredoxin reductase activity is regulated by selenium. *Anticancer Research*, *17*, 3377–3380.
154. El-Bayoumy, K. (2001). The protective role of selenium on genetic damage and on cancer. *Mutation Research*, *475*, 123–139.
155. Ip, C. (1998). Lessons from basic research in selenium and cancer prevention. *Journal of Nutrition*, *128*, 1845–1854.
156. Young, V. R., Nahapetian, A., & Janghorbani, M. (1982). Selenium bioavailability with reference to human nutrition. *American Journal of Clinical Nutrition*, *35*, 1076–1088.
157. Lu, J., Kaeck, M., Jiang, C., Wilson, A. C., & Thompson, H. J. (1994). Selenite induction of DNA strand breaks and apoptosis in mouse leukemic L1210 cells. *Biochemical Pharmacology*, *47*, 1531–1535.
158. Wilson, A. C., Thompson, H. J., Schedin, P. J., Gibson, N. W., & Ganther, H. E. (1992). Effect of methylated forms of selenium on cell viability and the induction of DNA strand breakage. *Biochemical Pharmacology*, *43*, 1137–1141.
159. Lu, J., Jiang, C., Kaeck, M., Ganther, H., Vadhanavikit, S., Ip, C., et al. (1995). Dissociation of the genotoxic and growth inhibitory effects of selenium. *Biochemical Pharmacology*, *50*, 213–219.
160. Gazi, M. H., Gong, A., Donkena, K. V., & Young, C. Y. (2007). Sodium selenite inhibits interleukin-6-mediated androgen receptor activation in prostate cancer cells via upregulation of c-Jun. *Clinica Chimica Acta*, *380*, 145–150.
161. Burk, R. F., Lawrence, R. A., & Lane, J. M. (1980). Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration. Effect of selenium deficiency. *Journal of Clinical Investigation*, *65*, 1024–1031.
162. Hill, K. E., & Burk, R. F. (1997). Selenoprotein P: Recent studies in rats and in humans. *Biomedical and Environmental Sciences*, *10*, 198–208.
163. Holben, D. H., & Smith, A. M. (1999). The diverse role of selenium within selenoproteins: A review. *Journal of the American Dietetic Association*, *99*, 836–843.
164. Yang, J. G., Hill, K. E., & Burk, R. F. (1989). Dietary selenium intake controls rat plasma selenoprotein P concentration. *Journal of Nutrition*, *119*, 1010–1012.
165. Gallegos, A., Berggren, M., Gasdaska, J. R., & Powis, G. (1997). Mechanisms of the regulation of thioredoxin reductase activity in cancer cells by the chemopreventive agent selenium. *Cancer Research*, *57*, 4965–4970.
166. Behne, D., Kyriakopoulos, A., Weiss-Nowak, C., Kalckloesch, M., Westphal, C., & Gessner, H. (1996). Newly found selenium-containing proteins in the tissues of the rat. *Biological Trace Element Research*, *55*, 99–110.
167. Singh, M., Lu, J., Briggs, S. P., McGinley, J. N., Haegle, A. D., & Thompson, H. J. (1994). Effect of excess dietary iron on the promotion stage of 1-methyl-1-nitrosourea-induced mammary carcinogenesis: Pathogenetic characteristics and distribution of iron. *Carcinogenesis*, *15*, 1567–1570.
168. Thirunavukkarasu, C., Prince Vijaya Singh, J., Thangavel, M., Selvendiran, K., & Sakthisekaran, D. (2002). Dietary influence of selenium on the incidence of N-nitrosodiethylamine-induced hepatoma with reference to drug and glutathione metabolizing enzymes. *Cell Biochemistry and Function*, *20*, 347–356.
169. Thirunavukkarasu, C., & Sakthisekaran, D. (2003). Sodium selenite, dietary micronutrient, prevents the lymphocyte DNA damage induced by N-nitrosodiethylamine and phenobarbital promoted experimental hepatocarcinogenesis. *Journal of Cellular Biochemistry*, *88*, 578–588.
170. Thompson, H. J., Wilson, A., Lu, J., Singh, M., Jiang, C., Upadhyaya, P., et al. (1994). Comparison of the effects of an organic and an inorganic form of selenium on a mammary carcinoma cell line. *Carcinogenesis*, *15*, 183–186.
171. Kim, T. S., Yun, B. Y., & Kim, I. Y. (2003). Induction of the mitochondrial permeability transition by selenium compounds mediated by oxidation of the protein thiol groups and generation of the superoxide. *Biochemical Pharmacology*, *66*, 2301–2311.
172. Ganther, H. E. (1968). Selenotrisulfides. Formation by the reaction of thiols with selenious acid. *Biochemistry*, *7*, 2898–2905.
173. Ganther, H. E. (1971). Reduction of the selenotrisulfide derivative of glutathione to a persulfide analog by glutathione reductase. *Biochemistry*, *10*, 4089–4098.
174. Kim, T. S., Jeong, D. W., Yun, B. Y., & Kim, I. Y. (2002). Dysfunction of rat liver mitochondria by selenite: Induction of mitochondrial permeability transition through thiol-oxidation. *Biochemical and Biophysical Research Communications*, *294*, 1130–1137.
175. Seko, Y., & Imura, N. (1997). Active oxygen generation as a possible mechanism of selenium toxicity. *Biomedical and Environmental Sciences*, *10*, 333–339.
176. Harrison, W. T. A., Stucky, G. D., Morris, R. E., & Cheetham, A. K. (1992). Synthesis and structure of aluminum selenite trihydrate,  $Al_2(SeO_3)_3 \cdot 3H_2O$ . *Acta Cryst*, *C48*, 1365–1367.

177. Xiao, D., An, H., Wang, E., & Xu, L. (2005). Synthesis and structure of a novel three-dimensional metal selenite containing multidirectional intersecting double helical chains:  $[\text{Fe}_2(\text{H}_2\text{O})_4(\text{SeO}_3)_2]$ . *Journal of Molecular Structure*, *740*, 249–253.
178. Cao, G., Sofic, E., & Prior, L. R. (1996). Antioxidant capacity of tea and common vegetables. *Journal of Agriculture and Food Chemistry*, *44*, 3426–3431.
179. Gey, K. F. (1990). The antioxidant hypothesis of cardiovascular disease: Epidemiology and mechanisms. *Biochemical Society Transactions*, *18*, 1041–1045.
180. Willett, C. W. (1994). Diet and health: What should we eat? *Science*, *264*, 532–537.
181. Ishikawa, K., Naganawa, R., Yoshida, H., Iwata, N., Fukuda, H., Fujino, T., et al. (1996). Antimutagenic effects of ajoene, an organosulfur compound derived from garlic. *Bioscience, Biotechnology, and Biochemistry*, *60*, 2086–2088.
182. Kyo, E., Uda, N., Suzuki, A., Kakimoto, M., Ushujima, M., Kasuga, S., et al. (1999). Prevention of psychological stress-induced immune suppression by aged garlic extract. *Phyto-medicine*, *5*, 259–267.
183. Mirhadi, S. A., Singh, S., & Gupta, P. P. (1991). Effect of garlic supplementation to cholesterol-rich diet on development of atherosclerosis in rabbits. *Indian Journal of Experimental Biology*, *29*, 162–168.
184. Morimitsu, Y., Morioka, Y., & Kawakishi, S. (1992). Inhibitors of platelet aggregation generated from mixtures of *Allium* species and/or *S*-alk(en)yl-L-cysteine sulfoxides. *Journal of Agriculture and Food Chemistry*, *40*, 368–372.
185. Prasad, K., Laxdal, V. A., Yu, M., & Raney, B. L. (1995). Antioxidant activity of allicin, an active principle in garlic. *Molecular and Cellular Biochemistry*, *148*, 183–189.
186. Siegers, C.-P., Robke, A., & Pentz, R. (1999). Effects of garlic preparations on superoxide production by phorbol ester activated granulocytes. *Phyto-medicine*, *6*, 13–16.
187. Singh, S. V., Pan, S. S., Srivastava, S. K., Xia, H., Hu, X., Zaren, H. A., et al. (1998). Differential induction of NAD(P)H:quinone oxidoreductase by anti-carcinogenic organosulfides from garlic. *Biochemical and Biophysical Research Communications*, *244*, 917–920.
188. Weber, N. D., Andersen, D. O., North, J. A., Murray, B. K., Lawson, L. D., & Hughes, B. G. (1992). In vitro virucidal effects of *Allium sativum* (garlic) extract and compounds. *Planta Medica*, *58*, 417–423.
189. Briggs, W. H., Xiao, H., Parkin, K. L., Shen, C., & Goldman, I. L. (2000). Differential inhibition of human platelet aggregation by selected *Allium* thiosulfates. *Journal of Agriculture and Food Chemistry*, *48*, 5731–5735.
190. Edwards, J. S., Musker, D., Collin, H. A., & Britton, G. (1994). The analysis of *S*-alk(en)yl-L-cysteine sulfoxides (flavor precursors) from species of *Allium* by high performance liquid chromatography. *Phytochemical Analysis*, *5*, 4–9.
191. Thomas, D. J., & Parkin, K. L. (1994). Quantification of alk(en)yl-L-cysteine sulfoxides and related amino acids in alliums by high-performance liquid chromatography. *Journal of Agriculture and Food Chemistry*, *42*, 1632–1638.
192. Yoo, K. S., & Pike, L. M. (1998). Determination of flavor precursor compound *S*-alk(en)yl-image-cysteine sulfoxides by an HPLC method and their distribution in *Allium* species. *Scientia Horticulturae*, *75*, 1–10.
193. Hirsch, K., Danilenko, M., Giat, J., Miron, T., Rabinkov, A., Wilchek, M., et al. (2000). Effect of purified allicin, the major ingredient of freshly crushed garlic, on cancer cell proliferation. *Nutrition and Cancer*, *38*, 245–254.
194. Rabinkov, A., Miron, T., Konstantinovski, L., Wilchek, M., Mirelman, D., & Weiner, L. (1998). The mode of action of allicin: Trapping of radicals and interaction with thiol containing proteins. *Biochimica et Biophysica Acta*, *1379*, 233–244.
195. Ito, Y., Nakamura, Y., & Nakamura, Y. (1997). Suppression of aflatoxin B1- or methyl methanesulfonate-induced chromosome aberrations in rat bone marrow cells after treatment with *S*-methyl methanethiosulfonate. *Mutation Research*, *393*, 307–316.
196. Nakamura, Y., Matsuo, T., Shimoi, K., Nakamura, Y., & Tomita, I. (1993). *S*-Methyl methane thiosulfonate, a new antimutagenic compound isolated from *Brassica oleracea* L. var. botrytis. *Biological and Pharmaceutical Bulletin*, *16*, 207–209.
197. Nakamura, Y., Matsuo, T., Shimoi, K., Nakamura, Y., & Tomita, I. (1996). *S*-Methyl methanethiosulfonate, bio-antimutagen in homogenates of *Cruciferae* and *Liliaceae* vegetables. *Bio-science, Biotechnology, and Biochemistry*, *60*, 1439–1443.
198. Kim, L. S., Axelrod, L. J., Howard, P., Buratovich, N., & Waters, R. F. (2006). Efficacy of methylsulfonylmethane (MSM) in osteoarthritis pain of the knee: A pilot clinical trial. *Osteoarthritis Cartilage*, *14*, 286–294.
199. Barrager, E., Veltmann, J. R., Jr., Schauss, A. G., & Schiller, R. N. (2002). A multicentered, open-label trial on the safety and efficacy of methylsulfonylmethane in the treatment of seasonal allergic rhinitis. *Journal of Alternative and Complementary Medicine*, *8*, 167–173.
200. Hucker, H. B., Miller, J. K., Hochberg, A., Brobyn, R. D., Riordan, F. H., & Calesnick, B. (1967). Studies on the absorption, excretion and metabolism of dimethylsulfoxide (DMSO) in man. *Journal of Pharmacology and Experimental Therapeutics*, *155*, 309–317.
201. Wong, K. K., Wang, G. M., Dreyfuss, J., & Jacques, E. C. (1971). Absorption, excretion, and biotransformation of dimethyl sulfoxide in man and miniature pigs after topical application as an 80% gel. *Journal of Investigative Dermatology*, *51*, 44–48.
202. Pryor, W. A., Jin, X., & Squadrito, G. L. (1994). One- and two-electron oxidations of methionine by peroxynitrite. *Proceedings of the National Academy of Sciences of the United States of America*, *91*, 11173–11177.
203. Schoneich, C., Zhao, F., Wilson, G. S., & Borchardt, R. T. (1993). Iron-thiolate induced oxidation of methionine to methionine sulfoxide in small model peptides. Intramolecular catalysis by histidine. *Biochimica et Biophysica Acta*, *1158*, 307–322.
204. Ali, F. E., Separovic, F., Barrow, C. J., Cherny, R. A., Fraser, F., Bush, A. I., et al. (2005). Methionine regulates copper/hydrogen peroxide oxidation products of Abeta. *Journal of Peptide Science*, *11*, 353–360.
205. Bush, A. I. (2000). Metals and neuroscience. *Current Opinion in Chemical Biology*, *4*, 184–191.
206. Lynch, T., Cherny, R. A., & Bush, A. I. (2000). Oxidative processes in Alzheimer's disease: The role of  $\text{A}\beta$ -metal interactions. *Experimental Gerontology*, *35*, 445–451.
207. Gao, J., Yin, D. H., Yao, Y., Sun, H., Qin, Z., Schoneich, C., et al. (1998). Loss of conformational stability in calmodulin upon methionine oxidation. *Biophysical Journal*, *74*, 1115–1134.
208. Squier, T. C., & Bigelow, D. J. (2000). Protein oxidation and age-dependent alterations in calcium homeostasis. *Frontiers Bioscience*, *5*, D504–D526.
209. Berlett, B. S., Levine, R. L., & Stadtman, E. R. (1998). Carbon dioxide stimulates peroxynitrite-mediated nitration of tyrosine residues and inhibits oxidation of methionine residues of glutamine synthetase: Both modifications mimic effects of adenylation. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 2784–2789.
210. Brot, N., & Weissbach, H. (1983). Biochemistry and physiological role of methionine sulfoxide residues in proteins. *Archives of Biochemistry and Biophysics*, *223*, 271–281.

211. Chao, C. C., Ma, Y. S., & Stadtman, E. R. (1997). Modification of protein surface hydrophobicity and methionine oxidation by oxidative systems. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 2969–2974.
212. Levine, R. L., Mosoni, L., Berlett, B. S., & Stadtman, E. R. (1996). Methionine residues as endogenous antioxidants in proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *93*, 15036–15040.
213. Vogt, W. (1995). Oxidation of methionyl residues in proteins: Tools, targets, and reversal. *Free Radical Biology and Medicine*, *18*, 93–105.
214. von Eckardstein, A., Walter, M., Holz, H., Benninghoven, A., & Assmann, G. (1991). Site-specific methionine sulfoxide formation is the structural basis of chromatographic heterogeneity of apolipoproteins A-I, C-II, and C-III. *Journal of Lipid Research*, *32*, 1465–1476.
215. Levine, R. L., Moskowitz, J., & Stadtman, E. R. (2000). Oxidation of methionine in proteins: Roles in antioxidant defense and cellular regulation. *IUBMB Life*, *50*, 301–307.
216. Moskowitz, J., Flescher, E., Berlett, B. S., Azare, J., Poston, J. M., & Stadtman, E. R. (1998). Overexpression of peptide-methionine sulfoxide reductase in *Saccharomyces cerevisiae* and human T cells provides them with high resistance to oxidative stress. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 14071–14075.
217. Tang, X. D., Daggett, H., Hanner, M., Garcia, M. L., McManus, O. B., Brot, N., et al. (2001). Oxidative regulation of large conductance calcium-activated potassium channels. *Journal of General Physiology*, *117*, 253–274.
218. Assmann, A., Briviba, K., & Sies, H. (1998). Reduction of methionine selenoxide to selenomethionine by glutathione. *Archives of Biochemistry and Biophysics*, *349*, 201–203.
219. Padmaja, S., Squadrito, G. L., Lemerrier, J. N., Cueto, R., & Pryor, W. A. (1996). Rapid oxidation of DL-selenomethionine by peroxynitrite. *Free Radical Biology and Medicine*, *21*, 317–322.
220. Amagase, H., Petesch, B. L., Matsuura, H., Kasuga, S., & Itakura, Y. (2001). Intake of garlic and its bioactive components. *Journal of Nutrition*, *131*, 955S–962S.
221. Corzo-Martinez, M., Corzo, N., & Villamiel, M. (2007). Biological Properties of Onions and Garlic. *Trends in Food Science and Technology*, *18*, 609–625.
222. Das, S. (2002). Garlic—a natural source of cancer preventive compounds. *Asian Pacific Journal of Cancer Prevention*, *3*, 305–311.
223. Chu, T. C., Ogidigben, M., Han, J. C., & Potter, D. E. (1993). Allicin-induced hypotension in rabbit eyes. *Journal of Ocular Pharmacology*, *9*, 201–209.
224. Gebhardt, R., & Beck, H. (1996). Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. *Lipids*, *31*, 1269–1276.
225. Kaye, A. D., Nossaman, B. D., Ibrahim, I. N., Feng, C. J., McNamara, D. B., Agrawal, K. C., et al. (1995). Analysis of responses of allicin, a compound from garlic, in the pulmonary vascular bed of the cat and in the rat. *European Journal of Pharmacology*, *276*, 21–26.
226. Makheja, A. N., & Bailey, J. M. (1990). Antiplatelet constituents of garlic and onion. *Agents Actions*, *29*, 360–363.
227. Chung, L. Y. (2006). The antioxidant properties of garlic compounds: Allyl cysteine, alliin, allicin, and allyl disulfide. *Journal of Medicinal Food*, *9*, 205–213.
228. Kumari, K., & Augusti, K. T. (1995). Antidiabetic effects of S-methylcysteine sulphoxide on alloxan diabetes. *Planta Medica*, *61*, 72–74.
229. Kumari, K., & Augusti, K. T. (2007). Lipid lowering effect of S-methyl cysteine sulfoxide from *Allium cepa* Linn in high cholesterol diet fed rats. *Journal of Ethnopharmacology*, *109*, 367–371.
230. Kumari, K., & Augusti, K. T. (2002). Antidiabetic and antioxidant effects of S-methyl cysteine sulfoxide isolated from onions (*Allium cepa* Linn) as compared to standard drugs in alloxan diabetic rats. *Indian Journal of Experimental Biology*, *40*, 1005–1009.
231. Kawamori, T., Tanaka, T., Ohnishi, M., Hirose, Y., Nakamura, Y., Satoh, K., et al. (1995). Chemoprevention of azoxymethane-induced colon carcinogenesis by dietary feeding of S-methyl methane thiosulfonate in male F344 rats. *Cancer Research*, *55*, 4053–4058.
232. Sugie, S., Okamoto, K., Ohnishi, M., Makita, H., Kawamori, T., Watanabe, T., et al. (1997). Suppressive effects of S-methyl methanethiosulfonate on promotion stage of diethylnitrosamine-initiated and phenobarbital-promoted hepatocarcinogenesis model. *Japanese Journal of Cancer Research*, *88*, 5–11.
233. Nakamura, Y. K., Kawai, K., Furukawa, H., Matsuo, T., Shimoi, K., Tomita, I., et al. (1997). Suppressing effects of S-methyl methanethiosulfonate and diphenyl disulfide on mitomycin C-induced somatic mutation and recombination in *Drosophila melanogaster* and micronuclei in mice. *Mutation Research*, *385*, 41–46.
234. Nagira, T., Narisawa, J., Teruya, K., Katakura, Y., Shim, S. Y., Kusumoto, K., et al. (2002). Suppression of UVC-induced cell damage and enhancement of DNA repair by the fermented milk, Kefir. *Cytotechnology*, *40*, 125–137.
235. Richmond, V. L. (1986). Incorporation of methylsulfonylmethane sulfur into guinea pig serum proteins. *Life Science*, *39*, 263–268.
236. Hucker, H. B., Ahmad, P. M., Miller, E. A., & Brobyn, R. (1966). Metabolism of dimethyl sulfoxide to dimethyl sulfone in the rat and man. *Nature*, *209*, 619–620.
237. Otsuki, S., Qian, W., Ishihara, A., & Kabe, T. (2002). Elucidation of dimethylsulfone metabolism in rat using a <sup>35</sup>S radioisotope tracer method. *Nutrition Research*, *22*, 313–322.
238. Magnuson, B. A., Appleton, J., & Ames, G. B. (2007). Pharmacokinetics and distribution of [<sup>35</sup>S]methylsulfonylmethane following oral administration to rats. *Journal of Agricultural and Food Chemistry*, *55*, 1033–1038.
239. Egorin, M. J., Rosen, D. M., Sridhara, R., Sensenbrenner, L., & Cottler-Fox, M. (1998). Plasma concentrations and pharmacokinetics of dimethylsulfoxide and its metabolites in patients undergoing peripheral-blood stem-cell transplants. *Journal of Clinical Oncology*, *16*, 610–615.
240. Engelke, U. F., Tangerman, A., Willemsen, M. A., Moskau, D., Loss, S., Mudd, S. H., et al. (2005). Dimethyl sulfone in human cerebrospinal fluid and blood plasma confirmed by one-dimensional (1)H and two-dimensional (1)H-(13)C NMR. *NMR in Biomedicine*, *18*, 331–336.
241. Lin, A., Nguy, C. H., Shic, F., & Ross, B. D. (2001). Accumulation of methylsulfonylmethane in the human brain: Identification by multinuclear magnetic resonance spectroscopy. *Toxicology Letters*, *123*, 169–177.
242. Rose, S. E., Chalk, J. B., Galloway, G. J., & Doddrell, D. M. (2000). Detection of dimethyl sulfone in the human brain by in vivo proton magnetic resonance spectroscopy. *Magnetic Resonance Imaging*, *18*, 95–98.
243. Cronin, J. R. (1999). Nutraceutical of the next century? *Alternative and Complementary Therapies*, *5*, 386–390.
244. Usha, P. R., & Naidu, M. U. (2004). Randomised, double-blind, parallel, placebo-controlled study of oral glucosamine, methylsulfonylmethane and their combination in osteoarthritis. *Clinical Drug Investigation*, *24*, 353–363.
245. Moskowitz, J., Berlett, B. S., Poston, J. M., & Stadtman, E. R. (1997). The yeast peptide-methionine sulfoxide reductase

- functions as an antioxidant in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 9585–9589.
246. Ruan, H., Tang, X. D., Chen, M. L., Joiner, M. L., Sun, G., Brot, N., et al. (2002). High-quality life extension by the enzyme peptide methionine sulfoxide reductase. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 2748–2753.
247. Moskovitz, J., Rahman, M. A., Strassman, J., Yancey, S. O., Kushner, S. R., Brot, N., et al. (1995). *Escherichia coli* peptide methionine sulfoxide reductase gene: Regulation of expression and role in protecting against oxidative damage. *Journal of Bacteriology*, *177*, 502–507.
248. St. John, G., Brot, N., Ruan, J., Erdjument-Bromage, H., Tempst, P., Weissbach, H., et al. (2001). Peptide methionine sulfoxide reductase from *Escherichia coli* and *Mycobacterium tuberculosis* protects bacteria against oxidative damage from reactive nitrogen intermediates. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 9901–9906.
249. Moskovitz, J., Bar-Noy, S., Williams, W. M., Requena, J., Berlett, B. S., & Stadtman, E. R. (2001). Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 12920–12925.
250. Sattler, W., Christison, J., & Stocker, R. (1995). Cholesterylester hydroperoxide reducing activity associated with isolated high- and low-density lipoproteins. *Free Radical Biology and Medicine*, *18*, 421–429.
251. Maier, K., Leuschel, L., & Costabel, U. (1991). Increased levels of oxidized methionine residues in bronchoalveolar lavage fluid proteins from patients with idiopathic pulmonary fibrosis. *American Review of Respiratory Disease*, *143*, 271–274.
252. Petropoulos, I., Mary, J., Perichon, M., & Friguet, B. (2001). Rat peptide methionine sulphoxide reductase: Cloning of the cDNA, and down-regulation of gene expression and enzyme activity during aging. *Biochemical Journal*, *355*, 819–825.
253. Gabbita, S. P., Aksenov, M. Y., Lovell, M. A., & Markesbery, W. R. (1999). Decrease in peptide methionine sulfoxide reductase in Alzheimer's disease brain. *Journal of Neurochemistry*, *73*, 1660–1666.
254. Michaelis, M. L., Bigelow, D. J., Schoneich, C., Williams, T. D., Ramonda, L., Yin, D., et al. (1996). Decreased plasma membrane calcium transport activity in aging brain. *Life Science*, *59*, 405–412.
255. Wells-Knecht, M. C., Lyons, T. J., McCance, D. R., Thorpe, S. R., & Baynes, J. W. (1997). Age-dependent increase in orthotyrosine and methionine sulfoxide in human skin collagen is not accelerated in diabetes. Evidence against a generalized increase in oxidative stress in diabetes. *Journal of Clinical Investigation*, *100*, 839–846.
256. Gellman, S. H. (1991). On the role of methionine residues in the sequence-independent recognition of nonpolar protein surfaces. *Biochemistry*, *30*, 6633–6636.
257. Armitage, B. (1998). Photocleavage of nucleic acids. *Chemical Reviews*, *98*, 1171–1200.
258. Mayo, D. J., Turner, D. P., Zucconi, B. E., & Predecki, A. H. (2007). DNA cleavage by photolysis of aryl sulfoxides. *Bioorganic & Medicinal Chemistry Letters*, *17*, 6116–6118.
259. Wauchope, O. R., Shakya, S., Sawwan, N., Liebman, J. F., & Greer, A. (2007). Photocleavage of plasmid DNA by dibenzothioephene S-oxide under anaerobic conditions. *Journal of Sulfur Chemistry*, *28*, 11–16.
260. Drake, E. N. (2006). Cancer chemoprevention: Selenium as a prooxidant, not an antioxidant. *Medical Hypotheses*, *67*, 318–322.
261. Poerschke, R. L., Franklin, M. R., & Moos, P. J. (2008). Modulation of redox status in human lung cell lines by organoselenocompounds: Selenazolidines, selenomethionine, and methylseleninic acid. *Toxicology in Vitro*, *22*, 1761–1767.
262. Singh, U., Null, K., & Sinha, R. (2008). In vitro growth inhibition of mouse mammary epithelial tumor cells by methylseleninic acid: Involvement of protein kinases. *Molecular Nutrition & Food Research*, *52*, 1281–1288.
263. Gundimeda, U., Schiffman, J. E., Chhabra, D., Wong, J., Wu, A., & Gopalakrishna, R. (2008). Locally generated methylseleninic acid induces specific inactivation of protein kinase C isoenzymes. *The Journal of Biological Chemistry*, *283*, 34519–34531.
264. Li, G.-H., Hu, H., Jiang, C., Schuster, T., & Lü, J. (2007). Differential involvement of reactive oxygen species in apoptosis induced by two classes of selenium compounds in human prostate cancer cells. *International Journal of Cancer*, *120*, 2034–2043.
265. Li, G.-x., Lee, H.-J., Wang, Z., Hu, H., Liao, J. D., Watts, J. C., et al. (2008). Superior in vivo inhibitory efficacy of methylseleninic acid against human prostate cancer over selenomethionine or selenite. *Carcinogenesis*, *29*, 1005–1012.
266. Krause, R. J., Glocke, S. C., Sicuri, A. R., Ripp, S. L., & Elfarra, A. A. (2006). Oxidative metabolism of seleno-L-methionine to L-methionine selenoxide by flavin-containing monooxygenases. *Chemical Research in Toxicology*, *19*, 1643–1649.
267. Schrauzer, G. N. (2000). Selenomethionine: A review of its nutritional significance, metabolism and toxicity. *Journal of Nutrition*, *130*, 1653–1656.
268. Krause, R. J., & Elfarra, A. A. (2009). Reduction of L-methionine selenoxide to seleno-L-methionine by endogenous thiols, ascorbic acid, or methimazole. *Biochemical Pharmacology*, *77*, 134–140.
269. Kajander, E. O., Harvima, R. J., Eloranta, T. O., Martikainen, H., Kantola, M., Karenlampi, S. O., et al. (1991). Metabolism, cellular actions, and cytotoxicity of selenomethionine in cultured cells. *Biological Trace Element Research*, *28*, 57–68.
270. Ziegler, D. M., Graf, P., Poulsen, L. L., Stahl, W., & Sies, H. (1992). NADPH-dependent oxidation of reduced ebselen, 2-selenylbenzamide, and of 2-(methylseleno)benzamide catalyzed by pig liver flavin-containing monooxygenase. *Chemical Research in Toxicology*, *5*, 163–166.