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Review article

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

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Selenium-enriched yeast (Se-yeast) is a common form of Se used to supplement the dietary intake of this important trace mineral. However, its availability within the European Union is under threat, owing to concerns expressed by the European Community (EC) Scientific Committee on Food that Se-yeast supplements are poorly characterised and could potentially cause the build up of Se in tissues to toxic levels. The present review examines the validity of these concerns. Diagrams of the biosynthesis and metabolism of Se 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 Se species in various commercial products are tabulated. In all cases described, selenomethionine is the largest single species, accounting for 54–74 % of total Se. Se-yeast is capable of increasing the activity of the selenoenzymes and its bioavailability has been found to be higher than that of inorganic Se sources in all but one study. Intervention studies with Se-yeast have shown the benefit of this form in cancer prevention, on the immune response and on HIV infection. Of about one dozen supplementation studies, none has shown evidence of toxicity even up to an intake level of 800 μg Se/d 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.

Selenium-enriched yeast: Speciation: Bioavailability: Toxicity

The trace mineral Se 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 Se metabolites that are thought to reduce the blood supply to tumours and kill cancer cells (Combs & Lü, 2001). Adequate dietary intakes of Se are therefore essential.

Se enters the food chain through plants and its concentration in foods is determined by a number of geological and geographical factors (Diplock, 1993; Fordyce *et al.* 2000; Johnson *et al.* 2000; Adams *et al.* 2002). These are: (i) soil Se species and concentration; (ii) pH, which determines to some extent the nature of the Se species; (iii) amounts of organic matter, iron hydroxides, Al compounds and clay that can bind Se, reducing its bioavailability to plants; (iv) amounts of S species (for example, from S fertilisers) that can compete with Se for absorption; (v) rainfall that can leech Se out of the soil; (vi) soil microbes that can convert insoluble forms of Se to soluble forms. In some parts of the world where Se is insufficiently available to plants, Se-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 Se 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 Se intake may not be adequate for full activity of protective selenoenzymes. Governments have set dietary reference values for Se intake based on their assessment of the amount of Se required to achieve optimal (or two-thirds optimal) activity of the antioxidant selenoenzyme glutathione peroxidase in plasma (Committee on Medical Aspects of Food Policy, 1991; World Health Organization/Food and Agriculture Organization/International

Abbreviations: EU, European Union; HCC, hepatocellular carcinoma; NPC, Nutritional Prevention of Cancer; PRECISE, Prevention of Cancer by Intervention with Selenium; SeMet, L-selenomethionine; SeCys, selenocysteine; Se-yeast, Selenium-enriched yeast.

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Atomic Energy Agency expert group, 1996; Food and Nutrition Board, Institute of Medicine, the National Academies with Health Canada Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2000). Table 1 shows daily intake levels of Se recommended by a number of national or international committees while Table 2 is a compilation of up-to-date Se-intake data for a number of countries. The intake of Se 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). However, in a number of European countries the intake of Se has declined owing to reduced importation of wheat from North America for bread-making that was relatively high in Se (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 Se intake appear to confer additional health benefits on the immune system, on cancer risk and on HIV symptoms and progression (Burbano *et al.* 2002; Rayman, 2002). Cancer protective intakes of Se 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 Se in serum, plasma or whole blood from sixty-nine countries. Using the minimum value of 70 μg Se/l in serum or plasma as a criterion of nutritional Se adequacy as described earlier (Nève, 1995), he estimated nutritional Se deficiency to be highly prevalent (>50 %) in twenty-one countries and moderately prevalent (10–50 %) in a further sixteen countries (Combs, 2001). He also points out that the extent of the problem may be greater, as there

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

	RDA, RNI, PRI or NR	
Country or region	Males	Females
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 and Australia (proposed levels)	65	55
Nordic countries, 1996	50	40
USA and Canada, 2000	55	55
UK (Committee on Medical Aspects of Food Policy, 1991)	75	60
World Health Organization/Food and Agriculture Organization/International Atomic Energy Agency (1996)	40	30

Table 2. Selenium intake data for a number of countries

Country	Se intake (μg/person per 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 & Neve (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	Becker (1989)
	38	Kumpulainen (1993)
Switzerland	70	Kumpulainen (1993)
UK	29–39	Ministry of Agriculture, Fisheries and Food (1997)
USA	106	Food and Nutrition Board (2000)
Venezuela	200–350	Combs & Combs (1986) (cited by Combs, 2001)

RNI, reference nutrient intake; PRI, population reference intake; NR, nutrient requirement.

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 Se in the reduction of viral virulence (Beck *et al.* 1995, 1998, 2001; Broome *et al.* 2004) and cancer risk (Giovannucci, 1998; Yoshizawa *et al.* 1998; Yu *et al.* 1999; Helzlsouer *et al.* 2000; Nomura *et al.* 2000; Brooks *et al.* 2001; van den Brandt *et al.* 2003; Li *et al.* 2004; Wei *et al.* 2004), it is not surprising that many individuals are interested in ensuring they have adequate Se status by supplementing their diets with Se.

Se supplements available in order of increasing cost include: the inorganic forms, i.e. sodium selenite, sodium hydrogen selenite and sodium selenate; organic forms, i.e. Se-enriched yeast (Se-yeast) and the seleno-amino acid, L-selenomethionine (SeMet), the major Se 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 European Union (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 Se but excluded organic forms such as SeMet and Se-yeast. Thus, organic forms of Se 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 before August 2003 for the postponement of this directive. In the latter case, a dossier supporting the safe use of any particular form of organic Se 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 that Se-yeast supplements were poorly characterised with regard to the nature and quantity of Se components and because of the fear that Se from SeMet could build up in body tissues to toxic levels. Given the relatively low cost, good bioavailability and wide popularity of Se-yeast supplements, which appear to be the most-commonly purchased form of single-nutrient Se 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 Se compounds by yeast and their metabolism in the human body.

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

Cereals and forage crops convert Se mainly into SeMet and incorporate it into protein in competition with methionine. In the same manner, *Saccharomyces cerevisiae* (baker's or brewer's yeast) may assimilate up to 3000 μg Se/g, 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). Fig. 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 Se compounds are also formed as described in detail by Whanger (2004).

The metabolic fate of SeMet and other organic Se compounds from the human diet [for example, 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 Hb 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 SeCys-bound tRNA from which SeCys is inserted co-translationally, at loci encoded by specific UGA codons, to give selenoproteins (Berry *et al.* 1991, 1993).

Excess Se 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.*

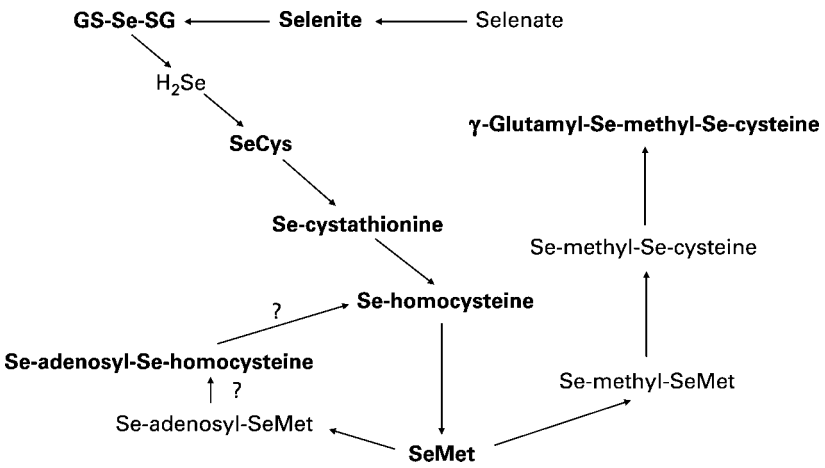

Fig. 1. Proposed pathways for the metabolism of selenium in plants. Compounds in bold have been identified in aqueous extracts of selenium-enriched yeast. GS-Se-SG, selenodiglutathione; SeCys, selenocysteine; SeMet, L-selenomethionine (adapted from Whanger, 2004).

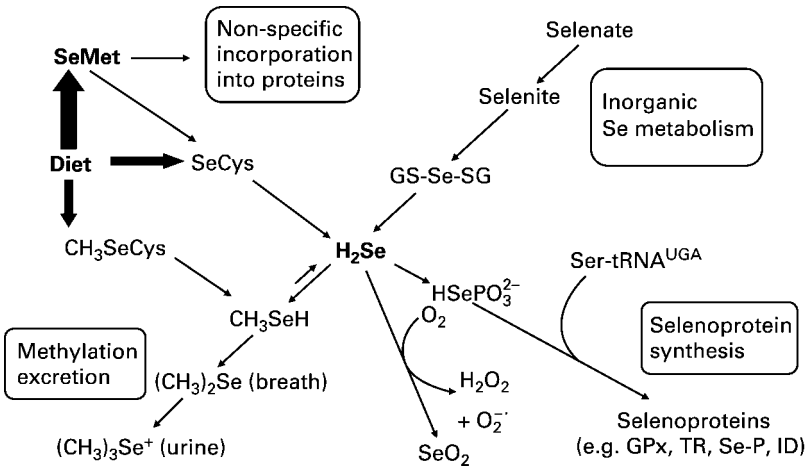

Fig. 2. The metabolic fate of L-selenomethionine (SeMet) and other organic selenium compounds from the human diet. SeCys, selenocysteine; GS-Se-SG, selenodiglutathione; CH_3SeCys, Se-methyl-Se-cysteine; GPx, glutathione peroxidase; TR, thioredoxin reductase; Se-P, selenoprotein P; ID, iodothyronine-5′-deiodinase (adapted from Combs, 2001).

2000, Whanger, 2004), is acted upon by another β-lyase to give CH_3SeH directly (Combs, 2001). There is some evidence for the direct formation of CH_3SeH from SeMet by the action of a γ-lyase, also known as methioninase (Nakamuro *et al.* 1997; Wang *et al.* 2002; Spallholz *et al.* 2004). [CH_3SeH and its precursor CH_3SeCys are believed to be potently anti-carcinogenic and anti-angiogenic (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 earlier. Oxidation of excess H_2Se can lead to the production of superoxide and other reactive oxygen species (Combs, 2001).

The ratio between the 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 selenium-enriched yeast production

Se-yeast is the product of the aerobic fermentation of *S. cerevisiae* in an Se-enriched medium. Different companies use different strains of *S. cerevesiae* and may describe them either as baker's or brewer's 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 Se salts (for example, sodium selenite) as the Se source. Control of pH, temperature, Se feeding profile and aeration allows optimal growth of the yeast strain and maximum biomass production. A Se-enriched yeast cream is produced that is then pasteurised thereby killing the yeast, and dried, frequently by spray drying. One company (Pharma Nord, Vejle, 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 Se-enriched medium, the Se becomes organically bound to the yeast. The amount bound should be greater than 90 %, typically 94 % (percentage of complexed organic Se found in three lots of LAMIN™ Se-yeast by KABS Laboratories, St Hubert, QC, Canada) in the case of one manufacturer (Lallemand, Montreal, Canada). In this case, Lallemand Research and Development reported that about 83 % of the Se 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 Se and the percentage of SeMet; measurement of particle size, moisture content and the level of toxic impurities (As, Cd, Pb, Hg) 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 Se is clearly not bound to the yeast; at worst, there may merely be a mixture of sodium selenite and yeast, the Se not being bound to the yeast (Uden *et al.* 2003). Thus a customer may not be getting what he or she has paid for, i.e. an organically bound Se 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 Se (Thomson *et al.* 1993; Combs, 2001). Sodium selenite, though a good Se 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 a US Food and Drug Administration-approved Se supplement for livestock, where Sel-Plex™ (Alltech, Lexington, KY, USA) is probably the market leader.

Species contained in selenium-enriched yeast: speciation

The wide use of Se-yeast in nutritional and intervention studies, such as the Nutritional Prevention of Cancer (NPC) trial in the USA 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 Se 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, before analysis using chromatographic and mass spectrometric techniques. Water has been used to extract high- and low-molecular-weight species (McSheehy *et al.* 2002; Polatajko *et al.* 2004) but the extraction efficiency is low, about 10–15 % of the total Se in the yeast. Proteolytic hydrolysis with non-specific enzymes, mostly Protease XIV, has given 60–80 % recovery of the total Se 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 Se compounds can then be achieved by a number of methods such as size-exclusion, cation or anion exchange HPLC (Lobinski *et al.* 2000; Larsen *et al.* 2004) or reversed-phase ion-pair HPLC (Lobinski *et al.* 2000; Uden *et al.* 2003). Intact Se-containing proteins from Se-yeast have also been successfully separated by two-dimensional gel electrophoresis (2-D SDS PAGE) (Chassaigne *et al.* 2004). Se-specific detection is generally by inductively coupled plasma MS. Alternatively, for volatile compounds and selenoaminoacid-volatile-derivatives, GC with detection by atomic emission has been used (Uden *et al.* 2003). The use of tandem MS on Se-containing fractions gives the possibility of the 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 Se species including selenopeptides (Encinar *et al.* 2003).

Owing to the differences in manufacturing method referred to earlier, and to the varying extraction techniques and analytical methods used in different laboratories, it is hardly surprising that the analysis of Se-yeast gives a variety of results. Analyses of commercially available Se-yeast preparations have shown organically bound Se to vary from 0 to 97 % of the total Se 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 Se can differ slightly. For example, the same batches of LALMIN™ Se-yeast (Lallemand, Montreal, 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 Se. This probably reflects 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 SeMet:total Se ratio 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. The values are: 58–65 % (see earlier) of total Se in LALMIN™ (Lallemand, Montreal, Canada); 60–70 % of total Se in SelenoExcell™ (Cypress Systems, Fresno, CA, USA); 54–60 % of total Se in SelenoPrecise™ (Pharma Nord, Vejle, Denmark) (Uden at al. 2003; Larsen *et al.* 2004); 62–74 % in Sel-Plex™ [R Power (Alltech, Kentucky, personal communication)], with the caveat that the quoted values are dependent on the extraction efficiency of the technique used by the analytical laboratory.

Good-quality Se-yeast preparations such as those described earlier should not contain more than 1 % of inorganic Se, 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 S 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 selenotrisulfide of glutathione and cysteinylglycine (GS-Se-SCG) (Lindemann & Hintelmann, 2002). Molecules identified in digested extracts as a percentage of eluted Se from enzymic hydrolysates of various commercially available Se-yeasts are given in Table 3. The data on the two commercial yeasts (believed to be Selenomax™, P Uden, personal communication) illustrate the difference in species and percentages that can occur in yeasts of different total Se content. It should be noted that SeMet-selenoxide or its hydrate are products of oxidation of SeMet and are probably analytical artifacts (Larsen *et al.* 2003; Uden *et al.* 2003) not present in the original samples.

While Table 3 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 earlier, 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 Se in a sample of Nutrition 21 (Selenomax™) Se-yeast (El-Bayoumy *et al.* 2002). However the authors of this paper

Table 3. Selenium species identified in aqueous extracts of commercial samples of selenium yeast following enzymic hydrolysis, expressed as a percentage of extracted selenium*

	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)	Commercial sample** (1200 μg Se/g dry yeast)	Commercial sample** (2100 μg Se/g dry yeast)
		Lobinski	Larsen				
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 or its 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 (personal communication)	H. Durand (personal communication)	Larsen *et al.* (2004)	R. Power (personal communication)	Kotrebai *et al.* (2000)	Kotrebai *et al.* (2000)

nd/nr, not determined or not reported.
* Percentages are dependent on the extraction efficiency of the technique used and the total Se content of the yeast; see p. 561.
† SelenoExcell, Cypress Systems, Fresno, CA, USA.
‡ Results from two different laboratories.
§ LALMIN, Lallemand, Montreal, Canada.
‖ SelenoPrecise, Pharma Nord, Vejle, Denmark.
¶ Sel-Plex, Alltech, Lexington, KT, USA.
** Commercial samples, purchased from Cypress Systems, under the trade name of Selenomax™ at that time (P Uden, personal communication, 2004).

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 (B Prokopczyk, personal communication). Furthermore the analysis for Se-methyl-Se-cysteine was performed on samples that were 2 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 in the human body to methyl selenol (CH_3SeH), a potent anti-carcinogen (Ip, 1998). Among the unidentified Se-yeast products, it is possible that there may be other organoselenium species that may also be more-potent anti-carcinogens than SeMet.

Archived samples of Se-yeast (Nutrition 21) tablets used in the NPC trial (Clark *et al.* 1996) have been analysed by two laboratories and have been found to contain a number of unknown Se compounds (Uden *et al.* 2003; Larsen *et al.* 2004). Uden *et al.* (2003) have identified not only SeMet-selenoxide (hydrate), the oxidation product of SeMet referred to earlier, 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 or 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 Se organically bound and about 55 to 75 % of it (allowing for extraction efficiency) in a form that hydrolyses to SeMet. Smaller amounts of other Se compounds, including at least one precursor of the potently anti-carcinogenic 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 selenium-enriched 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 Se 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 Se. 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 the human body, vitamin C appears to improve the bioavailability of Se from a mixed diet (Fairweather-Tait, 1997). Combs (1988) described vitamin A (high levels), vitamin E and antioxidants as enhancers of the bioavailability of dietary Se in animals. Substances that appear to reduce its bioavailability include guar gum, which reduces Se absorption in human subjects (Fairweather-Tait, 1997), heavy metals and high dietary S which are described as inhibitors of bioavailability in animals (Combs, 1988). As many Se compounds are absorbed by the same mechanism as their S analogues, the absorption of a number of Se species from Se-yeast may be reduced by high dietary S intake.

Absorption of dietary Se (organic Se) is generally believed to be good; about 80 % from food (Reilly, 1996). Absorption and retention of Se-yeast have been measured in twelve male subjects fed ^{77}Se-labelled SelenoPrecise™ yeast (Pharma Nord, Vejle, 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.* (2004) study used non-pasteurised brewer's yeast, flask-grown in the laboratory to enrich it with ^{77}Se and freeze-dried, rather than the commercially produced, pasteurised (dead), spray-dried, baker's yeast (SelenoPrecise™) used in the first bioavailability study. Temperature, time of fermentation and nature of Se source (selenious acid) were also different from those normally used in manufactured products [(H Durand (Lallemand), personal communication]. Forced aeration is not possible in flask-grown yeast resulting in low biomass production and low Se incorporation into amino acids [R Power (Alltech), personal communication]. Thus the Se concentration of the Fox *et al.* (2004) Se-yeast was reported as 145 μg/g dry yeast, almost 10-fold lower than the 1400 μg Se/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 [H Durand (Lallemand), personal communication]. It is hardly surprising, therefore, that the measured absorption and retention of the Se-yeast used in the study of Fox *et al.* (2004) 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 Se from Se-yeast is more bioavailable than from inorganic Se (with one exception; Fox *et al.* 2004, see later) and that increased Se 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 Se status for a time. Some examples of supplementation studies will now be given.

The bioavailability of Se from Se-yeast has been shown to resemble that of wheat Se rather than inorganic Se (selenate) in its effect on plasma Se (Levander *et al.* 1983). Three groups of ten men of low Se status were given 200 μg Se/d as Se-rich wheat, Se-rich yeast, or sodium selenate for 11 weeks. Twenty unsupplemented subjects served as controls. At 10 weeks after the supplements were discontinued, plasma Se and platelet glutathione

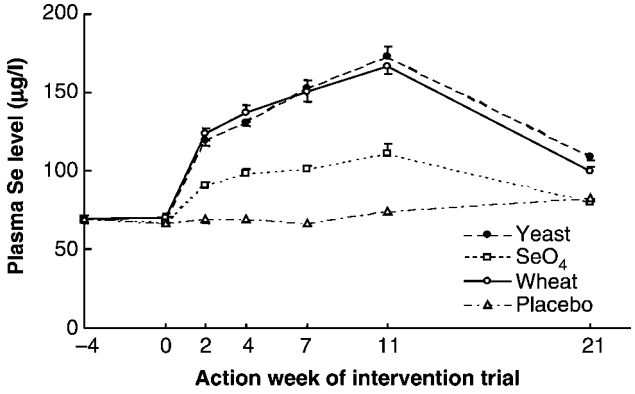

Fig. 3. Effect of selenium supplementation on plasma selenium level of middle-aged Finnish men of low selenium status. Mean values of eight to twenty subjects per group are shown, with standard errors of the mean represented by vertical bars. (--•--), Yeast; (--□--), SeO_4; (–○–), wheat; (-·Δ-·), placebo (from Levander *et al.* 1983).

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 Se both in terms of their capacity to raise plasma Se and in the rate of decline of Se status after the withdrawal of supplementation.

In a study carried out by Alfthan *et al.* (1991), men were supplemented with 200 μg Se/d in the form of selenate, selenite and Se-yeast. Se-yeast and selenite increased plasma Se 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 Se supplementation but the levels did plateau after 11 weeks and did not continue to increase indefinitely. Post-supplementation, the decrease in plasma and erythrocyte Se levels was far less pronounced in the Se-yeast group than in the groups supplemented with inorganic Se, taking longer to return to baseline levels.

Similarly, a 3-month Danish study of Se supplementation in human volunteers suggested that organic Se (SeMet and high Se-yeast) is more bioavailable than selenate and selenite (Clausen & Nielsen, 1988). Both the inorganic and the organic Se supplements gave rise to steady-state levels of glutathione peroxidase after 1 month but whole-blood levels of Se continued to rise for longer in those receiving organic Se. This did not happen, however, in the study of Schrauzer & White (1978), who found that a steady state of Se in whole blood was reached about 2 months after supplementing human subjects with 150 μg Se/d as Se-yeast.

Thirty-three New Zealand women aged 18–23 years received daily for 32 weeks, 200 μg Se as Se-yeast, or brewer's yeast mixed with selenate, or no added Se (placebo) in a double-blind trial (Thomson *et al.* 1993). Se-yeast was more effective in raising blood Se 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 *et al.* (1985) demonstrated that 100 μg Se/d as Se-yeast is more effective than the same dose of selenite in increasing maternal serum Se in breast-feeding mothers. The Se levels plateaued within 6 months of initiation of the yeast supplementation. In contrast to that finding, a Polish study showed no significant difference between the Se intake of breast-fed infants whose mothers were supplemented with Se-yeast or sodium selenite (200 μg/d) for 3 months (Trafikowska *et al.* 1998). Supplemental Se-yeast was, however, shown to be beneficial in preventing the longitudinal decline in milk Se content that occurred with advancing lactation (McGuire *et al.* 1993). When US women were supplemented with 200 μg Se/d as Se-yeast from 4 to 8 weeks postpartum, infant plasma Se concentration at the end of the supplemented period was 83 μg/l in the infants of supplemented mothers compared with 63 μg/l in the control infants (McGuire *et al.* 1993).

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

Not all studies have found yeast Se to be more bioavailable than inorganic Se. The study of Fox *et al.* (2004) supplemented thirty-five male volunteers with isotopically labelled Se 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 Se from these different sources. Absorption of yeast Se was lower than that of fish Se (60 %) and considerably lower than that of selenate (58 %). Retention of yeast Se was significantly lower than that of fish Se (69 %) and somewhat lower than that of selenate (95 %). Thus this recent study showed that yeast Se was less well absorbed and less well retained in the body than inorganic Se. However, as explained earlier, 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 Se status to normal levels in situations where inorganic Se cannot do so. One human and two animal examples are given. Administration of Se as Se-yeast and SeMet significantly increased plasma Se levels up to the normal range in rheumatoid arthritis patients when selenite supplementation was unable to do so (Peretz *et al.* 1992; Heinle *et al.* 1997).

Selenite and selenate have a limited capacity to increase the concentration of Se in milk from dairy cows. This is a problem in Sweden where suckling calves are at risk of Se deficiency. The following two studies carried out in Sweden illustrate that Se-yeast is much more effective than inorganic Se in increasing the concentration of Se in milk. First, in the study of Ortman & Pehrson (1999), when dairy cows were supplemented with Se as sodium selenite, selenate or Se-yeast, Se concentration rose to a plateau in plasma at 4 weeks from 50 μg/l (control) to 75 μg/l (selenite), 80 μg/l (selenate) and 90 μg/l (Se-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 Se concentration in milk (Ortman & Pehrson, 1999). Second, Pehrson *et al.* (1999) supplemented the diet of suckler cows with Se-yeast rather than sodium selenite. This improved the Se 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.

The contrast between the bioavailability of Se-yeast and SeMet is illustrated by a study by McGuire *et al.* (1993). The impact of providing SeMet or Se-yeast on the Se status of lactating and non-lactating women was studied. Plasma Se declined in unsupplemented lactating women but not in non-lactating women. SeMet increased plasma Se in both lactating and non-lactating women whereas Se-yeast increased plasma Se only in non-lactating women. Plasma glutathione peroxidase activity decreased with the duration of lactation in unsupplemented women and SeMet or Se-yeast supplementation prevented the decline. Milk Se declined markedly for 20 weeks after parturition in unsupplemented women. SeMet significantly increased milk Se concentrations whereas Se-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 Se concentrations than inorganic Se. However its apparently lower bioavailability compared with SeMet (McGuire *et al.* 1993) presumably reflects the fact that the yeast has to be degraded and that not all the Se in Se-yeast is in the form of SeMet. Reported whole-body half-lives of SeMet and selenite in human subjects are 252 and 102 d, respectively, implying that SeMet is retained 2·5 times longer in the body than selenite (Schrauzer, 2000). Accordingly, human volunteers or animals supplemented with Se-yeast can maintain higher activities of selenoenzymes during Se depletion for longer periods than those supplemented with inorganic Se owing to the recycling of SeMet following catabolism from protein stores. These factors are an advantage in situations of low to marginal Se status, such as exist notably in some European countries or in groups such as preterm infants. Yeast Se is also more effective than inorganic Se in its ability to transfer Se to breast-fed infants or suckling animals, thereby preventing the risk of deficiency.

Use of selenium-enriched 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 Se other than Se-yeast. It is thus impossible to know if other forms, i.e. inorganic Se, 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.

Nutrition intervention trials in Linxian, China. National Cancer Institute-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 9 years later, results from the same study showed significant inverse associations between baseline serum Se and death from oesophageal squamous cell carcinoma (relative risk 0·83; 95 % CI 0·71, 0·98) and gastric cardia cancer (relative risk 0·75; 95 % CI 0·59, 0·95) (Wei *et al.* 2004). The authors have suggested that population-wide Se supplementation in the region of China with low serum Se and high incidences of these cancers should be seriously considered.

Nutritional Prevention of Cancer trial. The NPC trial was designed to test the hypothesis that Se 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/d (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 re-analysis 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 Se concentrations (< 121·6 μg/l).

Supplementation of hepatitis B antigen carriers. In the Qidong county, about 40 miles north of Shanghai, the incidence of hepatocellular carcinoma (HCC) is particularly high. In this region about 15 % adults carry the hepatitis B surface antigen and these individuals are 200 times more likely to develop HCC. In a study where 226 hepatitis B antigen carriers were randomised to either 200 μg Se as Se-yeast or placebo, no case of HCC occurred in the supplemented group after 4 years, while seven subjects in the placebo group had developed HCC (Yu *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.

Influence of selenium-enriched yeast supplementation on biomarkers of oxidative damage. A double-blind randomised, placebo-controlled trial of Se-yeast was carried out in thirty-six healthy adult males, 19–43 years of age, for a period of 9 months. Beneficial effects were seen in the Se-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).

Breast cancer. 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 Se as Se-yeast for 3 months gave a significant reduction in pain ($P<0{\cdot}01$) and joint involvement ($P<0{\cdot}05$) (Peretz *et al.* 1992).

Patients with rheumatoid arthritis were supplemented with 600 μg Se/d as 'selenomethionine-containing yeast' (presumably Se-yeast) enriched with vitamin E for 8 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

Se appears to be able to reverse the age-related decline in immune response in the elderly. In a group of twenty-two institutionalised elderly subjects supplemented with 100 μg Se/d as Se-yeast or placebo for 6 months, response to mitogen challenge was restored to the level of that in healthy young individuals in those receiving the Se (Peretz *et al.* 1991).

HIV progression

HIV-positive men and women (*n* 186) with plasma Se concentration in the 'adequate' range (>85 μg/l) were supplemented with 200 μg Se/d as 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 2 years before and during the trial. A marked decrease in total admission rates (relative risk 0·38; $P=0{\cdot}002$) and percentage of hospitalisations due to infection/100 patients, for those receiving Se, was observed ($P=0{\cdot}01$). The cost of hospitalisation decreased by 58 % in the Se group compared with 30 % in the placebo group ($P=0{\cdot}001$). In the final analysis, Se therapy continued to be a significant independent factor associated with lower risk of hospitalisation (relative risk of hospitalisation, placebo *v.* Se 2·44; 95 % CI 1·31, 4·53; $P=0{\cdot}01$). Furthermore, the number of subjects with CD4 cell counts >50 declined by 46 % in the placebo group but only by 25 % in the Se-treated group ($P=0{\cdot}01$). It was concluded that Se supplementation was a beneficial adjuvant treatment in HIV-1-infected patients (Burbano *et al.* 2002).

Further studies using selenium-enriched yeast

Se-yeast (Cypress Systems, Fresno, CA, USA) 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.* 2003*a*), the Preprostatectomy trial (Marshall, 2001) and the 'Watchful Waiting' trial (Stratton *et al.* 2003*b*). In the UK, the SELINA (Selenium in Asthma) trial has been underway since 2002 using a dose of 100 μg/d (SelenoPrecise™; Pharma Nord, Vejle, Denmark) or placebo (co-principal investigators SO Shaheen and MP Rayman).

Further studies using Se-yeast are planned though funding has not yet been secured; the SPRINT (Selenium in Pregnancy Intervention) trial investigating the effect of 60 μg/d *v.* placebo on the risk of pre-eclampsia in the UK (principal investigator MP Rayman; Rayman *et al.* 2003), and the international Prevention of Cancer by Intervention with Selenium (PRECISE) 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 Se are more acutely toxic than organic forms such as Se-yeast. There are data on toxicity of Se-yeast from two animal studies while a number of human intervention studies show that the chronic administration of Se-yeast up to 800 μg/d provides no evidence of toxicity.

Acute toxicity: animal studies

Acute toxicity studies with Se-yeast have been carried out on rats (Vinson & Bose, 1987). The LD_{50} for Se-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 (Fig. 4). A further study comparing the toxicity of a selenite and an 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 Se/g diet over an 8-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 Se, 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 Se may be more toxic during long-term consumption as they can be

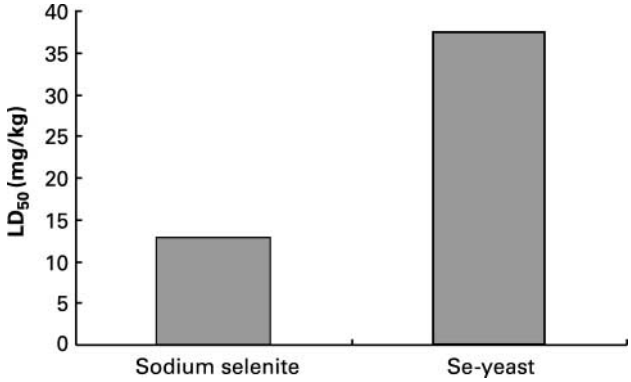

Fig. 4. LD_{50} for sodium selenite and selenium-enriched yeast (Se-yeast) in rats.

incorporated into tissue proteins rather than be 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 Professor Kim Overvad of the University of Aarhus. These studies will now be described, followed by a description of a medium-term human study with high-dose Se-yeast and a short-term study in lactating women.

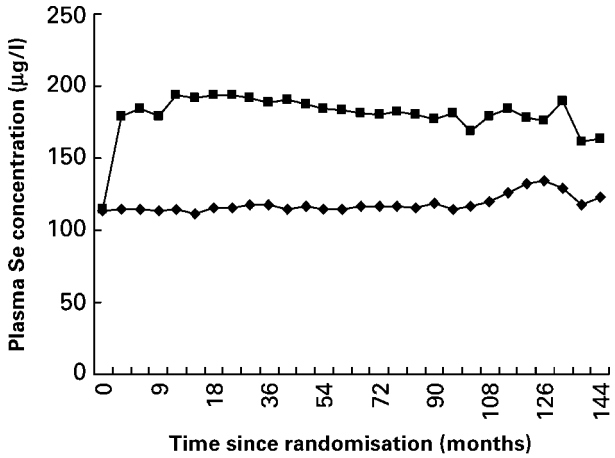

Fig. 5. Mean plasma selenium in selenium-treated (–■–) and control (–◆–) groups in the Nutritional Prevention of Cancer trial (adapted from Clark *et al.* 1996) (units on the *x* axis are not evenly spaced).

University of Arizona, Arizona Cancer Center studies

In the NPC trial, a total of 1312 subjects whose baseline intake was about 90 μg Se/d were randomly allocated to receive supplements of 200 μg Se as Se-yeast (Nutrition 21; brewer's 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 Se group. A total of thirty-five subjects complained of adverse effects, most of which involved gastrointestinal upset, resulting in their withdrawal from treatment. Of these, twenty-one were in the Se-group and fourteen in the placebo group. Within each group, those reporting adverse effects did not have significantly different plasma Se concentrations from those not reporting such effects.

Longer-term follow-up data on Se status from this trial than previously published (Clark *et al.* 1996) are shown in Fig. 5 (M Reid and J Marshall, personal communications). It can be seen that the administration of 200 μg Se/d as Se-yeast gives a rapid increase in plasma Se up to about 3 months, after which the increase levels off reaching a clear plateau at about 190 μg/l by 1 year. This plasma Se 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 Se/d as high-Se yeast from Cypress Systems (Fresno, CA, USA) (Marshall, 2001). These are: (i) the Negative Biopsy trial, where 700 men with persistently-elevated prostate-specific antigen are randomly allocated to placebo or 200 or 400 μg Se/d with a follow-up period of 57 months (Stratton *et al.* 2003*a*); (ii) the Preprostatectomy trial, where 110 men with localised prostate cancer are supplemented with 200 or 400 μg Se/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 or 800 μg Se/d as Se-enriched yeast (Stratton *et al.* 2003*b*). There have been no safety concerns in this trial even after supplementation with 800 μg Se/d as Se-yeast for 3 years or more (Stratton *et al.* 2003*b*) and according to Marshall (2001), no evidence of any toxicity in any of these trials. This is consistent with the no observed adverse effect level (NOAEL) of 819 (SD 129) μg/d from dietary Se determined by Yang & 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 Se levels achieved were 492 (SD 188) and 640 (SD 491) μg/l, respectively. While the 3200 μg/d group reported more Se-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 Se-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 with 200 μg Se as Se-yeast or placebo yeast daily for 4 years. No side effects were reported (Yu *et al.* 1997).

Studies from the PRECISE Study Group at the University of Surrey

A pilot study for the UK PRECISE 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 years, were randomised from June 2000. The study had four treatment groups; placebo, 100, 200, and 300 μg Se/d as Se-yeast (SelenoPrecise™; Pharma Nord, Vejle, Denmark). Randomisation was stratified to give groups that were equivalent in terms of numbers, age and sex.

Up to the end of January 2003, sixteen subjects (3·2 %) had withdrawn because of abdominal or stomach problems and sixteen (3·2 %) had withdrawn because of other adverse effects. Of the thirty-two subjects who reported adverse effects, seven (22 %) were on placebo, eight (25 %) were taking 100 μg Se, seven (22 %) were taking 200 μg Se and ten (31 %) were taking 300 μg Se. None of the subjects in the 300 μg group developed hair or nail problems, known signs of Se toxicity. χ^2 Tests on withdrawals due to adverse events show that there was no significant difference in: the number of abdominal or 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 Se status of those suffering adverse effects significantly different from the mean of their treatment group ($P>0·05$). Although mean plasma Se 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 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 years, were randomised to one of four treatment groups; placebo, 100, 200, and 300 μg Se/d as Se-yeast (SelenoPrecise™; Pharma Nord, Vejle, Denmark).

Up to the end of August 2003, twenty-three subjects (4·6 %) had withdrawn because of adverse effects. Of the twenty-three subjects who reported adverse effects, eight (35 %) were on placebo, three (13 %) were taking 100 μg Se, eight (35 %) were taking 200 μg Se and four (17 %) were taking 300 μg Se (S Cold, personal communication). Neither the distribution nor the severity of side-effects correlated with the intake of Se. The χ^2 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 whole-blood Se concentration in thirteen subjects from the 300 μg/d treatment group 2 years after randomisation was 441 (SD 132) μg/l (Larsen *et al.* 2004), similar to the level reported by Yang *et al.* (1983) to be safe, i.e. without evidence of selenosis. Thus, from this study there is no evidence that Se 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 pilot studies have been combined into a single table (Table 4). χ^2 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 Se/d.

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 Se/d	8	0	8
200 μg Se/d	7	7	14
300 μg Se/d	10	3	13

Medium-term human study with high-dose selenium-enriched yeast

Patients with rheumatoid arthritis were supplemented with 600 μg Se/d as 'selenomethionine-containing yeast' (presumably Se-yeast) enriched with vitamin E for 8 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 Se plateaued at 500 μg/l at 4 months.

Short-term study with selenium-enriched yeast in lactating women

A Polish study supplemented lactating mothers for 3 months with 200 μg Se/d 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 1 month. There was no significant difference between the Se intake of these infants and of those whose mothers were supplemented with 200 μg Se/d as sodium selenite. The infant Se intake achieved was 4- to 5-fold lower than the upper limit of 45–60 μg/d recommended for infants of 0–12 months (Food and Nutrition Board, Institute of Medicine, the National Academies with Health Canada Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2000). At the end of 3 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 μg/l that are associated with safe intakes of Se (Yang *et al.* 1983; Yang & Zhou, 1994) leads to the conclusion that there is considerable leeway between the supplementation level of 200 μg/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 Se 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 Se toxicity at younger ages (Food and Nutrition Board, Institute of Medicine, the National Academies with Health Canada Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2000). This is also consistent with the observation of Kim & Mahan (2001) that nursing pigs are capable of buffering against an excess of milk Se (organic) by storing more Se in body tissues.

Summary of toxicity data

The EC Scientific Committee on Food expressed the concern in 2002 that organic Se, for example, SeMet or Se-yeast, could build up in body tissues to toxic levels. The evidence presented here is reassuring in that respect, showing that Se reaches a plateau in plasma after 11 weeks to 6 months, depending on the subjects and the study (Kumpulainen *et al.* 1985; Alfthan *et al.* 1991; Aaseth *et al.* 1998), and in whole blood after about 2 months, following Se-yeast supplementation (Schrauzer & White, 1978). In line with the results of the latter study, Se levels plateaued in erythrocytes after 6 weeks of SeMet supplementation (Luo *et al.* 1987). There are no published studies on tissue levels of Se following long-term administration of Se-yeast though there are some in progress that will determine tissue levels of Se in muscle biopsies from human volunteers that have been taking organic Se for varying lengths of time up to 20 or 30 years (D Behne, personal communication). However, there is at present no evidence that Se-yeast supplementation causes a continuing rise in tissue Se. 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 Se and supports the assertion that this form of Se-supplementation is no more hazardous than supplementation with inorganic Se. This conclusion is supported by the observation that thousands of individuals living in South Dakota are, and have been, exposed to high intakes of organic Se for over a century without adverse effects; no evidence of Se 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 Se (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 Se into selenoproteins and prevent toxicity mediated through reactive oxygen species (see Fig. 2) from excessive intakes (Brown *et al.* 2000).

Bearing in mind the fact that Se recommendations for infants are currently not achieved in 30 % of breast-milk samples measured worldwide (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.

Selenium-enriched yeast in the fortification of foods

Should some of the studies currently underway indicate a significant benefit of a higher Se intake, Se-yeast may be an excellent fortificant for foods. It provides a biological barrier between a cheap Se source (inorganic Se) 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 [N Renard (Lallemand) and S Moesgaard (Pharma Nord), personal communications].

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 Se content of bread. Se-yeast (LALMIN™; Lallemand, Montreal, Canada) is stable to baking showing an excellent correlation (R^2 0·999) between the expected amount of total Se per slice and the quantity expected [N Renard (Lallemand), personal communication]. 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 Se in raising the concentration in milk and cheese (Malbe *et al.* 1995; Ortman & Pehrson, 1999).

Benefits and drawbacks of selenium-enriched 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 Se is likely to be perceived as its variability with respect to its Se content and speciation. Furthermore, we are still some way away from full identification of all the Se 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 Se 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 Se intake that exist in various parts of the world. The problem in countries of the EU is not Se toxicity, but marginal or deficient Se 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 Se insufficient to optimise plasma glutathione peroxidase activity (Rayman, 2000). Se-yeast, as the most inexpensive organic Se supplement, could be particularly appropriate in

these situations. Concerns about Se toxicity are more properly reserved for regions of the world where Se intakes are naturally high, such as some parts of China, Venezuela and the Great Plains of the USA where supplementation with Se-yeast, SeMet or indeed any form of Se would be inadvisable.

Se-yeast appears to be a less-effective anti-carcinogen in animal studies than Se-methyl-Se-cysteine, the form produced in Se-enriched garlic and broccoli, and slightly less effective than selenite (Ip, 1998; Ip *et al.* 2000). However, it is none-the-less the only form of Se 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 Se-enriched foods, of which Se-yeast may be considered an example, contain mixtures of different Se compounds that are metabolised uniquely and can therefore affect multiple pathways (for example, 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 Se compound in high-Se wheat is SeMet, in rats injected with a chemical carcinogen, a high-Se-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 Se, with the inorganic forms only entering the diet as supplements or contaminants while Schrauzer (2001) has stated that Se 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 Se (being apparently handled similarly to high-Se wheat; Levander, 1983) that is readily available for fortification or supplementation.

Conclusion

The evidence presented here has shown Se-yeast to be a safe, bioavailable form of Se that mimics food-form Se, 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 Se 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 Se 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 (for example, the rather-conservative European Community tolerable upper intake level of 300 μg/d) are adhered to (EC Scientific Committee on Food, 2000).

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