The use of high-selenium yeast to raise selenium status: how does it measure up?

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Abstract

Selenium-enriched yeast (Se-yeast) is a common form of selenium used to supplement dietary intake of this important trace mineral. However, its availability within the EU is under threat owing to concerns expressed by the EC Scientific Committee on Food that Se-yeast supplements are poorly characterised and could potentially cause the build up of selenium in tissues to toxic levels. This review examines the validity of these concerns. Diagrams of the biosynthesis and metabolism of selenium compounds show which species can be expected to occur in Se-yeast preparations. Se-yeast manufacture is described together with quality control measures applied by reputable manufacturers. The way in which speciation of Se-yeast is achieved is explained and results on amounts of selenium species in various commercial products are tabulated. In all cases described, selenomethionine is the largest single species, accounting for 54-74% of total selenium. Se-yeast is capable of increasing the activity of the selenoenzymes and its bioavailability has been found to be higher than that of inorganic selenium sources in all but one study. Intervention studies with Se-yeast have shown the benefit of this form in cancer prevention, immune response and HIV infection. Of around one dozen supplementation studies, none has shown evidence of toxicity even up to an intake level of 800 μg/d selenium over a period of years. It is concluded that Se-yeast from reputable manufacturers is adequately characterised, of reproducible quality, and that there is no evidence of toxicity even at levels far above the EC Tolerable Upper Intake Level of 300 μg/d.

Introduction

The trace mineral selenium is a crucial nutrient for human health. It is a component of a number of important selenoproteins and enzymes required for such functions as antioxidant defence, reduction of inflammation, thyroid hormone production, DNA synthesis, fertility and reproduction (Rayman, 2000). It can also be converted in the body to selenium metabolites that are thought to reduce the blood supply to tumours and kill cancer cells (Combs & Lü, 2001). Adequate dietary intakes of selenium are therefore essential.

Selenium enters the food chain through plants and its concentration in foods is determined by a number of geological and geographical factors:- (i) soil selenium species and concentration, (ii) pH, which determines to some extent the nature of the selenium species; (iii) amounts of organic matter, iron hydroxides, aluminium compounds and clay that can bind selenium reducing its bioavailability to plants, (iv) amounts of sulphur species (e.g. from sulphur fertilisers) that can compete with selenium for absorption; (v) rainfall that can leech selenium out of the soil and (vi) soil microbes that can convert insoluble forms of selenium to soluble forms (Diplock, 1993; Fordyce *et al.* 2000; Johnson *et al.* 2000; Adams *et al.* 2002). In some parts of the world where selenium is insufficiently available to plants, selenium-deficiency diseases have been identified such as Keshan disease, an endemic cardiomyopathy found in the North East of China (Keshan Disease Research Group, 1979) that formerly caused many deaths. Supplementation with selenium has greatly reduced the incidence of the condition (Reilly, 1996).

There are other regions of the world, such as the south island of New Zealand and parts of China and Europe, where selenium intake may not be adequate for full activity of protective selenoenzymes. Governments have set dietary reference values for selenium intake based on their assessment of the amount of selenium required to achieve optimal (or ⅔ optimal) activity of the antioxidant selenoenzyme glutathione peroxidase in plasma (Committee on Medical Aspects of

Food Policy, 1991; WHO/FAO/IAEA expert group, 1996; Food and Nutrition Board, 2000). Table 1 shows daily intake levels of selenium recommended by a number of national or international committees while Table 2 is a compilation of up-to-date selenium-intake data for a number of countries. While the intake of selenium in New Zealand appears to have increased in the last decade as a result of importation of Australian wheat (Thomson & Robinson 1996; Vannoort *et al*. 2000), that in a number of European countries has declined owing to reduced importation of wheat from N. America for bread-making that was relatively high in selenium (Rayman 1997; Adams *et al.* 2002). Comparison of the values in Table 2 with those of Table 1 shows that recommended daily intakes are not now achieved in the majority of European countries together with parts of China. These recommended intake levels (Table 1), however, do not take into account the fact that higher levels of selenium intake appear to confer additional health benefits on the immune system, on cancer risk and on HIV symptoms and progression (Rayman 2002, Burbano *et al.* 2002). Cancer protective intakes of selenium have been calculated separately by two groups (Combs *et al.* 2001; Thomson & Paterson, 2001) to be in the region of 75-125 or 96-120 μg/d, considerably higher than currently-recommended intakes.

Combs (2001) has compiled a table of reported concentrations of selenium in serum, plasma or whole-blood from 69 countries. Using the minimum value of 70 μg selenium/L in serum or plasma as a criterion of nutritional selenium adequacy as described above (Nève, 1995), he estimated nutritional selenium deficiency to be highly prevalent (>50%) in 21 countries and moderately prevalent (10-50%) in a further 16 countries (Combs, 2001). He also points out that the extent of the problem may be greater, as there is little or no information for most of Africa, South America and central and south Asia. Against this background and with new research evidence regularly appearing for the role of selenium in the reduction of viral virulence (Beck *et al.* 1995, 1998, 2001) and cancer risk (Li *et al.* 2004; Yoshizawa *et al.* 1998; Yu M-W *et al.* 1999; Helzlsouer *et al.* 2000; Nomura *et al.* 2000; Brooks *et al.* 2001; van den Brandt *et al.* 2003; Wei *et al.* 2004), it is not

surprising that many people are interested in ensuring they have adequate selenium status by supplementing their diets with selenium.

Selenium supplements available in order of increasing cost include:- the inorganic forms, sodium selenite, sodium hydrogen selenite and sodium selenate; organic forms, high-selenium yeast (Se-yeast) and the seleno-amino acid, L-selenomethionine (SeMet), the major selenium species in Se-yeast. Although all these supplements have been available without any apparent associated problems for many years, the European Parliament and Council of the EU issued a directive in 2002 (Directive 2002/46/EC) on permitted food supplements, specifying a so-called "positive list", which included inorganic forms of selenium but excluded organic forms such as selenomethionine and Se-yeast. Thus organic forms of selenium will no longer be able to be sold in the EU from August 2005, or from January 2010 in the case of countries that asked prior to August 2003 for postponement of this directive. In the latter case, a dossier supporting the safe use of any particular form of organic selenium has to be received by the European Commission by July 2005 and then approved by the European Food Safety Authority. This decision was based on the opinion of the EC Scientific Committee on Food (SCF) that Se-yeast supplements were poorly characterised with regard to nature and quantity of selenium components and because of the fear that selenium from SeMet could build up in body tissues to toxic levels. Given the relatively low cost, good bioavailbility and wide popularity of Se-yeast supplements, which appear to be the most-commonly purchased form of single-nutrient selenium supplement in Europe, it seems timely to examine the validity of the concerns raised by the EU in relation to Se-yeast. Before discussing these issues, it is helpful to have an idea of the different species involved in the biosynthesis of selenium compounds by yeast and their metabolism by humans as laid out below.

Biosynthesis and metabolism of selenium compounds with special reference to Se-yeast

Cereals and forage crops convert selenium mainly into SeMet and incorporate it into protein in competition with methionine. In the same manner, *Saccharomyces cerevisiae* (bakers' or brewers' yeast) may assimilate up to 3000 μg/g selenium, the major product being SeMet which is incorporated into yeast proteins or physically associated with macromolecules, especially cell-wall constituents (Demirci, 1999; Polatajko *et al.* 2004). Figure 1 shows the biosynthetic pathways in plants and yeast that relate to the formation of SeMet. SeMet formed biosynthetically is the L-isomer and where SeMet from yeast is mentioned hereafter, it should be understood to be L-SeMet. A number of other organic selenium compounds are also formed as described in detail by Whanger (2004).

The metabolic fate of SeMet and other organic selenium compounds from the human diet [e.g. selenocysteine (SeCys), Se-methyl-Se-cysteine (CH_3SeCys)] is shown in Fig. 2 (adapted from Combs, 2001). SeMet from Se-yeast and food proteins can be incorporated non-specifically into proteins such as albumin and haemoglobin in place of methionine. Alternatively it can be trans-selenated to SeCys which is then converted to hydrogen selenide (H_2Se) by a β-lyase. The H_2Se formed may be converted to selenophosphate ($HSePO_3^{2-}$) by selenophosphate synthetase. Selenophosphate 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 (Berry *et al.* 1991, 1993).

Excess selenium is detoxified by successive methylation of H_2Se, yielding methyl selenol (CH_3SeH), dimethyl selenide [$(CH_3)_2Se$] and trimethyl selenonium ion [$(CH_3)_3Se^+$], the latter two of which are excreted in breath and urine respectively. Se-methyl-Se-cysteine (CH_3SeCys), apparently present to a small extent in Se-yeast (Bird et al. 1997; Kotrebai et al. 2000; Larsen *et al.*

2001) and in some foods, notably Allium and Brassica vegetables (Kotrebai *et al.* 2000, Whanger, 2004), is acted upon by another β-lyase to give CH_3SeH directly (Combs, 2001). There is some evidence for direct formation of CH_3SeH from SeMet by action of a γ-lyase, also known as methioninase (Nakamuro *et al.* 1997; Spallholz *et al.* 2004; Wang *et al.* 2002). [CH_3SeH and its precursor Se-methyl-Se-cysteine are believed to be potently anticarcinogenic and antiangiogenic (Ip, 1998; Whanger, 2004).]

Oxidised inorganic forms (selenate, selenite), undergo reductive metabolism yielding H_2Se, the starting point for the manufacture of selenoproteins as described above. Oxidation of excess H_2Se can lead to the production of superoxide and other reactive oxygen species (Combs, 2001).

The ratio between incorporation of SeMet into body proteins and the formation of alternative more-active metabolic products will depend on the amount of dietary methionine [though this is generally only an issue in animal studies where diets may be methionine-poor (Sunde 1997)] and presumably on the history of SeMet intake. After a sufficient period of ingestion of SeMet (or Se-yeast), a state of equilibrium may be reached wherein turnover from the general protein pool is able to supply SeMet metabolites (Schrauzer 2001) at a level associated with health effects (Rayman 2000).

Manufacture and quality control of Se-yeast production

Se-yeast is the product of the aerobic fermentation of *Saccharomyces cerevisiae* in a selenium-enriched medium. Different companies use different strains of *Saccharomyces cerivesiae* and may describe them either as bakers' or brewers' yeast. The medium is usually beet or cane molasses to which are added vitamins, nutritional salts and other growth factors to ensure maximal biomass, and measured amounts of selenium salts (e.g. sodium selenite) as the selenium source. Control of pH, temperature, selenium feeding profile and aeration allow optimal growth of the yeast strain and

maximum biomass production. A selenium-enriched yeast cream is produced that is then pasteurised thereby killing the yeast, and dried, frequently by spray-drying. One company (Pharma Nord, Denmark) does not use molasses because of the inherent variability of such a material but instead prefers to grow the yeast on pure glucose syrup with appropriate pharmacopoeia-grade additives in order to produce a pharmaceutical-grade, species-constant Se-yeast.

As a result of the fermentation in the selenium-enriched medium, the selenium becomes organically bound to the yeast. The amount bound should be greater than 90%, typically 94% (percentage of complexed organic selenium found in three lots of LAMIN™ Se-yeast by KABS Laboratories, St. Hubert, QC, Canada) in the case of one manufacturer (Lallemand, Canada). In this case, Lallemand R&D reported that around 83% of the selenium in the yeast was bound to yeast proteins, including cell-wall proteins.

Reputable manufacturers of Se-yeast ensure that their material is of good quality by carrying out the following checks or analyses on a routine basis:- assessment of the purity of the yeast strain (by biochemical and genetic identification techniques), the percentage of complexed organic selenium and the percentage of selenomethionine; measurement of particle size, moisture content and of the level of toxic impurities (arsenic, cadmium, lead, mercury) and microbiological contaminants which must meet required purity criteria.

Unfortunately not all material sold as Se-yeast is produced according to these stringent criteria. Sometimes 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 (Uden *et al.* 2003). Thus a customer may not be getting what he/she has paid for i.e. an organically-bound selenium source that contains a good proportion of SeMet. While this may not matter in most cases, there is an issue of bioavailability. SeMet has the capacity to be

assimilated non-specifically into normal body proteins in place of methionine thus acting as a potential reservoir for selenium (Combs, 2001; Thomson *et al.* 1993). Sodium selenite, though a good selenium substrate for the formation of selenoproteins, cannot be stored for later use (Alfthan *et al.* 1991).

It should be noted that Se-yeast is not just manufactured for human consumption. There is a burgeoning market in Se-yeast as an FDA-approved selenium supplement for livestock, where Sel-Plex™ (Alltech) is probably the market leader.

Species contained in Se-yeast – speciation

The wide use of Se-yeast in nutritional and intervention studies such as the Nutritional Prevention of Cancer Trial in the US that showed a significant reduction in cancer incidence and mortality with a 200 μg Se-yeast supplement (Clark *et al.* 1996), has spurred analysts on to identify the selenium species, other than SeMet, that are present in Se-yeast. Such "speciation" studies are difficult, requiring a combination of state-of-the-art techniques and advanced instrumentation.

The main difficulty is how to preserve the identity of the selenocompounds during their extraction from the yeast, prior to analysis using chromatographic and mass spectrometric techniques. Water has been used to extract high- and low-molecular-weight species (Polatajko *et al.* 2004; McSheehy *et al.* 2002) but the extraction efficiency is low, around 10-15 % of the total selenium in the yeast. Proteolytic hydrolysis with non-specific enzymes, mostly Protease XIV, has given 60-80 % recovery of the total selenium content in the dry yeast, mostly as SeMet. However with this method, it is not possible to distinguish between species that have undergone degradation and those that have not.

Separation of selenium compounds can then be achieved by a number of methods such as size-exclusion, cation or anion exchange high performance liquid chromatography (HPLC) (Lobinski *et al.* 2000; Larsen *et al*. 2004) or reversed-phase ion-pair HPLC (Lobinski *et al*. 2000; Uden *et al.* 2003). Intact selenium-containing proteins from Se-yeast have also been successfully separated by two-dimensional gel electrophoresis (2-D SDS PAGE) (Chassaigne et al. 2004). Selenium-specific detection is generally by inductively coupled plasma mass spectrometry (ICP-MS). Alternatively, for volatile compounds and selenoaminoacid-volatile-derivatives, gas chromatography with detection by atomic emission (AED) has been used (Uden *et al.* 2003). The use of tandem mass spectrometry on selenium-containing fractions gives the possibility of identification of species for which standards do not yet exist. Matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) MS with electrospray MS have been used successfully to identify selenium species including selenopeptides (Encinar *et al.* 2003).

Owing to the differences in manufacturing method referred to above, and to the varying extraction techniques and analytical methods used in different laboratories, it is hardly surprising that analysis of Se-yeast gives a variety of results. Analyses of commercially-available Se-yeast preparations have shown organically-bound selenium to vary from 0-97% of the total selenium content (Uden *et al.* 2003). Even for the same yeast batch, results can vary somewhat between laboratories. Depending on the extraction technique, the yield and therefore the ratio of molecules such as SeMet to total selenium can differ slightly. For example, the same batches of LALMIN™ selenium yeast (Lallemand, Canada) analysed by two laboratories gave percentages of SeMet as 58% (Danish Veterinary and Food Administration Laboratory) and 65% (Ultra Traces Analyses Aquitaine, UT2A, Pau, France) of total selenium, probably reflecting the difference in extraction efficiencies, calculated as 78% in the first case and 93% in the second case. Both laboratories however found very little variation in the ratio of SeMet to total selenium in different batches (1.6% and 1.5% RSD) indicating the reproducible quality of the Se-yeast.

In Se-yeast digests from reputable manufacturers, SeMet is the major single component: 58-65% (see above) of total selenium in LALMIN™ (Lallemand, Canada); 60-70% of total selenium in SelenoExcell™ (Cypress Systems, Fresno, California); 54-60% of total selenium in SelenoPrecise™ (Pharma Nord, Denmark) (Uden *at al.* 2003; Larsen *et al.* 2004), 62-74% in Sel-Plex™ (personal communication Dr. Ronan Power, Alltech, Kentucky 2004), with the caveat that the quoted figures are dependent on the extraction efficiency of the technique used by the analytical laboratory.

Good-quality Se-yeast preparations such as those described above should not contain more than 1% of inorganic selenium, the remainder having been converted to organic species or washed from the Se-yeast. Intermediates formed in the biosynthesis or metabolism of SeMet (see Fig. 1) such as Se-homocysteine, Se-cystathionine, and γ-glutamyl-Se-methyl-Se-cysteine have also been identified in addition to analogues of sulphur metabolites such as Se-adenosyl-Se-homocysteine (Bird *et al.* 1997; Uden *et al.* 2003). The metabolite Se-methyl-Se-cysteine has also been reported in digested Se-yeast extracts on the basis of its matching retention time with a standard of that substance (Bird *et al.* 1997; Kotrebai *et al.* 2000; Larsen *et al.* 2001). Aqueous yeast extracts have also shown the presence of selenodiglutathione (GS-Se-SG), a metabolite of selenite, and the mixed selenotrisulphide of glutathione and cysteinylglycine (GS-Se-SCG) (Lindemann & Hintelmann, 2002). Molecules identified in digested extracts as a percentage of eluted selenium from enzymatic hydrolysates of various commercially available Se-yeasts are given in Table 3. The data on the two commercial yeasts (believed to be Selenomax, personal communication, Prof. Peter Uden 2004) illustrate the difference in species and percentages that can occur in yeasts of different total selenium content. Note that SeMet-selenoxide or its hydrate are products of oxidation of SeMet and are probably analytical artefacts (Larsen *et al.* 2003; Uden *at al.* 2003) not present in the original samples.

While the table shows the wide variety of compounds that have been identified in Se-yeast, direct comparison between samples may be misleading owing to the application of different methods of analysis in different laboratories.

As explained above, a number of Se-yeasts appear to contain small amounts of Se-methyl-Se-cysteine (0.5%) or its precursor, γ-glutamyl-Se-methyl-Se-cysteine (0.5%). One study has reported Se-methyl-Se-cysteine to account for as much as 25% of the total yeast selenium in a sample of Nutrition 21 (Selenomax™) Se-yeast (El-Bayoumy *et al.* 2002). However the authors of this paper point out that the imputed presence of Se-methyl-Se-cysteine was based on co-chromatography with a synthetic standard and that the method they used was, at best, only semi-quantitative (personal communication, Bogdan Prokopczyk, 2003). Furthermore the analysis for Se-methyl-Se-cysteine was performed on samples that were two-years old. The presence of Se-methyl-Se-cysteine and γ-glutamyl-Se-methyl-Se-cysteine is of interest as these species are believed to be metabolised in animals and humans to methyl selenol (CH_3SeH), a potent anticarcinogen (Ip, 1998). Among the unidentified Se-yeast products, it is possible that there may be other organoselenium species that may also be more-potent anticarcinogens than SeMet.

Archived samples of Se-yeast (Nutrition 21) tablets used in the Nutritional Prevention of Cancer Trial (Clark *et al.* 1996) have been analysed by two laboratories and have been found to contain a number of unknown selenium compounds (Larsen *et al.* 2004; Uden *at al*. 2003). Uden and co-workers (2003) have identified not only SeMet-selenoxide (hydrate), the oxidation product of SeMet referred to above, but also a hitherto unreported Se-S bonded product, S-(selenomethyl)-cysteine. It is of course not known if these compounds were present in the yeast tablets at the time of the trial nor if they have any health effects, though it seems possible that SeMet-selenoxide is equivalent in action to SeMet (Uden *et al.* 2003). In any case it is readily reduced back to SeMet by glutathione (Arteel *et al.* 1999).

In conclusion, it seems fair to say that Se-yeast products from reputable manufacturers contain almost all their selenium organically bound and around 55 to 75% of it (allowing for extraction efficiency) in a form that hydrolyses to SeMet. Smaller amounts of other selenium compounds, including at least one precursor of the potently anticarcinogic methyl selenol, and an oxidation product of SeMet, SeMet-selenoxide (hydrate) have also been identified in the enzymic extracts. Apparent substantial qualitative and quantitative differences between Se-yeasts may be at least partly a result of different techniques and analytical procedures applied in different laboratories.

Bioavailability of Se-yeast

Bioavailability is conventionally defined as the fraction of ingested nutrient that is utilised for normal physiological functions (Fox *et al.* 2004). As there is no direct method of measuring bioavailability, absorption and retention of the nutrient are taken as indirect measures of bioavailability (Fox *et al.* 2004). Absorption of selenium is not homeostatically regulated and is not believed to be affected by nutritional status.

A few dietary factors are known to affect the bioavailability of selenium. Protein is said to enhance its bioavailability (Combs, 1988), presumably because methionine from protein competes with SeMet for incorporation into body proteins thus making SeMet more bioavailable for short-term use. In humans, vitamin C appears to improve the bioavailability of selenium from a mixed diet (Fairweather-Tait, 1997). Combs (1988) describes vitamin A (high levels), vitamin E and antioxidants as enhancers of the bioavailability of dietary selenium in animals. Substances that appear to reduce its bioavailability include guar gum, which reduces selenium absorption in humans (Fairweather-Tait, 1997), heavy metals and high dietary sulphur which are described as inhibitors of bioavailability in animals (Combs, 1988). As many selenium compounds are absorbed by the same

mechanism as their sulphur analogues, the absorption of a number of selenium species from Se-yeast may be reduced by high dietary sulphur intake.

Absorption of dietary selenium (organic selenium) is generally believed to be good - around 80% from food (Reilly, 1996). Absorption and retention of Se-yeast have been measured in 12 male subjects fed ^{77}Se-labelled SelenoPrecise™ yeast (Pharma Nord, Denmark), as 90% and 75% respectively (Sloth *et al.* 2003). Using the same methodology, a different form of Se-yeast gave a lower absorption and retention - 53.5% and 59.3% respectively (Fox *et al.* 2004). The reasons for the different results between the two studies may relate to the strain of the yeast and more-importantly to the difference between the processes used to prepare the Se-yeast. The Fox *et al.* study used non-pasteurised brewers' yeast, flask-grown in the laboratory to enrich it with ^{77}Se and freeze-dried, rather than the commercially produced, pasteurised (dead), spray-dried, bakers' yeast (SelenoPrecise™) used in the first bioavailability study. Temperature, time of fermentation and nature of selenium source (selenious acid) were also different from those normally used in manufactured products (personal communication Dr. Henri Durand, Lallemand, 2004). Forced aeration is not possible in flask-grown yeast resulting in low biomass production and low selenium incorporation into amino acids (personal communication Dr. Ronan Power, Alltech, 2004). Thus the selenium concentration of the Fox *et al.* Se-yeast was reported as 145 μg/g dry yeast, almost ten-fold lower than the 1400 μg selenium/g of SelenoPrecise™. Furthermore, the heat treatment (pasteurisation) to which commercial Se-yeast products are subjected kills the yeast and causes the disintegration of the yeast cells thereby improving their digestibility (personal communication Dr. Henri Durand, Lallemand, 2004). It is hardly surprising, therefore, that the measured absorption and retention of the Se-yeast used in the study of Fox and colleagues was lower than that of a commercial product and it is unlikely to be representative of Se-yeasts on the market.

A number of supplementation studies have indicated that the selenium from Se-yeast is more bioavailable than from inorganic selenium (with one exception, Fox *et al.* 2004, see below) and that increased selenium status is retained for a longer period after supplementation has ceased. This is undoubtedly due to the non-specific incorporation of SeMet from digested yeast proteins into tissue proteins such as skeletal muscle, erythrocytes and plasma albumin from which it can subsequently be released by catabolism to maintain increased selenium status for a time. Some examples of supplementation studies are given below.

The bioavailability of selenium from Se-yeast has been shown to resemble that of wheat selenium rather than inorganic selenium (selenate) in its effect on plasma selenium (Levander *et al.* 1983). Three groups of ten men of low selenium status were given 200 μg selenium/day as selenium-rich wheat, selenium-rich yeast, or sodium selenate for 11 weeks. Twenty unsupplemented subjects served as controls. Ten weeks after the supplements were discontinued, plasma selenium and platelet glutathione peroxidase were higher in the wheat and yeast groups than in the selenate group (see Fig. 3). Fig. 3 shows the remarkable similarity in the behaviour of wheat and yeast selenium both in terms of their capacity to raise plasma selenium and in the rate of decline of selenium status after withdrawal of supplementation.

In a study carried out by Alfthan and colleagues (1991), men were supplemented with 200 μg selenium/d in the form of selenate, selenite and Se-yeast. Se-yeast and selenite increased plasma selenium after 11 weeks from 110 μg/L to peak values of 170 and 125 μg/L, respectively. Thus the effect of Se-yeast supplementation was more pronounced than that of inorganic selenium supplementation but the levels did plateau after 11 weeks and did not continue to increase indefinitely. Post-supplementation, the decrease in plasma and erythrocyte selenium levels was far less pronounced in the Se-yeast group than in the groups supplemented with inorganic selenium, taking longer to return to baseline levels.

Similarly, a three-month Danish study of selenium supplementation in humans, suggested that organic selenium (selenomethionine and high Se-yeast) is more bioavailable than selenate and selenite (Clausen & Nielsen, 1988). Both the inorganic and the organic selenium supplements gave rise to steady-state levels of glutathione peroxidase after one month but whole-blood levels of selenium continued to rise for longer in those receiving organic selenium. This did not happen, however, in the study of Schrauzer and White (1978), who found that a steady state of selenium in whole blood was reached about two months after supplementing human subjects with 150 μg/d of Se-yeast.

Thirty-three New Zealand women aged 18-23 years received daily for 32 weeks, 200 μg selenium as Se-yeast, or brewer's yeast mixed with selenate, or no added selenium (placebo) in a double-blind trial (Thomson *et al.* 1993). Se-yeast was more effective in raising blood selenium concentrations than selenate, but both were equally effective in raising glutathione peroxidase activities in whole blood, erythrocytes and plasma, indicating a similar bioavailability of the two forms in terms of increasing selenoenzyme activity.

Kumpulainen demonstrated that 100 μg/d of Se-yeast is more effective than the same dose of selenite in increasing maternal serum selenium in breast-feeding mothers (Kumpulainen *et al.* 1985). The selenium levels plateaued within six months of initiation of the yeast supplementation. In contrast to that finding, a Polish study showed no significant difference between the selenium intake of breast-fed infants whose mothers were supplemented with 200 μg/d of Se-yeast or sodium selenite for three months (Trafikowska *et al*. 1998). Supplemental Se-yeast was, however, shown to be beneficial in preventing the longitudinal decline in milk selenium content that occurred with advancing lactation (McGuire et al. 1993). When US women were supplemented with 200 mcg/d Se-yeast from 4 to 8 weeks post-partum, infant plasma selenium concentration at the end of the

supplemented period was 83 μg/L in the infants of supplemented mothers compared to 63 μg/L in the control infants (McGuire *et al.* 1993).

Pre-term infants experience significant selenium depletion (Daniels, 1996). The impact of Se-yeast on the serum selenium concentration of pre-term infants living in a low selenium area (Hungary) was determined (Bogye *et al.* 1998). Although the bioavailability of selenium in the form of yeast selenium was higher than that of other selenium compounds used for pre-term infants, no complications or side-effects owing to enteral yeast selenium supplementation were observed.

Not all studies have found yeast selenium to be more bioavailable than inorganic selenium. The study of Fox and colleagues (2004) supplemented thirty-five male volunteers with isotopically-labelled selenium as selenate (^{82}Se, reference dose) and trout fish (raw or cooked, ^{74}Se) or yeast (^{77}Se) in order to compare the apparent absorption and retention of selenium from these different sources. Absorption of yeast selenium was lower than that of fish selenium (60%) and considerably lower than that of selenate (58%). Retention of selenium yeast was significantly lower than that of fish selenium (69%) and somewhat lower than that of selenate (95%). Thus this recent study showed that yeast selenium was *less-well-absorbed* and *less-well-retained* in the body than inorganic selenium. However, as explained above, this yeast was not typical of manufactured Se-yeasts which may explain the different results obtained.

There is some evidence that Se-yeast can raise selenium status to normal levels in situations where inorganic selenium cannot do so. One human and two animal examples are given. Administration of selenium as Se-yeast and SeMet significantly increased plasma selenium levels up to the normal range in rheumatoid arthritis patients when selenite supplementation was unable to do so (Heinle *et al.* 1997, Peretz *et al.* 1992).

Selenite and selenate have a limited capacity to increase the concentration of selenium in milk from dairy cows. This is a problem in Sweden where suckling calves are at risk of selenium deficiency. The following two studies carried out in Sweden illustrate that Se-yeast is much more effective than inorganic selenium in increasing the concentration of selenium in milk. (i) When dairy cows were supplemented with selenium as sodium selenite, selenate or Se-yeast, selenium concentration rose to a plateau in plasma at four weeks from 50 μg/l (control) to 75 μg/l (selenite), 80 μg/l (selenate) and 90 μg/l (selenium yeast). Milk concentration rose from 14 μg/l in the control group to 16.4, 16.4 and 31.2 μg/l in the selenite, selenate and Se-yeast groups respectively showing that Se-yeast was much more effective in raising the selenium concentration in milk (Ortman & Pehrson, 1999). (ii) Supplementation of the suckler cows' diet with Se-yeast rather than sodium selenite improved the selenium status of their calves (otherwise considered to be marginal). In practice, such supplementation would probably eliminate the existing risk of nutritional muscular degeneration in suckling calves (Pehrson *et al.* 1999).

The contrast between the bioavailability of Se-yeast and SeMet is illustrated by a study by McGuire and colleagues (1993). The impact of providing SeMet or Se-yeast on the selenium status of lactating and non-lactating women was studied. Plasma selenium declined in un-supplemented lactating women but not in non-lactating women. SeMet increased plasma selenium in both lactating and non-lactating women whereas Se-yeast increased plasma selenium only in non-lactating women. Plasma glutathione peroxidase activity decreased with duration of lactation in unsupplemented women and SeMet or Se-yeast supplementation prevented the decline. Milk selenium declined markedly for 20 weeks after parturition in unsupplemented women. SeMet significantly increased milk selenium concentrations whereas selenium yeast prevented a decline.

In summary, the studies quoted show that Se-yeast is both able to increase selenoenzyme activity and furthermore, unlike selenite or selenate, can be stored as SeMet in tissues, giving it a slower

whole-body turnover rate and allowing it to support greater tissue selenium concentrations than inorganic selenium. However its apparently lower bioavialbility compared to SeMet (McGuire *et al.* 1993) presumably reflects the fact that the yeast has to be degraded and that not all the selenium in Se-yeast is in the form of SeMet. Reported whole-body half-lives of SeMet and selenite in humans were 252 and 102 days respectively, implying that SeMet is retained 2.5 times longer in the body than selenite (Schrauzer, 2000). Accordingly, humans or animals supplemented with Se-yeast can maintain higher activities of selenoenzymes during selenium depletion for longer periods than those supplemented with inorganic selenium owing to the recycling of SeMet following catabolism from protein stores. These factors are an advantage in situations of low to marginal selenium status, such as exist notably in some European countries or in groups such as pre-term infants. Yeast selenium is also more effective than inorganic selenium in its ability to transfer selenium to breast-fed infants or suckling animals, thereby preventing the risk of deficiency.

Use of Se-yeast in intervention studies

A number of human intervention studies, as summarised below, have used Se-yeast as the intervention agent to good effect.

Cancer prevention studies

No controlled human cancer prevention studies have been carried out with any form of selenium other than Se-yeast. It is thus impossible to know if other forms i.e. inorganic selenium, or even SeMet, would have the same anti-cancer effects that have been shown for Se-yeast. The following intervention studies have used Se-yeast, the first in combination with other agents.

i. NCI sponsored trials in China for the prevention of oesophageal cancer observed significant reductions in total mortality and stomach-cancer mortality in the intervention arm containing Se-yeast, β-carotene, and vitamin E (Blot *et al*. 1993). Some nine years later, results from the same study showed significant inverse associations between baseline serum selenium

and death from esophageal squamous cell carcinoma (RR: 0.83; 95% CI: 0.71-0.98) and gastric cardia cancer (0.75; 0.59-0.95) (Wei *et al.* 2004). The authors have suggested that population-wide selenium supplementation in the region of China with low serum selenium and high incidences of these cancers should be seriously considered.

ii. The Nutritional Prevention of Cancer (or NPC) Trial, was designed to test the hypothesis that selenium supplementation could reduce the risk of cancer (Clark *et al.* 1996). In 1312 subjects with a history of non-melanoma skin cancer who were randomised in a double-blind manner to placebo or 200 μg Se/day (as Se-enriched yeast), there was no effect on the primary end-point of non-melanoma skin cancer. However, those receiving Se showed secondary end-point effects of 50% lower total cancer mortality ($p = 0.002$) and 37% lower total cancer incidence ($p = 0.001$) with 63% fewer cancers of the prostate, 58% fewer cancers of the colon and 46% fewer cancers of the lung. In a reanalysis of the data to include a further 25 months of blinded treatment and follow-up, Se-yeast supplementation significantly reduced total cancer incidence by 25% and prostate cancer incidence by 52% but although lung and colorectal cancers were reduced (by 26% and 54% respectively), these effects did not reach significance (Duffield-Lillico *et al.* 2002). The protective effect was stronger in males and those with lower baseline plasma selenium concentrations (<121.6 μg/L).

iii. In the Qidong county, around 40 miles north of Shanghai, the incidence of hepatocellular carcinoma (HCC) is particularly high. In this region around 15% of adults carry the Hepatitis B surface antigen and these people are 200 times more likely to develop HCC. In a study where 226 Hepatitis B antigen carriers were randomised to either 200 μg of Se-yeast or placebo, no case of HCC occurred in the supplemented group after four years, while seven subjects in the placebo group had developed HCC (Yu SY *et al.* 1997). However, as full details of the methodology of this study are not available, it is difficult to assess whether its protocol was sufficiently well-controlled or robust to be confident in its conclusions.

iv. A double-blind randomised, placebo-controlled trial of Se-yeast was carried out in 36 healthy adult males, 19-43 years of age, for a period of nine months. Beneficial effects were seen in the selenium-supplemented group that were not seen in the control group, namely a 32% increase in blood glutathione (GSH) levels ($p<0.05$) and a significant decrease in prostate specific antigen ($p<0.001$), suggesting a protective effect against prostate cancer with Se-yeast supplementation (El-Bayoumy et al. 2002).

v. Se-yeast was shown to be effective in reducing the tumour yield in an animal model of breast cancer induced by methylnitrosourea (Ip *et al.* 2000)

Rheumatoid arthritis

In a double-blind randomised controlled trial in a small group of rheumatoid arthritis patients, supplementation with 200 μg selenium as Se-yeast for three months, gave a significant reduction in pain ($p < 0.01$) and joint involvement ($p< 0.05$) (Peretz *et al.* 1992).

Patients with rheumatoid arthritis were supplemented with 600 μg/d "selenmomethionine-containing yeast" (presumably Se-yeast) enriched with vitamin E for eight months in a double-blind manner. Significant alleviation of articular pain and morning stiffness was observed and no adverse effects were seen (Aaseth *et al.* 1998).

Immune response in the elderly

Selenium appears to be able to reverse the age-related decline in immune response in the elderly. In a group of 22 institutionalised elderly subjects supplemented with 100 μg Se-yeast/d or placebo for six months, response to mitogen challenge was restored to the level of that in healthy young people in those receiving the selenium (Peretz *et al.* 1991).

HIV-progression

186 HIV-positive men and women with plasma selenium concentration in the "adequate" range (>85 μg/L), were supplemented with 200 μg/day Se-yeast (Selenomax® Nutrition 21) from 1998-2000 in a randomised, double-blind, placebo-controlled clinical trial (Burbano *et al.* 2002). In-patient hospitalisations, hospitalisation costs, and rates of hospitalisation were determined two years before and during the trial. A marked decrease in total admission rates (RR = 0.38; p = 0.002) and percent of hospitalisations due to infection/100 patients, for those receiving selenium was observed (p = 0.01). The cost of hospitalisation decreased by 58% in the selenium group compared to 30% in the placebo group (p = 0.001). In the final analysis, selenium therapy continued to be a significant independent factor associated with lower risk of hospitalisation (RR of hospitalisation, placebo *vs.* selenium 2.44, 95% CI 1.31-4.53, p = 0.01). Furthermore, the number of subjects with CD4 cell counts >50 declined by 46% in the placebo group but only by 25% in the selenium-treated group (p = 0.01). It was concluded that selenium supplementation was a beneficial adjuvant treatment in HIV-1-infected patients (Burbano *et al.* 2002).

Further studies using Se-yeast

Se-yeast (Cypress Systems) is currently being used in further prostate cancer studies at the Arizona Cancer Center at doses of 200-800 μg/d:- the Negative Biopsy Trial (Stratton *et al.* 2003a), the Preprostatectomy Trial (Marshall, 2001) and the Watchful Waiting Trial (Stratton et al 2003b). In the UK, the SELINA (SELenium IN Asthma) trial has been underway since 2002 using a dose of 100 μg/d (SelenoPrecise™, Pharma Nord) or placebo (Co-Principal Investigators Shaheen and Rayman).

Further studies using Se-yeast are planned though funding has not yet been secured: the SPRINT Trial (Selenium in PRegnancy INTervention) investigating the effect of 60 μg/d *vs*. placebo on the

risk of pre-eclampsia in the UK (Principal Investigator Rayman, Rayman *et al.* 2003), and the international PRECISE (PREvention of Cancer by Intervention with Selenium) trial which will use doses of 0, 100, 200 and 300 μg/d in 42,000 volunteers recruited through the internet.

Toxicity

It is generally accepted that inorganic forms of selenium are more acutely toxic than organic forms such as Se-yeast. There is data on toxicity of Se-yeast from two animal studies while a number of human intervention studies show that chronic administration of Se-yeast provides no evidence of toxicity.

Acute toxicity and animal studies

Acute toxicity studies with Se-yeast have been carried out on rats (Vinson & Bose, 1987). The LD_{50} for selenium yeast was 37.3 mg/kg compared with 12.7 mg/kg for sodium selenite demonstrating that Se-yeast is considerably less acutely toxic than sodium selenite (Figure 4). A further study comparing the toxicity of a selenite and a Se-yeast diet in rats also showed that Se-yeast was much less toxic than selenite (Spallholz & Raftery, 1987). Severe hepatotoxicity, cardiotoxicity and splenomegaly were observed in rats fed selenite at levels of 16 μg/g dietary selenium over an eight-week period whereas animals fed high-Se-yeast at the same level showed no such symptoms. Although the livers of animals fed Se-yeast showed up to 50% greater deposition of selenium, there was no corresponding toxicity, as evidenced by histological examination (Spallholz & Raftery, 1987).

Chronic toxicity - human studies

It has been suggested that organic forms of selenium may be more toxic during long-term consumption as they can be incorporated into tissue proteins rather than excreted rapidly. Signs of

selenosis are hair loss, brittle, thickened and stratified nails, garlic breath and skin lesions (Whanger *et al.* 1996).

Long term supplementation studies with Se-yeast have been carried out by four research groups:- Arizona Cancer Center, University of Arizona, first led by Dr. Larry Clark and later (following his death), by Dr. Jim Marshall with Dr. Mary Reid; Cancer Institute, Chinese Academy of Medical Sciences, Beijing by Dr. Shu Yu Yu and colleagues; The UK PRECISE study group at the University of Surrey in collaboration with the Institute of Cancer Research led by Dr. Margaret Rayman; The Danish PRECISE study group at Odense University Hospital led by Dr. Søren Cold in collaboration with Prof. Kim Overvad of the University of Aarhus. These studies are described below followed by a description of a medium-term human study with high dose Se-yeast and a short-term study in lactating women.

University of Arizona, Arizona Cancer Center studies: In the Nutritional Prevention of Cancer (NPC) Trial, a total of 1312 subjects whose baseline intake was around 90 μg Se/d, were randomly allocated to receive supplements of 200 μg of selenium as Se-yeast (Nutrition 21, brewers' yeast) or placebo yeast for a mean of 4.5 years (Clark *et al.* 1996). After a mean of 6.5 years of follow-up, significant reductions in total cancer mortality (down by 50%) and total cancer incidence (down by 37%) were seen in the selenium group. A total of 35 subjects complained of adverse effects, most of which involved gastrointestinal upset, resulting in their withdrawal from treatment. Of these, 21 were in the selenium-group and 14 in the placebo group. Within each group, those reporting adverse effects did not have significantly different plasma selenium concentrations from those not reporting such effects.

Longer-term follow-up data on selenium status from this trial than previously published (Clark *et al.* 1996) is shown in Figure 5 (personal communication, Dr. Mary Reid and Dr. Jim Marshall,

2003). It can be seen that administration of 200 μg/d Se-yeast gives a rapid increase in plasma selenium up to about three months, after which the increase levels off reaching a clear plateau at around 190 μg/L by one year. This plasma selenium level is well below that associated with toxicity (1054-1854 μg/L in whole blood, generally 23% higher than in plasma, Yang *et al.* 1989)

Currently there are three trials underway using doses up to 800 μg/d of high-Se yeast from Cypress Systems (California) (Marshall, 2001):- (i) the Negative Biopsy Trial, where 700 men with persistently-elevated prostate-specific antigen are randomly allocated to placebo or 200 μg or 400 μg selenium/d with a follow-up period of 57 months (Stratton *et al.* 2003a); (ii) the Preprostatectomy Trial, where 110 men with localised prostate cancer are supplemented with 200 μg or 400 μg selenium/d between biopsy and prostatectomy; (iii) the Watchful Waiting Trial, where men with localised prostate disease and a life expectancy of < 10 years receive 200 μg or 800 μg selenium/d as Se-enriched yeast (Stratton *et al.* 2003b). There have been no safety concerns in this trial even after supplementation with 800 μg Se-yeast/d for three years or more (Stratton *et al.* 2003b) and according to Marshall (2001), no evidence of any toxicity in any of these trials. This is consistent with the NOAEL of 819±129 μg/d from dietary selenium determined by Yang and Zhou (1994).

In an arm of the Watchful Waiting Trial that was discontinued in 2001, eight subjects were randomised to a dose of 1600 μg Se-yeast/d and 16 subjects to a dose of 3200 μg/d (Reid *et al.* 2004). Subjects were on these doses for average periods of almost 12 months. Mean plasma selenium levels achieved were 492 (SD 188) and 640 (SD 491) μg/L respectively. While the 3200 μg/d group reported more selenium-related side effects such as garlic breath, brittle nails and hair, stomach upset and dizziness than the 1600 μg/d group, blood chemistry and haematology results were all within normal limits. No severe or serious selenium-related toxicity was observed in either

group but these doses were discontinued because of the lack of safety information in the literature relating to these doses (Reid *et al.* 2004).

Studies from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing: Two hundred and twenty six Hepatitis Surface-Antigen Positive subjects were provided 200 μg Se yeast or placebo yeast daily for four years. No side-effects were reported (Yu SY *et al.* 1997).

Studies from the PRECISE Study Group at the University of Surrey: A pilot study for the UK PRECISE (PREvention of Cancer by Intervention with SElenium) Trial, funded by the Cancer Research Campaign, was initiated in October 1999. In this double-blind placebo-controlled pilot study, 500 male and female volunteers, aged 60-74, were randomised from June 2000. The study had four treatment groups: placebo, 100, 200, and 300 μg/day of selenium as Se-yeast (SelenoPrecise™, Pharma Nord, Vejle, Denmark). Randomisation was stratified to give groups that were equivalent in terms of numbers, age and gender.

Up to the end of January 2003, 16 subjects (3.2%) had withdrawn because of abdominal or stomach problems and 16 (3.2%) had withdrawn because of other adverse effects. Of the 32 subjects who reported adverse effects, 7 (22%) were on placebo, 8 (25%) were taking 100 μg of selenium, 7 (22%) were taking 200 μg of selenium and 10 (31%) were taking 300 μg of selenium. None of the subjects in the 300 μg group developed hair or nail problems, known signs of selenium toxicity. Chi-squared tests on withdrawals due to adverse events show that there was no significant difference in:- the number of abdominal/stomach problems in the four treatment groups ($X^2 = 0.5$, df = 3, p = 0.92); the number of other adverse events in the four groups ($X^2 = 0.57$, df = 3, p = 0.57); the number of total adverse effects by treatment group ($X^2 = 0.75$, df = 3, p = 0.86).

Nor was the mean selenium status of those suffering adverse effects significantly different from the mean of their treatment group ($p>0.05$). Although mean plasma selenium rose significantly in all treatment groups except placebo demonstrating good bioavailability of the Se-yeast, at 227 μg/g (233 μg/L) (Larsen *et al.* 2004), the level attained in the 300 μg/d treatment group was well within safe limits.

Thus from this study there is *no evidence of toxic effects* associated with Se intakes of 340 μg/d (300 μg/d from Se-yeast and approximately 40 μg/d from diet) over a maximum period of 2 years and 8 months, and plasma levels of 233 μg/L.

Studies from the PRECISE study group at the Odense University Hospital: A pilot study for the Danish PRECISE (PREvention of Cancer by Intervention with SElenium) Trial, was initiated in December 1998 (Larsen *et al.* 2004). In this double-blind placebo-controlled pilot study, as in the UK cohort, 500 male and female volunteers, aged 60-74, were randomised to one of four treatment groups: placebo, 100, 200, and 300 μg/day of selenium as Se-yeast (SelenoPrecise™ Pharma Nord, Vejle, Denmark).

Up to the end of August 2003, 23 subjects (4.6%) had withdrawn because of adverse effects. Of the 23 subjects who reported adverse effects, 8 (35%) were on placebo, 3 (13%) were taking 100 μg of selenium, 8 (35%) were taking 200 μg of selenium and 4 (17%) were taking 300 μg of selenium (personal communication, Dr. Søren Cold, 2004). Neither the distribution nor the severity of side effects correlated with the intake of selenium. The Chi squared test applied to withdrawals due to adverse effects shows that there was no significant difference in the number of total adverse effects by treatment group ($X^2 = 3.61$, $df = 3$, $p = 0.31$).

Mean (SD) *whole-blood* selenium concentration in 13 subjects from the 300 μg/d treatment group two-years after randomisation was 441 (132) μg/L (Larsen *et al.* 2004), similar to the level reported by Yang and colleagues (1983) to be safe, i.e. without evidence of selenosis. Thus from this study there is *no evidence* that selenium intakes up to 343 μg/d (300 μg/d from the Se-yeast supplement and approximately 43 μg/d from diet) over a maximum period of 4 years and 8 months, and whole-blood levels of 441 μg/L, are associated with toxic effects.

As the UK and Danish PRECISE Pilot Studies used the same protocol with respect to randomisation and treatment, withdrawals owing to adverse effects up to 24 months from randomisation in both pilots have been combined into a single table (Table 4). Chi squared tests on these data show clearly that there is no difference in adverse effects between the treatment groups ($X^2 = 1.83$, df = 3, p = 0.61) showing that Se-yeast supplementation is without toxic effects even after chronic dosage at 300 μg/d.

Medium term human study with high dose Se-yeast: Patients with rheumatoid arthritis were supplemented with 600 μg/d "selenmomethionine-containing yeast" (presumably Se-yeast) enriched with vitamin E for eight months in a double-blind manner. Though a significant alleviation of articular pain and morning stiffness was observed, no adverse effects were reported (Aaseth *et al.* 1998). Plasma selenium plateaued at 500 μg/L at four months.

Short-term study with Se-yeast in lactating women: A Polish study supplemented lactating mothers for three months with 200 μg/d selenium as Se-yeast or sodium selenite (Trafikowska *et al.* 1998). Supplementation with Se-yeast resulted in an increase in infant intake from 6.1 μg/d to a level of 11.3-12.8 μg/d that reached a plateau after one month. There was no significant difference between the selenium intake of these infants and of those whose mothers were supplemented with 200 μg/d as sodium selenite. The infant selenium intake achieved was four- to five-fold lower than

the Upper Limit of 45-60 μg/d recommended for infants of 0-12 months (Food and Nutrition Board 2000). At the end of three months, the infants supplemented with Se-yeast had plasma concentrations of 87 μg/L and whole blood concentrations of 102 μg/L. Comparison of the latter concentration with mean whole-blood values of 440-968 mcg/L that are associated with safe intakes of selenium (Yang *et al.* 1983, Yang & Zhou, 1994) leads to the conclusion that there is considerable leeway between the supplementation level of 200 μg g/d and the maximum level of Se-yeast that can safely be consumed without harm to the infant.

On this point, Dorea (2002) has commented that selenium appears in breast milk as a component of specific selenoproteins and seleno-amino-acids in milk proteins that are well-tolerated by breast-fed infants even in high amounts. This is consistent with the lack of evidence of increased sensitivity to selenium toxicity at younger ages (Food and Nutrition Board, 2000) and with Kim and Mahan's observation that nursing pigs are capable of buffering against excess of milk selenium (organic) by storing more selenium in body tissues (Kim & Mahan, 2001).

Summary of toxicity data

The EU Scientific Committee on Food (SCF) expressed the concern in 2002 that organic selenium e.g. SeMet or Se-yeast could build up in body tissues to toxic levels. The evidence presented here is reassuring in that respect, showing that selenium reaches a plateau in plasma after 11 weeks to 6 months, depending on the subjects and the study (Alfthan *et al.* 1991; Aaseth *et al.* 1998; Kumpulainen *et al.* 1985), and in whole blood after about two months, following selenium yeast supplementation (Schrauzer & White, 1978). In line with results of the latter study, selenium levels plateaued in erythrocytes after six weeks of selenomethionine supplementation (Luo *et al.* 1987). There are no published studies on tissue levels of selenium following long-term administration of Se-yeast though there are some in progress that will determine tissue levels of selenium in muscle biopsies from human volunteers that have been taking organic selenium for varying lengths of time

up to 20 or 30 years (personal communication, Deitrich Behne 2004). However, there is at present no evidence that Se-yeast supplementation causes a continuing rise in tissue selenium. According to Schrauzer (2001), at constant intakes of SeMet (as from Se-yeast), a steady state is established which is maintained indefinitely and over a large range of intakes owing to the release of SeMet from body proteins during protein turnover which occurs continuously. In any case, the large number of intervention studies quoted that have used Se-yeast in doses of 200, 300, 400 and even 800 μg/d for lengthy periods (up to 12 years in the case of the 200 μg dose) without any indication of toxic effects, implies that there cannot be a continuing rise in tissue selenium and supports the assertion that this form of Se-supplementation is no more hazardous than supplementation with inorganic selenium. This conclusion is supported by the observation that thousands of people living in South Dakota are, and have been, exposed to high intakes of organic selenium for over a century without adverse effects: no evidence of selenium toxicity was found in subjects whose intakes were as high as 724 μg/d (Longnecker et al. 1991).

In fact, the obligatory conversion of organic selenium (such as Se-yeast) into an inorganic form (H_2Se) may be an important regulator of Se bioavailability in that it may confer protection against excessive incorporation of selenium into selenoproteins and prevent toxicity mediated through reactive oxygen species (see Figure 2), from excessive intakes (Brown *et al.* 2000).

Bearing in mind the fact that selenium recommendations for infants are currently not achieved in 30% of breast-milk samples measured world-wide (Dorea. 2002) and that infants in Europe are more at risk of not meeting recommendations than infants from most other countries (Dorea, 2002), Se-yeast, as an inexpensive supplement, should help prevent inadequacy in this vulnerable group.

Se-yeast in the fortification of foods

Should some of studies currently underway indicate a significant benefit of a higher selenium intake, Se-yeast may be an excellent fortificant for foods. It provides a biological barrier between a cheap selenium source – inorganic selenium – and the individual consumer, reducing the risk of overdose. Evaluation of Se-yeast products with regard to shelf life indicates good long-term stability, 36 months or more (personal communications Nadine Renard, Lallemand, 2004 & Sven Moesgaard, Pharma Nord 2004).

Se-yeast is a pasteurised (dead), generally spray-dried, finely divided powder that has none of the unpleasant smell associated with SeMet and could readily be added to flour to increase the selenium content of bread. Se-yeast (LALMIN™, Lallemand) is stable to baking showing an excellent correlation ($R^2 = 0.999$) between the expected amount of total selenium per slice and the quantity expected (personal communication, Nadine Renard, Lallemand, 2004). By the same token, Se-yeast would be suitable for other food products, particularly those in powder form or drinks.

The use of Se-yeast to supplement the diet of cows has been shown to be considerably more effective than inorganic selenium in raising the concentration in milk and cheese (Malbe *et al.* 1995; Ortman & Pehrson, 1999).

Benefits and drawbacks of Se-yeast as a selenium source

There are a number of drawbacks associated with Se-yeast. A few individuals are allergic to yeast for whom this form of supplement would clearly be unsuitable, but this is not a common problem. The major drawback to this source of selenium is likely to be perceived as its variability with respect to its selenium content and speciation. Furthermore, we are still some way away from full identification of all the selenium species that can occur in Se-yeast. However, if it is purchased

from reputable manufacturers operating good quality control and quality assurance schemes, the Se-yeast will be of reproducible nature and Se-Met content.

The fear that selenium from SeMet in Se-yeast proteins could build up in body tissues to toxic levels appears to be unsubstantiated. The ability to be stored in the organism and reversibly released by normal metabolic processes to counteract periods of insufficient intake is an advantage in areas of low selenium intake that exist in various parts of the world. The problem in countries of the EU is not selenium toxicity, but marginal or deficient selenium status that has worsened in some countries in recent years as a result of a fall in imports of North American wheat following their accession to the EU (Rayman, 1997). In Eastern Europe in particular, many populations have mean levels of serum or plasma selenium insufficient to optimise plasma glutathione peroxidase activity (Rayman, 2000). Se-yeast, as the most inexpensive organic selenium supplement, could be particularly appropriate in these situations. Concerns about selenium toxicity are more properly reserved for regions of the world where selenium intakes are naturally high, such as some parts of China, Venezuela and the Great Plains of the US where supplementation with selenium-yeast, SeMet or indeed any form of selenium would be inadvisable.

While Se-yeast appears to be a less-effective anticarcinogen in animal studies than Se-methyl-Se-cysteine (CH_3SeCys), the form produced in selenium enriched garlic and broccoli, and slightly less effective than selenite (Ip 1998; Ip et al 2000), it is none-the-less the only form of selenium to date to have shown efficacy in human anti-cancer intervention studies (Clark *et al.* 1996; Yu *et al.* 1997; Blot *et al.* 1993). It is also a better precursor for selenoprotein synthesis than Se-methyl-Se-cysteine which is largely converted directly to methyl selenol (Ip, 1998).

It should be noted that selenium-enriched foods, of which Se-yeast may be considered an example, contain mixtures of different selenium compounds that are metabolised uniquely and can therefore

affect multiple pathways (e.g. to inhibit carcinogenesis). Furthermore, they have been shown to have equal or higher chemopreventive activity in animal models than purified compounds (Davis & Finley, 2003): though the major selenium compound in high-selenium wheat is SeMet, in rats injected with a chemical carcinogen, a high-selenium wheat diet significantly reduced aberrant colon crypts and aberrant crypt foci while pure SeMet was unable to do so (Finley & Davis, 2001).

Reilly (1996) has pointed out that foods normally contain organic forms of selenium, with the inorganic forms only entering the diet as supplements or contaminants while Schrauzer (2001) has stated that selenium should be supplemented in the form or forms in which it occurs in major staple foods. Se-yeast is the nearest product to food-form selenium, being apparently handled similarly to high-selenium wheat (Levander, 1983), that is readily available for fortification or supplementation.

Conclusion

The evidence presented here has shown Se-yeast to be a safe, bio-available form of selenium that mimics food-form selenium, suitable for use in food supplements, that can act both as a precursor for selenoprotein synthesis and as a human anti-cancer agent. When manufactured by reputable manufacturers, the product is of reproducible quality and defined SeMet content. Even over long periods of supplementation at levels of 300, 400 or even 800 μg/d, Se-yeast has shown no evidence of toxicity.

The ability of selenium from Se-yeast to be stored in the organism and reversibly released by normal metabolic processes to counteract periods of insufficient intake is likely to be particularly valuable in areas of low-selenium intake such as exist in Eastern Europe.

There is therefore no justification for disallowing the sale of Se-yeast in Europe, particularly if the guidelines relating to Upper Safe Limits (e.g. the rather-conservative EC Tolerable Upper Intake Level of 300 μg/d) are adhered to (EC Scientific Committee on Food, 2000).

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Table 1. Current recommended selenium intakes for adults (μg/d) (EC Scientific Committee on Food 2003; Thomson 2004)

Country/Region	RDA/RNI/PRI/NR	
	M	F
Australia, 1990	85	70
Belgium, 2000	70	70
DACH (Germany, Austria, Switzerland), 2000	30-70	30-70
EC Scientific Committee on Food, 2003	55	55
France, 2001	60	50
Italy, 1996	55	55
Japan, 1999	55-60	45
New Zealand/Australia (proposed levels)	65	55
Nordic Countries, 1996	50	40
USA and Canada, 2000	55	55
UK, COMA,1991	75	60
WHO/FAO/IAEA, 1996	40	30

Table 2. Selenium intake data for a number of countries

Country	Selenium intake (μg/person/d)	Reference
Australia	57-87	Fardy et al. 1989
Austria	48	Simma & Pfannhauser 1998 (cited by Combs, 2001)
Belgium	28-61	Robberecht et al. 1994
Czech Republic	10-25 (estimate)	Kvícala et al. 1996
Canada	98-224	Gissel-Nielsen 1998 (cited by Combs, 2001)
China	7-4990	Combs, 2001
Croatia	27	Klapec et al. 1998 (cited by Combs, 2001)
Denmark	38-47	Danish Government Food Agency, 1995
France	29-43	Lamand et al. 1994
Germany	35	Alfthan G & Neve J, 1996
Japan	104-199	Miyazaki et al. 2001
Netherlands	39-54	van Dokkum 1995
	67	Kumpulainen, 1993
New Zealand	55-80	Vannoort et al. 2000
Poland	30-40 (calculated)	Wasowicz et al. 2003
Serbia	30	Djujic et al. 1995
Slovakia	38	Kadrabová et al 1998
Sweden	31	Swedish National Food Administration, 1989
	38	Kumpulainen, 1993
Switzerland	70	Kumpulainen, 1993
UK	29-39	MAFF, 1997
USA	106	Food and Nutrition Board 2000
Venezuela	200-350	Combs & Combs, 1986 (cited by Combs, 2001)

Table 3. Selenium species identified in aqueous extracts of commercial samples of selenium yeast following enzymatic hydrolysis, expressed as a percentage of extracted selenium (N.B. Percentages are dependent on the extraction efficiency of the technique used and the total selenium content of the yeast - see text.)

	Yeasts					
Se-Species	SelenoExcell† 1250 µg Se/g dry yeast	LALMIN† 2119 µg Se/g dry yeast	SelenoPrecise† 1327 µg Se/g dry yeast	Sel-Plex† 1800 µg Se/g dry yeast	Selenomax† 1200 µg Se/g dry yeast	Selenomax† 2100 µg Se/g dry yeast
SeMet	84%	69% (75%)*	81%	83%	61%	60%
selenite	0.1%	<1% (nd/nr)*	nd/nr	0.3%	15%	1%
γ-glutamyl-Se-methyl-Se-cysteine	0.5%	nd/nr (nd/nr)*	nd/nr	nd/nr	0.5%	0.5%
Se-methyl-Se-cysteine	nd/nr	nd/nr (nd/nr)*	nd/nr	nd/nr	0.5%	0.5%
Se-adenosyl-Se-homocysteine	0.5%	nd/nr (nd/nr)*	nd/nr	nd/nr	5%	2%
Se-cystathionine	nd/nr	nd/nr (nd/nr)*	nd/nr	nd/nr	1%	0.5%
Se-lanthionine	nd/nr	nd/nr (nd/nr)*	nd/nr	nd/nr	nd/nr	0.5%
Se-cystine	nd/nr	nd/nr (nd/nr)*	nd/nr	nd/nr	0.5%	0.5%
Se-cysteine	nd/nr	3% (nd/nr)*	nd/nr	5%	nd/nr	nd/nr
SeMet-selenoxide /hydrate	nd/nr	nd/nr (nd/nr)*	0.4%	nd/nr	nd/nr	nd/nr
Methaneseleninic acid	nd/nr	nd/nr (nd/nr)*	nd/nr	nd/nr	nd/nr	nd/nr
Sum of identified species	85.1%	72% (75%)*	81.4%	88.3%	84%	67%
Laboratory	P. Uden	R. Lobinski (E. Larsen)	E. Larsen	R. Lobinski	P. Uden	P. Uden
Reference	Uden *et al.* 2003	H. Durand (pers. comm.)	Larsen *et al.* 2004	R. Power, pers. comm. 2004	Kotrebai *et al.* 2000	Kotrebai *et al.* 2000

nd/nr = not determined/not reported

*results from two different laboratories, the second in parentheses

†SelenoExcell, formerly Selenomax, Cypress Systems, Fresno, California; LALMIN, Lallemand, Montreal, Canada; SelenoPrecise, Pharma Nord, Vejle, Denmark; Sel-Plex, Alltech, Lexington, Kentucky

Nutrition 21, manufacturer of Selenomax, was asked for similar information but did not respond.

Table 4. Summary data from UK and Danish PRECISE Pilot Studies (1000 randomised subjects) on withdrawals up to 24 months from randomisation.

Treatment group	UK Pilot study withdrawals	Danish Pilot study withdrawals	Total number of withdrawals
placebo	7	6	13
100 μg/d	8	0	8
200 μg/d	7	7	14
300 μg/d	10	3	13

Fig. 1. Proposed pathways for the metabolism of selenium in plants (adapted from Whanger 2004). Compounds in bold have been identified in aqueous extracts of Se-yeast.

Fig. 2. The metabolic fate of SeMet and other organic selenium compounds [e.g. selenocysteine (SeCys), Se-methyl-Se-cysteine (CH_3SeCys)] from the human diet (adapted from Combs 2001).

Fig. 3. Effect of selenium supplementation on plasma selenium level of middle-aged Finnish men of low selenium status. Each point represents the mean ± SEM of 8 to 20 subjects per group (from Levander et al. 1983).

Fig. 4. LD_{50} for sodium selenite and Se-yeast in rats.

Fig. 5. Mean plasma selenium in selenium-treated and control groups in the NPC trial (figure adapted from that of Clark et al. 1996) (N.B. Units on the x axis are not evenly spaced).