Food chain selenium and human health: spotlight on speciation

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Abstract

There is a growing appreciation that it is not just the total intake of dietary selenium (Se) that is important to health but that the species of Se ingested may also be important. This review attempts to catalogue what is known about Se species in food sources and supplements and the health effects in which they are implicated. The biosynthetic pathways involved in Se assimilation by plants and the way in which Se species are metabolized in animals are presented in order to give an insight into the species likely to be present in plant and animal foods. Known data on the species of Se in the food chain and in food supplements are tabulated along with their concentrations and the analytical methodology used. The latter is important since identification that is only based on retention time matching with authentic standards must be considered as tentative: for evidence of structural confirmation, fragmentation of the molecular ion in addition to MS data is required. Bioavailability, as normally defined, is higher for organic Se species. Health effects, both beneficial and toxic, thought to be associated with specific Se species are described. Potent anti-tumour effects have been attributed to the low-molecular-weight species, *Se*-methyl-selenocysteine and its γ-glutamyl-derivative, found in a number of edible plants of the *Allium* and *Brassica* families. There remain considerable gaps in our knowledge of the forms of Se that naturally occur in foods. Without adequate knowledge of Se speciation, false conclusions may be drawn when assessing Se requirements for optimal health.

The extent of the literature on the essential trace element selenium (Se) appears to have increased exponentially over the last decade reflecting the tremendous growth of interest in this nutrient since it was shown by Clark and co-workers to reduce cancer risk in their landmark trial[1]. Though the form of Se used in that trial was high-Se yeast, when large-scale funding was obtained from the National Cancer Institute for a follow-up randomized trial of the effect of supplemental Se on prostate cancer risk (SELECT), the decision was taken to use selenomethionine (SeMet) owing to the perceived importance of being able to define the specific form of Se that might be associated with an important health effect[2]. Thus we are no longer satisfied with knowing simply the amount of Se that may be associated with benefit but seek to know the species of Se to which that alleged benefit may be attributed. Furthermore, we have come to realize that different species of an element (*viz.* arsenic) can have very different health effects. This review therefore attempts to pull together what is known about the species of Se in foods and supplements, the pathways by which they are synthesized, their apparent bioavailability as found in different food sources as this has implications for Se requirements, and the health effects that can be ascribed to specific Se species.

Biosynthesis and metabolism of dietary Se species

A consideration of Se speciation in plant and animal food sources requires some understanding of the biosynthetic pathways involved in Se assimilation by plants and how these species are metabolized in animals. Such knowledge enables us to predict to some extent the Se species likely to be contained in foods. The biosynthetic pathways for Se in plants, some of which are assumed by analogy with S pathways, are shown in **Figure 1** (adapted from references[3-8]). The relative dominance of the pathways differs for Se-accumulators and non-accumulators.

The major species in plant sources of Se are:- selenate (translocated directly from the soil and less-readily bound to soil components than selenite); SeMet (biosynthesised) and a smaller amount of SeCys (biosynthesised); Se-containing proteins (where SeMet and SeCys have been incorporated non-specifically in place of methionine and cysteine); *Se*-methyl-selenocysteine and γ-glutamyl-*Se*-methyl-selenocysteine (considered as detoxification products, notably formed in Se-accumulators and plants of the *Brassica* and *Allium* families). Plants can volatilize significant amounts of Se as dimethylselenide (non-accumulators) and dimethyldiselenide (accumulators)[6]. To avoid an over-complicated figure, the enzymes implicated in these pathways are not shown, with the exception of SeCys methyltransferase, the enzyme notably present in Se-accumulators and responsible for the methylation of SeCys to the characteristic methylated metabolites which are believed to have anti-cancer properties.

While a study of these pathways suggests Se species that may be expected in foods from plant sources, it should be noted that compounds formed and their relative quantities differ not only between Se-accumulators and non-accumulators but also between species.

There is much less information on the species of Se in dietary sources of animal origin[9]. When inorganic Se is given to animals, SeCys is the main seleno-compound formed but when animals eat Se-containing foods of plant origin, protein-bound SeMet will also be formed from the non-specific incorporation of plant-derived SeMet in place of methionine. Selenotrisulphide (-SeS-), glutathione selenopersulphide (GSSeH) and metallic selenides have also been reported in tissues[10]. The presence of some of these compounds can be explained by the metabolic pathway of dietary Se in animals which resembles that in humans as described below.

Most of what we know about the metabolism of dietary (or supplement) Se in humans is inferred from studies in rats and mice. A simplified version of the metabolic pathway is shown in **Figure 2** and clearly illustrates the central role of hydrogen selenide (H_2Se) (adapted from[11,12])[13,14]. SeMet catabolised from proteins can be trans-selenated to SeCys (by analogy with the trans-sulphuration pathway). SeCys, either from this source or directly from the diet, is then converted to H_2Se by selenocysteine β-lyase. Alternatively, SeMet can undergo α,γ-elimination catalysed by a γ-lyase to yield CH_3SeH, though the relative importance of this route in humans is not known[13,15,16]. CH_3SeH is also produced by a β-lyase from plant sources containing *Se*-methyl-selenocysteine and γ-glutamyl-*Se*-methyl-selenocysteine. Utilisation of selenate or selenite (plant sources or supplements) for selenoprotein synthesis, or excretion of excess, first requires reduction to the central Se metabolite, H_2Se, *via* interaction with the tripeptide, glutathione (GSH). The H_2Se so formed may be converted to selenophosphate ($HSePO_3^{2-}$) which then reacts with tRNA-bound serinyl residues to give selenocysteine-bound tRNA from which selenocysteine is inserted co-translationally, at loci encoded by specific UGA codons, to give selenoproteins[17,18]. As CH_3SeH can be demethylated to H_2Se in an equilibrium reaction, both it and its precursors can also act as Se sources for selenoprotein synthesis[13]. Oxidation of excess H_2Se can lead to the production of superoxide and other reactive oxygen species with associated toxic effects[11].

Surplus Se is transformed to methylated metabolites mostly for excretion into urine. Excretion of Se is either from H_2Se through a methylated selenosugar (1β-methylseleno-*N*-acetyl-D-galactosamine) in urine or by further methylation of CH_3SeH to dimethyl selenide [$(CH_3)_2Se$] which is exhaled in breath, and trimethyl selenonium ion [$(CH_3)_3Se^+$] excreted in urine[19-21]. Though 1β-methylseleno-*N*-acetyl-D-galactosamine is the most significant urinary metabolite in most individuals, $(CH_3)_3Se^+$ is a major product from *Se*-methyl-selenocysteine[13,21].

Se in food sources and dietary supplements: speciation and concentration

Table 1 shows the Se species apparently identified in foods and dietary supplements and their concentrations or relative concentrations in some cases. In terms of identification, it must be borne in mind that many of these studies were carried out when the available analytical strategies that combined both elemental and molecular mass spectrometry were less-well developed than is currently the case. In the case of most foods, however, it is the only data we have and can help focus the direction of further studies. Column 5 shows the methodology used for Se species identification. Readers should be aware, however, that identification that is only based on retention time matching with authentic standards by HPLC-ICP-MS is tentative and that ESI-MS (electrospray ionisation mass spectrometry) data alone does not provide enough evidence of structural confirmation. To obtain this, fragmentation of the molecular ion has to be performed[28]. The table contains some speciation data that have been obtained in this way e.g. by ICP-MS combined with MS/MS data obtained by MALDI (matrix assisted laser desorption/ionization) or ESI MS/MS (electrospray ionisation mass spectrometry with fragmentation of the precursor/molecular ion)[27,30,31,32,33,37,47,48,56,57,58,61,62]. Those wishing to understand more about speciation-analysis methodology are referred to critical reviews of recent analytical developments for the Se speciation analysis of foods, supplements and bio-samples[28,73].

Most quantitative data in this table have been calculated from the peak area for a particular Se species expressed as a percentage of the total area of eluted Se peaks. However, accurate measurements by isotope dilution mass spectrometry or standard additions are also reported for methylated Se compounds such as SeMet and γ-glutamyl-*Se*-methyl-selenocysteine[25,27,29,72]. Ideally, full mass balance data (i.e. total selenium, total extracted selenium, selenium species, sum of species, extraction efficiency) should be considered together with recovery results from spiking experiments or analysis of "speciated" certified reference materials for validation of speciation methodologies.

The total Se concentration has been reported in the table where possible, as it can affect the distribution of Se between species, as in the case of Se-enriched garlic and yeast[23]. As the concentration of Se in Se-enriched foods is considerably higher that in the corresponding natural foods, such foods must be treated with caution, though the amounts in which they are eaten (e.g. garlic) may reduce the risk of toxicity.

It is noteworthy that while wheat, other grains and soy contain predominantly SeMet with lesser amounts of SeCys and selenate, the major seleno-aminoacids found in *Allium* and *Broccoli* species are *Se*-methyl-selenocysteine and γ-glutamyl-*Se*-methyl-selenocysteine. The latter two compounds are characteristic of the Se species produced by Se-accumulator plants which avoid the

toxic effects of incorporation of excessive amounts of SeCys and SeMet into their proteins by accumulating non-protein selenoamino acids or their γ-glutamyl derivatives[6]. Other nonprotein selenoaminoacids that have been identified in selenium accumulator plants are selenocystathionine, *Se*-methyl-selenomethionine, γ-glutamyl-selenocystathionine, selenopeptides and selenohomocysteine[9] though of these, only selenocystathionine has been fully identified in foods (Table 1).

Given that Brazil nuts are potentially the richest food source of Se, and the tree that produces them, Bertholletia excelsa, is regarded as a Se-accumulator, it might be expected that the major Se species would be Se-methyl-selenocysteine or gamma-glutamyl-Se-methyl-selenocysteine, as described above. Instead the major species in Brazil nuts appears to be SeMet [44-46]. This may to some extent be an illustration of the differences in concentration and speciation found between different plant tissues, Brazil nuts being seeds rather than fleshy leaves or florets as in the case of garlic or broccoli[3,6]. However, it may also be due to more general differences in Se metabolism between plant species (personal communication, Dr Martin Broadley, 2007).

Considerably less information is available on Se species in animal foods than is available for plant foods. Although the Se content of fish and other sea-foods has been reviewed by Reilly[74], normally ranging from 0.1-1.0 μg/g fresh weight, there is little information on specific Se species in fish. Several studies have found that sea-food Se appears to be less bioavailable than that from other dietary sources, the implication being that the molecular form of at least some of the fish Se is such that it is not utilisable for selenoprotein synthesis [40,75,76]. Though it has been suggested that an explanation for this lower bioavailability may be interaction with mercury (Hg) in sea-food, the molar concentration of Se exceeds that of Hg by one or two orders of magnitude except in the case of sea-mammals (cetaceans) suggesting that this is an unlikely explanation[77-79]. While Se and Hg undoubtedly have very high affinity for one another[80], there is as yet no published data identifying Se-Hg species in sea-food. However, according to Dr Nick Ralston (personal communication 2007) it appears that inorganic HgSe is present in the muscle meat of blue marlin as has already been shown in organs of mammals[81]. SeMet was the only compound identified in fish samples of high Se content in a speciation study[67] though other studies found from 4-47% of total fish Se in the form of selenate [68-70]. This is an area ripe for further speciation studies.

Recently, new Se-containing glutathione (GSH) species, *S*-selenomethyl-glutathione and glutathione-*S*-selenoglutathione (GS-SeG) have been identified in aqueous extracts of Se-yeast[33]. As shown in Figure 3, bonding of Se to GSH *via* a non-enzymatic reaction occurs in metabolism at the point where selenite enters the pathway to SeCys[6]. Alternatively, as glutathione is a tripeptide of γ-glutamine, cysteine and glycine, it seems possible that the formation of these Se-containing

GSH species could result from the incorporation of selenocysteine (or methylated selenocysteine) in place of cysteine in the biosynthetic pathway to glutathione.

While on the subject of Se-yeast, we should make it clear that it is not a defined form of Se. There is considerable variability in products described as Se-yeast which is reflected in the species composition. Se-yeast is produced by fermenting yeast in a Se-enriched medium when the Se becomes organically bound to yeast components. With reputable manufacturers, the percentage of Se that is organically bound should be greater than 90% and more than 80% should be bound to yeast proteins, including cell-wall proteins[12]. However, in some products, the percentage of sodium selenite is such that most of the selenium is clearly not bound to the yeast: at worst, there may merely be a mixture of sodium selenite and yeast, the selenium not being bound to the yeast[24]. Such products dupe the consumer as they do not conform to the normal understanding of Se-yeast as containing Se in an organic form. While they may be capable of increasing the production of selenoproteins, they will be less-good at increasing plasma Se and acting as a storage form of Se in the body (see below) thereby maintaining Se status[82].

Se in food sources and dietary supplements: bioavailability

Bioavailability of a nutrient is conventionally defined as that fraction of ingested nutrient that is utilised for normal physiological functions[83]: absorption and retention of the nutrient are taken as indirect measures of bioavailability as these are measurable[83] though they cannot address *functional* bioavailability which is that most likely to be relevant to health.

Absorption of Se is not homeostatically regulated and is not believed to be affected by nutritional status. Absorption of dietary Se is generally believed to be good - around 80% from food[74]. Guar gum is thought to reduce its absorption in humans[84] as is high dietary sulphur, probably because of competition between chemically similar sulphur and Se species[74, 85]. Absorption of SeMet is active and uses the same enzyme transport system as does methionine[74]. Absorption and retention of a commercially-produced Se-yeast in which 66% of the Se present was in the form of SeMet (SelenoPrecise™), were measured as 90% and 75% respectively (see[12])[86].

A number of supplementation studies have compared the bioavailability of different forms of Se to humans, i.e. Se-rich wheat, Se-enriched yeast, SeMet, sodium selenate and sodium selenite (see review[12]). Organic forms of Se (wheat Se, SeMet and high Se-yeast) were found to be more bioavailable than selenate and selenite in that they were more effective in raising blood Se concentrations (suggesting better absorption and retention), though all forms were able to increase selenoenzyme (glutathione peroxidase) activity. This difference is undoubtedly due to the ability of SeMet from digested organic Se sources to be incorporated in place of methionine into tissue proteins such as skeletal muscle, erythrocytes and plasma albumin where it can act as a Se store

though it becomes available to the body only upon turnover of tissue proteins[87]. Organic Se (Se-yeast) was also more effective than inorganic forms in its ability to transfer Se to breast-fed infants or suckling animals, thereby reducing the risk of deficiency in the offspring[12] . Foods that contain high proportions of SeMet, such as Brazil nuts and wheat, are good bioavailable sources of the element[88,89]. Though the Se content of mushrooms is higher than that of most other vegetables[74], its bioavailability is said to be very low[90]. However, our own recent work on Se-enriched mushrooms shows SeMet to be the major Se species and bioavailability to be good[57]. A speciation effect may be responsible for the bioavailability of Se from fish being inconsistent[91]: one study has shown a daily intake of 115 μg Se from fish to be unable to increase Se status[76].

There is good evidence that the increased Se status attained after supplementation with organic forms of Se is retained for a longer period after supplementation has ceased than is the case with selenite or selenate[12]. Reported whole-body half-lives of SeMet and selenite in humans were 252 and 102 days respectively, implying that Se administered as SeMet is retained 2.5 times longer in the body than is selenite[85]. Accordingly, foods or supplements containing SeMet can maintain the activities of selenoenzymes during Se depletion for longer periods of time than those containing inorganic Se owing to the recycling of SeMet catabolised from protein stores[85].

No bioavailability data exist for *Se*-methyl-selenocysteine or γ-glutamyl-*Se*-methyl-selenocysteine.

Health effects associated with specific Se species in foods and supplements

While the nutritionally essential functions of Se are understood to be fulfilled by the selenoproteins, dietary Se can be metabolized to small molecular weight species that have more recently generated interest because of putative anti-cancer effects. In contrast to such beneficial effects, at a sufficiently-high dose level, Se metabolites can also cause toxicity.

Species-related beneficial effects

Though supplementation with Se or a good Se intake or status has been associated with health benefits, there is little or no evidence to connect such benefits with particular Se species. We know from studies in transgenic mice that selenoproteins are important for the cancer-protective effects of selenium[92] and it seems likely that antioxidant selenoproteins may be of benefit in counteracting diseases of oxidative stress. However, selenoproteins can be synthesised more or less efficiently from many different Se species, though if consumed in foods, they are digested and must be resynthesised as shown in Fig. 2.

In mice with genetically impaired selenoprotein expression, the presence of low molecular weight selenocompounds has been shown to reduce colon cancer risk[92]. Such low-molecular weight selenocompounds may be an *in vivo* source of the methylated metabolite, CH_3SeH which is believed to be responsible for the potent anti-carcinogenic and anti-angiogenic effects of Se shown in the rat mammary tumour model and in cells in culture[5,60,93-97]. As shown in Fig 2 and explained above, CH_3SeH can be formed directly from the low-molecular weight selenocompounds, *Se*-methyl-selenocysteine, by the action of a β-lyase[11] and SeMet by the action of a γ-lyase, also known as methioninase[13,15,16,97-99].

Se-methyl-selenocysteine and its γ-glutamyl-derivative are found in a number of edible plants, including garlic, onions and broccoli and others of the *Allium* and *Brassica* families, particularly when grown in Se-enriched conditions[5,23,60]. Se-enriched plants such as broccoli and garlic have been shown to have potent anti-tumour effects in animals that are attributed to the presence of these species[60,96]. Though these species have not yet been tested in human interventions, a number of groups are planning pharmacokinetic studies as a prelude to human trials (Dr Clement Ip, personal communication, 2006). Small amounts of both *Se*-methyl-selenocysteine and γ-glutamyl-*Se*-methyl-selenocysteine have also been identified in Se-yeast which may possibly be relevant to the anti-cancer effects seen in human trials with Se-yeast[26,27]. *Se*-methyl-selenocysteine has been commercially available for some time and can be bought over-the-counter as a supplement.

Though there is as yet no evidence of it, it appears possible that Se analogues of anti-cancer sulfur compounds such as diallyldisulphide and ajoene may also be isolable from Se-enriched garlic or onions. As diallylselenide was found to be more than 300-times more effective than diallylsulfide in protecting against carcinogen-induced mammary adenocarcinoma in rats[97], attempts to find such species may be worthwhile.

Species-related toxic effects

More is known about species-related toxic effects of Se than about species-related beneficial effects. The toxicity of Se and the mechanisms by which it exerts its toxic effects depend on its form though there is little species-specific data on the toxicity of Se in humans.

It is likely that a number of different mechanisms are involved. According to Spallholz and colleagues[97, 98], Se compounds that can easily form the anion, RSe^-, generate superoxide in the presence of thiols such as GSH, resulting in redox cycling, cell-cycle arrest and apoptosis. Spallholz ascribes the toxic (and indeed the carcinostatic) effects of Se to this oxidative-stress mechanism. Superoxide has been shown to be generated from selenite and diselenides such as selenocystamine in the presence of reduced GSH *in vitro*, though not from selenate, SeMet or *Se*-

methyl-selenocysteine[97]. Neither SeMet nor *Se*-methyl-selenocysteine is very toxic to cells in culture nor to animals or humans in line with their inability to generate superoxide, although both are capable of conversion to CH_3SeH by enzymatic systems either *in vitro* or *in vivo*[97].

Selenodiglutathione (GSSeSG), an intermediate in the formation of superoxide from selenite and GSH, has been found to be even more toxic than selenite itself[98,99]. However, in contradiction to Spallholz's belief, Harrison and colleagues[100] did not find that the growth inhibition observed with this compound resulted from induction of an oxidative-stress mechanism, at least not of the type observed with oxidants such as H_2O_2. Supporting an oxidative-stress mechanism, selenite-induced redox cycles have been suggested to be responsible for oxygen-dependent DNA fragmentation in Se toxicity to hepatocyte model systems[101] and high levels of selenite have been shown to induce the formation of 8-hydroxy-2-deoxyguanosine in rat liver DNA[102].

Other suggested mechanisms of Se toxicity include inhibition of Se methylation, the major detoxification pathway for Se, allowing the accumulation of hepato-toxic selenides, notably H_2Se. For instance, in mice, high doses of SeCys have been shown to cause hepatic toxicity by depressing Se methylation through the inactivation of methionine adenosyltransferase, the enzyme responsible for *S*-adenosyl methionine (SAM) synthesis[103].

Although it has been suggested that organic forms of Se may be more toxic than inorganic forms during long-term consumption as they can be incorporated into tissue proteins rather than be excreted rapidly[104], there is no evidence that this is the case[40]. Long term supplementation studies with Se-yeast at doses of 200, 300, 400 and even 800 μg Se/d for lengthy periods (up to 12 years in the case of the 200 μg dose) have been carried out by a number of research groups without any indication of toxic effects (for references, see[12]). Furthermore men with prostate cancer tolerated doses of 1600 and 3200 μg Se/d for almost 12 months "without any obvious Se-related serious toxicity"[105]. Thus these results imply that uncontrolled accumulation of tissue Se does not occur.

Though there is no direct evidence in humans, it is generally accepted on the basis of animal studies that inorganic forms of Se are more *acutely* toxic than organic forms, selenite being slightly more toxic than selenate[40]. Though of equivalent toxicity to SeCys in animals, sodium selenite is considerably more acutely toxic than SeMet, dimethyl selenide, trimethyl selenonium ion, selenoethers, selenobetaine or Se-yeast, the major Se component of which is SeMet[40]. From LD50 determinations, selenite was found to be four-fold more toxic than SeMet when administered to mice intravenously[106] and three-fold more toxic than Se-yeast when given orally to rats[107].

Chronic toxicity of SeCys is equivalent to that of selenite and both are more toxic than SeMet (the L-isomer of which is more toxic than the D-isomer) and other organic Se compounds in animal studies[40]. Comparison of selenite and Se-yeast diets in rats showed that Se-yeast was much less toxic than selenite: although the livers of animals fed Se-yeast showed up to 50% greater

deposition of Se, there was no corresponding toxicity, as evidenced by histological examination[108]. Se-yeast also seems to be less toxic than L-SeMet: after two weeks of feeding 30 μg Se/g diet, survival in mallard ducklings was 36% for L-SeMet and 88% for Se-yeast[109]. Human studies have also shown a lower chronic toxicity of organically-bound Se though there is limited data on the toxicity of individual compounds[40]. However, SeMet is known to be the main Se species present in the diet of Chinese who developed chronic selenosis from consumption of corn and rice grown in the Enshi area of China[39].

The toxicity of the Se-accumulators to livestock has been linked to the high levels of *Se*-methyl-selenocysteine found in these species[110]. Se-accumulator plants are able to circumvent the toxicity that would result from the non-specific integration of the selenoamino acids SeCys and SeMet into proteins by converting the precursor, SeCys, into the non-protein amino acids *Se*-methyl-selenocysteine, γ-glutamyl-*Se*-methyl-selenocysteine and selenocystathionine[8]. The potent toxicity of Se-accumulator plants to grazing animals is probably more a reflection of the extremely high concentrations of Se that can build up in these plants – up to 10 to15 mg Se/g dry weight even on non-seleniferous soils[8] – rather than the toxicity of *Se*-methyl-selenocysteine *per se*. According to Dr Clement Ip (personal communication 2006) who has worked with *Se*-methyl-selenocysteine for many years, it should be a safer compound than SeMet based on its biochemistry: though both compounds are equally well absorbed, *Se*-methyl-selenocysteine is converted to excretable metabolites more rapidly resulting in lower tissue retention of selenium. Comparison of the NOAEL in male and female rats for *Se*-methyl-selenocysteine (1.0 and 0.5 mg/kg/day, respectively) with that for selenite (0.14 and 0.2 mg/kg/day, respectively) suggests that *Se*-methyl-selenocysteine is less toxic at least than selenite[111] (Clement Ip, personal communication 2006). Results from Hasegawa and colleagues[103] similarly suggest that methylated forms of Se are generally less-toxic than non-methylated compounds. This postulated lower toxicity may be highly relevant to the potential for use of *Se*-methyl-selenocysteine in human cancer prevention studies.

Conclusion

The development of state-of-the-art analytical methods that combine elemental and molecular mass spectrometric detection, to investigate different chemical forms of Se in food has made possible the identification of a variety of Se species in foods and supplements. However, this is such a difficult and exacting area of research that to date, we have only scratched the surface. It is difficult to maintain the integrity of species through the extraction process. Though we may know the identity of some Se species present in foods, there is no case where we know the identity of all the Se species: only where we have mass balance, can we ensure that all species have been captured. We

need to take food processing and preparation into account so that we are actually investigating the species that will be consumed (e.g. Japanese soup stock made from shiitake mushrooms[56]).

There remain considerable gaps in our knowledge of the forms of Se that naturally occur in foods:- for instance we know little about species of Se, other than selenomethionine, in fish, normally considered a good source of the element, or indeed what Se-Hg species may be present; we need to know full speciation of Se in Se yeast because of its frequent use in human intervention studies; and perhaps most importantly, there is a need to know to which Se species beneficial or detrimental health effects can be attributed.

We need to continue to develop speciation methodology, and to further investigate biosynthetic and metabolic pathways in order to have a steer on what species we should be searching for. Where we do suspect we know the identity of an active species (e.g. *Se*-methyl-selenocysteine), we need single-species trials to prove efficacy or relative efficacy to help us towards a better understanding of how dietary Se should be supplemented.

Finally, there is a clear need for analytical chemists to present the data in a form that is understandable to and usable by consumers, nutritionists and legislators. Without adequate knowledge of Se speciation, false conclusions may be drawn when assessing Se requirements for optimal health. Furthermore, the ability to identify and accurately quantify Se species with powerful anti-cancer or other valuable effects will be essential for the development of plant breeding programmes to optimise the biosynthesis of such species if clear proof of their health effects should be forthcoming.

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Figure legends

Figure 1. Biosynthetic pathways elucidated for Se in higher plants (some by analogy with S pathways) (adapted from references[3-8]). Note: reactions vary from species to species so that compounds formed and their relative quantities differ between species and strains. Abbreviations: APSe adenosine-5′-phosphoselenate; DMDSe dimethyl-diselenide (volatile); DMSe dimethyl selenide (volatile); DMSeP dimethyl-selenonio-propionate [$CH_3Se^+(CH_3)_2CH_2CH_2COO^-$]; GSH glutathione; $GSSeO_3^{2-}$ glutathione-*S*-selenite; GSSeSG selenodiglutathione; GSSeH glutathione-selenopersulphide; $GSSe^-$ glutathione-conjugated selenide; γ-GMeSeCys γ-glutamyl-*Se*-methyl-selenocysteine; γ-GSeCysth γ-glutamyl-selenocystathionine; $MeMet^+$ *S*-methyl methionine; MeSeH methyl selenol; MeSeCysSeO *Se*-methyl-selenocysteine selenoxide; MeSeCys *Se*-methyl-selenocysteine; $MeSeMet^+$ *Se*-methyl-selenomethionine; MeTHF methyl-tetrahydrofolate; SAM *S*-adenosyl methionine; *Se*-ASeMet Se-adenosyl-selenomethionine; Se-Homocysteine selenohomocysteine; SeCysth Selenocystathionine.

Figure 2. Metabolic pathway of dietary Se in humans (adapted from[11,12])[13, 14]. Abbreviations: SeMet, selenomethionine; SeCys, selenocysteine; γ-glutamyl-CH_3SeCys, γ-glutamyl-*Se*-methyl-selenocysteine; CH_3SeCys, *Se*-methyl-selenocysteine; H_2Se, hydrogen selenide; CH_3SeH, methyl selenol; $(CH_3)_2Se$ dimethyl selenol; $(CH_3)_3Se^+$ trimethyl selenonium ion; GSSeSG selenodiglutathione; $HSePO_3^{2-}$, selenophosphate; SeO_2, selenium dioxide.