

Selenium-enriched yeast as source for selenium added for nutritional purposes in foods for particular nutritional uses and foods (including food supplements) for the general population ¹

Scientific Opinion of the Panel on Food Additives,

Flavourings, Processing Aids and Materials in Contact with Food

(Question No EFSA-Q-2005-078, EFSA-Q-2005-119, EFSA-Q-2005-186, EFSA-Q-2006-215, EFSA-Q-2006-216, EFSA-Q-2006-217)

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SUMMARY

Following a request from the Commission, the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Foods (AFC Panel) was asked to deliver a scientific opinion on the safety and bioavailability of selenium-enriched yeast as a source for selenium when added for nutritional purposes in foods for particular nutritional uses and foods (including food supplements) for the general population.

The Scientific Committee for Food (SCF) has previously given an opinion on the Tolerable Upper Intake Level of selenium and has also provided an opinion on selenium in relation to the essential requirements for infant formulae and follow-on formulae.

The present opinion deals only with the safety and bioavailability of one particular source of selenium, selenium-enriched yeast, to be used in foods for particular nutritional uses and in foods (including food supplements) for the general population. The safety of selenium itself, in terms of amounts that may be consumed, is outside the remit of this Panel.

¹ For citation purposes: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission on Selenium-enriched yeast as source for selenium. *The EFSA Journal* (2008) 766, 1-43.

Selenium in selenium-enriched yeast produced using sodium selenite as a source is typically in the form of the seleno-amino acid selenomethionine, accounting for approximately 60-85% of total selenium species in the selenium-enriched yeast product. Selenocysteine is the second most abundant identified species, approximating to 2-4% of total selenium species. Inorganic selenium (IV) ion is normally found at less than 1% of total, confirming that virtually all of the selenium present in the product is organically bound. The remaining proportion is the sum of minor species.

The conclusions of the present opinion relate only to selenium-enriched yeasts in compliance with the following product characteristics:

Selenium-enriched yeasts produced by culture in the presence of sodium selenite as selenium source and containing, in the dried form as marketed, not more than 2.5 mg selenium/g. The predominant organic selenium species present in the yeast is selenomethionine which constitutes between 60 and 85% of the total selenium in the product. The content of other organic selenium compounds including selenocysteine does not exceed 10%.

While levels of inorganic selenium in selenium-enriched yeast normally do not exceed 1%, since inorganic selenium in the form of sodium selenite, sodium selenate and sodium hydrogen selenite has been reviewed by the SCF and has been also permitted in PARNUTS and fortified foods, inorganic selenium from a selenium-enriched yeast source is not a safety issue.

Like other forms of selenium salts and organoselenium compounds, selenomethionine is readily absorbed from the gastrointestinal tract. In a number of studies in humans and animals, in particular those on selenium-deficient diets, the bioavailability of selenium from selenium-enriched yeasts and the bioavailability of selenomethionine has been shown to be approximately 1.5 to 2-fold higher than that of inorganic forms of selenium.

Following absorption, selenomethionine is metabolised to other functional forms of selenium (e.g. selenocysteine) or diverted into pathways of methionine metabolism and stored as selenoproteins. The half-life of L-selenomethionine (252 days) is longer than that of inorganic selenite (102 days), indicating that once absorbed, selenomethionine is incorporated into a long term body pool. The steady-state is reached after 6-12 months of supplementation with selenium in the form of selenomethionine or selenium-enriched yeast. The selenium is incorporated into tissue proteins such as skeletal muscle, liver, erythrocytes and plasma albumin from which it can subsequently be released by catabolism to maintain increased selenium status, indicating that selenomethionine is extensively utilized and re-utilized. Recent data in human volunteers show that in response to an increase in dietary selenium intake from sources such as selenium-enriched yeast, plasma selenium concentration increases to a new steady-state level that is maintained for many years if the level of selenium intake is unchanged.

Selenium is known to be chronically toxic and selenosis has been reported in humans and in food animals in seleniferous areas. Intakes in the range of 3200-6990 µg/day (mean 4990 µg/day) are associated with chronic selenosis, with no selenosis observed in the intake range of 240-1510 µg /day (mean 750 µg/day). Investigations into the health effects of high dietary intakes of selenium in populations living in the seleniferous areas of South Dakota, Venezuela and China have indicated that the highest long-term daily intake that can be ingested without the development of toxicity in most individuals is approximately 800 µg while prolonged intakes of daily selenium doses of 1000 µg or greater may cause adverse reactions.

Data in lactating women supplemented with selenium or exposed to high dietary selenium levels suggest that the mother acts as a buffer, protecting the infant against excess intake of selenium. Even at a maternal selenium intake of 300 µg/day (the recommended EC Tolerable Upper Intake Level) milk selenium concentration only rises to 60 µg/l giving an infant intake

of 39-47 µg/day which is below the UL of 60 µg/day for children aged 1-3 years recommended by the SCF and the UL of 45-60 µg/day for infants aged 0 – 12 months recommended by the Food and Nutrition Board..

Despite the higher bioavailability of selenium from organic sources such as selenium-enriched yeast, the toxicity of these organic forms has been shown in a number of studies in experimental animals to be lower than that of inorganic selenite or selenate. This suggests that the increased bioavailability may be counterbalanced by lower toxicity. A number of recent clinical studies carried out with selenium-enriched yeast show no evidence of toxicity following selenium intakes up to 343 µg/day, and whole-blood levels of 441 selenium µg/l, for a period of over 4 years.

The Panel thus concludes that the reservations expressed by the SCF in 1999, when the Committee initially considered selenium-enriched yeasts for use in foods for particular nutritional purpose (PARNUTS), namely that there was uncertainty about the range of selenium-enriched yeast preparations which could be used and the range of selenium compounds they may contain, and differences in the way in which the body handles organic compared with inorganic forms, have been addressed via the additional data considered in this opinion. These data include (i) the general product characteristics for the majority of the selenium-enriched yeast products covered by this opinion, (ii) the additional data that have become available on the identity and speciation of the organo-selenium compounds contained in the yeast, (iii) the available information indicating that on repeated supplementation/exposure to a selenium-rich source such as yeast, levels of selenium reach a steady-state within 2-4 months, rather than increasing indefinitely in plasma and in tissues such as muscle, (iv) the results of recent clinical trials with selenium enriched yeast.

The quantity of selenium-enriched yeast to be added to food supplements is in the range of 30-200 mg of enriched yeast, providing 50-200 µg selenium per day, although the majority of products identified by the petitioners would provide 100 µg selenium or less per day. Limited information was provided on use levels in foods for particular nutritional purposes, although one petitioner indicated that levels to be added to food would provide an intake of less than 50 µg selenium/day.

Average dietary intake of selenium by the European population has been estimated to lie in the range of 27-70 µg/day, with higher intakes seen in Finland, where selenium is added to agricultural fertilizers. Assuming a mean dietary selenium intake in the range of 30-70 µg/day, consumption of an additional food supplement containing 100 µg Se would result in a total daily Se intake of 130-170 µg/day in an adult at an average level of dietary exposure. Intake from the proposed use of supplements containing selenium-enriched yeast would thus be approximately 2-3 times the average dietary intake from food. In the case of a food supplement containing 200 µg Se, the total daily Se intake would be between 230-270 µg/day in an adult at an average level of dietary exposure, with higher intakes being anticipated in individuals from selenium-rich areas where consumption of selenium-rich cereals may contribute to a higher intake. For these individuals, the Tolerable Upper Intake Level for selenium established by the SCF in 2000 could be exceeded.

Assuming a use level of selenium-enriched yeast in foods for particular nutritional uses and for fortification purposes that would provide an intake of less than 50 µg selenium/day, the total daily selenium intake of an adult exposed at the average level of dietary exposure from these sources plus dietary sources would lie in the range of 80-120 µg/day.

Having considered the general product characteristics for the selenium-enriched yeast products covered by this opinion, and the additional data that have become available on the identity and speciation of the organo-selenium compounds contained in the yeast, the Panel concludes that

five of the selenium-enriched yeasts included in this opinion are sufficiently well characterised in terms of the selenium content and the nature of the selenium compounds contained therein, enabling the broad product characteristics for the product outlined above to be defined.

For the two remaining products: biotransformed yeast produced using selenium dioxide as a source and selenium-enriched yeast produced using selenium-aminoate, the Panel considered that insufficient information was provided on the selenium species likely to be present in these products. The Panel also noted that in the case of the products from these two petitioners, the selenium source used in the manufacturing process was not sodium selenite. The Panel considered therefore that it was not possible to conclude that the profile of the selenium species in these two selenium-enriched yeast products is likely to be similar to those reported for the other five products, with selenomethionine accounting for approximately 60 - 85% of the total selenium. Due to deficiencies in the bioavailability and safety data provided on the selenium species likely to be present in these products, the Panel was unable to evaluate their safety.

The Panel notes that the quantity of yeast ingested as a result of the use of supplements containing selenium-enriched yeast will be small (up to 200 mg daily) and that the cellular constituents of the yeast are anticipated to be endogenous in the human body. The Panel also concludes that the quantity of yeast ingested as a result of the use of supplements containing selenium-enriched yeast is unlikely to present an allergenic risk, and that individuals with yeast sensitivity will be alerted to the presence of yeast protein via the labelling of these products.

On the basis of the data provided by the petitioners and information in the literature on the bioavailability, metabolism and toxicity of selenium-enriched yeast and selenomethionine, from dietary sources and in the form of dietary supplements, the Panel concludes that the use of selenium-enriched yeast, complying with the general product characteristics outlined above, as a source of selenium when used in foods for particular nutritional uses and in foods (including food supplements) for the general population does not present a safety concern at the proposed intake levels.

Key words:

Selenium, selenium-enriched yeast, selenomethionine, biotransformed yeast, PARNUTS, food supplements

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BACKGROUND AS PROVIDED BY THE COMMISSION

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients.

The Commission has received a request for the evaluation of selenium-enriched yeast added for nutritional purposes to foodstuffs. The relevant Community legislative measures are:

- Commission Directive 2001/15/EC of 15 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses².
- Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements³.
- Regulation No 1925/2006/EC of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods⁴.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion, based on its consideration of the safety and bioavailability of selenium-enriched yeast added for nutritional purposes in foods for particular nutritional uses and foods (including food supplements) intended for the general population.

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² OJ No L 52, 22.2.2001, p.19.

³ OJ L 183, 12.7.2002, p.51.

⁴ OJ L 404, 30.12.2006, p.26.

ASSESSMENT

1. Introduction

The present opinion deals only with the safety and bioavailability of a particular source of selenium, selenium-enriched yeast, intended to be used in foods for particular nutritional uses and foods (including food supplements) for the general population. The safety of selenium itself, in terms of amounts that may be consumed, is outside the remit of this Panel.

The Scientific Committee for Food (SCF) has previously given an opinion on the Tolerable Upper Intake Level (UL) of selenium (SCF, 2000) and has also provided an opinion on selenium in relation to the essential requirements for infant formulae and follow-on formulae (SCF, 1993). In addition, in 1994, the SCF considered the acceptability of selenomethionine and of selenium-enriched yeasts as sources of selenium to be used in the manufacture of infant formulae (SCF, 1994), and in 1999 issued a further opinion on the use of organic selenium compounds for nutritional purposes which have been proposed for use in the manufacture of foods for particular nutritional purposes (PARNUTS) (SCF, 1999).

This opinion is based on information contained in six dossiers on selenium-enriched yeasts provided to EFSA covering seven selenium-enriched yeasts.

2. Technical data

2.1. Chemistry

Selenium-enriched yeast is mainly defined in terms of its selenomethionine content (see Section 2.3.1).

2.2. Identification of the yeast strain

The yeast strain used by the manufacturers to produce selenium-enriched yeast in all cases was *Saccharomyces cerevisiae*. The majority of petitioners provided details of the yeast strain and the source and maintenance of the cultures to ensure stability and high reproducibility of the product. One petitioner provided a description of identification of the source by Polymerase Chain Reaction (PCR) and comparison of DNA profiles to previous band patterns to ensure that the strain has not changed. All petitioners report that selenium-enriched yeast is not produced from a genetically modified organism (GMO), a genetically modified microorganism (GMM) nor is it produced by using ingredients or substrates derived from a GMO.

2.3. Product characteristics of the yeast product

According to the information provided by the majority of the petitioners, the final dried selenium-enriched yeast powdered product contains an average selenium (Se) content of approximately 2 mg Se/g. The contents reported by the different petitioners lay in the range 1.0-2.4 mg Se/g. Detailed product characteristics were provided for the majority of the individual products, including water content (5-7%), protein (normally in the range 40-50%, but in one case 28%), carbohydrate (11-48%), fat (2-8%) and residual ash (5-10%). Several petitioners provided quantitative data for amino acid, vitamin and mineral content and profile.

Information on heavy metal content was also provided, in the range of < 5 mg/kg for lead, mercury, cadmium and arsenic, with most of the products having levels considerably below 5 mg/kg. The Panel noted the importance of compliance with these product characteristics for heavy metals, given the capacity of the yeast to bind metals present in the growth medium.

Microbiological product characteristics were also provided, typically showing the following profile:

	Product characteristics
Total Plate Count	< 1000 - < 3 000 CFU/g
Yeast and moulds	< 100 - <300 CFU/g
Coliforms	< 10 /g
<i>Salmonella</i> spp.	Negative in 125g
<i>P. aeruginosa</i>	< 10 in 10g
<i>E. coli</i>	Negative in 10g
<i>S. aureus</i> (coagulase +)	Negative in 10g

Certificates of analysis for specific batches of product, showing compliance with these product characteristics, were provided by the majority of petitioners.

2.3.1. Chemical speciation

As indicated above, the dried selenium-enriched yeast powdered product contains an average selenium (Se) content of approximately 2 mg Se/g (reported range 1 – 2.4 mg Se/g). For two products petitioners provided detailed information regarding the speciation of the selenium species in selenium-enriched yeast. Characterisation of the selenium species is carried out following enzymatic (proteolytic and/or pectinolytic) digestion and extraction followed by high performance liquid chromatography (HPLC) separation and detection by inductively coupled plasma mass spectrometry (ICP-MS). Methodological differences between laboratories may however prevent direct comparison of reported results.

One petitioner reported that selenomethionine is the major species identified in the proteolytic extract, accounting for approximately 60-84% of total selenium species in the selenium-enriched yeast product (Rayman, 2004). Selenocysteine is the second most abundant identified species, approximating to 2-4% of total selenium species. Inorganic selenium (IV) ion is normally found at less than 1% of total, confirming that virtually all of the selenium present in the product is organically bound. The remaining proportion is the sum of minor species. Data provided by the petitioner for another product were comparable to these, with 54 – 84% of total selenium being identified as selenomethionine (Lobinski, 2003; Larsen *et al.*, 2004). Table 1 provides a comparison of the composition/selenium speciation of a range of selenium-enriched yeast products on the market, as reported in the literature. The Panel noted that four of the products covered by this opinion correspond to products 1, 2, 3, 4 in Table 1.

Table 1. **Composition of commercial selenium-enriched yeast products as reported in the literature (after Rayman, 2004). The results given are for total extracted selenium species identified in aqueous extracts of yeast following enzymic hydrolysis, expressed as a percentage of total extracted selenium***

Product (Selenium conc.)	Seleno- methionine (% of total extracted Se species)	Other organic Se compounds (% of total extracted Se species)	Inorganic selenium (% of total extracted Se)	Source of Selenium	Reference
Product 1 (1327 mg Se/kg dry yeast)	81	5.1	0.3	Sodium selenite	Larsen <i>et al.</i> (2004)
Product 2 (1250 mg Se/kg dry yeast)	84	1.0	0.5	Sodium selenite	Uden <i>et al.</i>, (2004)
Product 3a** (2119 mg Se/kg dry yeast)	69	3.0	< 1	Sodium selenite	Lobinski (2004)
Product 3b** (2119 mg Se/kg dry yeast)	75	nd	nd	Sodium selenite	Larsen <i>et al.</i>, (2004)
Product 4 (1800 mg Se/kg dry yeast)	83	5.0	0.3	Sodium selenite	Lobinski (2003)
Product 5*** (1200 mg Se/kg dry yeast)	61	7.5	15	Sodium selenite	Kotrebai <i>et al.</i>, (2000)
Product 6 (2100 mg Se/kg dry yeast)	60	4.5	1	Sodium selenite	Kotrebai <i>et al.</i>, (2000)

* Percentages are dependent on the extraction efficiency of the technique used and the total Se content of the yeast (Rayman, 2004);

** Results for the same product from two different laboratories (3a and 3b)

*** The Panel noted that this sample, dating from the late 1990's, had a much higher inorganic selenium content than current selenium yeasts.

Further analytical characterisation carried out by one petitioner indicated that part of the selenomethionine is present in peptide fragments of the original yeast protein, due to incomplete proteolytic digestion, while 1 - 5% may be present in the solid residue after enzymatic hydrolysis, and not extracted. Unidentified selenium-containing compounds detected by HPLC are thought to be intermediates in the biological pathway of synthesis of selenomethionine such as Se-methyl homoselenocysteine, selenium-containing peptides due to incomplete hydrolysis of the original proteins and/or oxidation products of selenomethionine or products resulting from its reaction with matrix constituents.

While similar details on speciation were not provided for all products, the Panel concluded that, given the similarity in the manufacturing process for five of the selenium-enriched yeasts included in this opinion (see section 2.4) and the fact that the yeast species used was the same in all cases, the profile of the selenium species in these five selenium-enriched yeast products is likely to be similar and that selenomethionine accounts for approximately 60 - 85% (rounded from the maximum of 84% cited in Table 1) of the total selenium. Detailed speciation information was provided for three of the products (products 1, 2, 3 in Table 1), a fourth is also included in table 1 (product 4), while for a fifth product (not included in Table 1) the data provided by the petitioner was limited to the information that the product typically contains 1200 mg Se/kg and 1900 mg/kg selenomethionine (64%).

Of the two remaining products, one petitioner provided only the information that the yeast contained not less than 2 mg Se/g and not more than 2.4 mg Se/g. No information was provided

on speciation. For the other product of these two products, the petitioner provided the information that an aqueous extract of the yeast contained predominantly selenite and selenate, but that these species accounted for only 10% of the total selenium measured in the yeast. The remainder of the selenium was assumed to be bound within the organic cell matter of the yeast cellular matter. The Panel noted that this information related to an aqueous extract, whereas the information on selenium speciation provided by other petitioners related to enzymatic digests of the yeasts. Nevertheless, the Panel considered that insufficient information was provided on the selenium species likely to be present in this product. The Panel also noted that in the case of these two products, the selenium source used in the manufacturing process was not sodium selenite (see section 2.4). The Panel considered therefore that it was not possible to conclude that the profile of the selenium species in these two selenium-enriched yeast products is likely to be similar to those reported for the other five products, with selenomethionine accounting for approximately 60 - 85% of the total selenium.

2.4. Manufacturing Process

Adequate information has been provided by the majority of petitioners on the production process for selenium yeast, which involves culture of the yeast under optimum fermentation conditions in the presence of sources of carbon, nitrogen, oxygen and phosphorus and a defined source of selenium. Five selenium-enriched yeasts were reported by the petitioners to be produced using sodium selenite as a source of selenium. For the remaining two products, one petitioner indicated that the source of selenium is a selenium amino acid chelate (“selenium-aminoate”) and one reported the use of selenium dioxide as source. Following harvest, the selenium-enriched yeast cream is pasteurised and then spray dried or dried by other appropriate drying methods. One petitioner reports that the inactivated and spray dried product is blended with inactive dehydrated baker’s yeast (*Saccharomyces cerevisiae*) to standardize selenium content.

2.5. Methods of analysis in food

Analytical methods given by the petitioners for the determination of total selenium in the selenium-enriched yeast and in the food matrix utilize enzymatic hydrolysis, followed by extraction and high-performance liquid chromatography (HPLC) coupled with element-specific detection for the analysis of seleno amino acids by atomic absorption spectrometry (AAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES).

2.6. Reaction and fate in foods to which the source is added

Information was provided by a number of petitioners on the stability of the selenium-enriched yeast product, showing little or no change in total selenium or percentage selenium bound to yeast over the period evaluated (2-3 years), although data provided by one petitioner showed some decrease (25-30%) in the selenomethionine: total selenium ratio on blending of the yeast with other components prior to tablet manufacturing for food supplements. Limited information on reaction and fate in foods was provided, although several petitioners noted that since selenium is a stable element, interactions with components of food and changes in total selenium content of the food (other than that contributed by the addition of the product itself) are not to be expected. One petitioner provided the results of a study performed to evaluate the

effect of baking on stability of selenium in bread, showing that levels of selenium remained constant during the baking process.

2.7. Case of need and intended levels of use

Selenium-enriched yeast is intended to be used as a source for selenium in foods for particular nutritional uses (PARNUTS) and foods for the general population including food supplements. All petitioners indicate that the yeast is to be used as an ingredient in a wide range of food supplements in the form of tablets and capsules. One petitioner refers to use additionally in effervescent powders and liquids.

The quantity of the different sources of selenium-enriched yeasts to be added to fortified foods, PARNUTS and food supplements will be determined by individual formulators. Petitioners indicated that this would be in the range of 30 – 200 mg of enriched yeast, providing 50 - 200 µg selenium per day, although the majority of products identified by the petitioners would provide 100 µg selenium or less per day. One petitioner indicated that the recommended intake for children under 11 years would be 50 µg per day. Limited information was provided on use levels in foods for particular nutritional purposes (PARNUTS), one petitioner indicated that levels to be added to food would provide an intake of less than 50 µg selenium/day.

2.8. Exposure

Currently selenium-enriched yeast is used in food supplements and foods for particular nutritional uses in a number of countries in the European Union. Exposure to its major component, selenomethionine, also commonly occurs via food, since selenomethionine is the major selenium compound in plants. Animals fed on forage or plant foods will incorporate some selenomethionine into proteins in place of methionine so both animal and plant food sources will contain a substantial proportion of their selenium as selenomethionine. Highest levels of selenium, as selenomethionine, are found in nuts, offal, fish, eggs and poultry. Selenium (as selenomethionine) is also found in bread and cereals but the amount is dependant on where the crop is grown. Selenium levels in cereals are higher in USA (0.44 mg/kg) than in Europe (0.04 mg/kg). Average intake of selenium by the European population has been estimated to lie in the range 27-70 µg/day, with higher intakes seen in Finland, where selenium is added to agricultural fertilisers (SCF, 2000; Rayman, 2004). Table 2 provides information on average selenium intake levels in some European countries and comparison with levels in other parts of the world.

Table 2. Average selenium intake levels in some European countries and comparison with levels in other parts of the world (SCF, 2000; Rayman, 2004)

Country	Average selenium intake (µg/person/day)	Source of data*
Austria	48	Sima & Pfannhauser, 1998 (cited by Combs, 2001)
Belgium	28-61	Robberecht <i>et al.</i> , 1994
Czech Republic	10-25 (estimate)	Kvícala <i>et al.</i> , 1996
Croatia	27	Klapec <i>et al.</i> , 1998 (cited by Combs, 2001)
Denmark	38-47	Danskernes kostvaner, 1995. Danish Institute for Food and Veterinary Research unpublished results.
Finland	100-110	SCF, 2000
France	29-43	Lamand <i>et al.</i> , 1994
Germany	35	Alfthan, G. & Nève, J., 1996
Netherlands	39-54 67	van Dokkum, 1995 Kumpulainen, 1993
Poland	30-40 (calculated)	Wasowicz <i>et al.</i> , 2003
Serbia	30	Djujic <i>et al.</i> , 1995
Slovakia	38	Kadrabová <i>et al.</i> , 1998
Sweden	31 38	Becker, 1989 Kumpulainen, 1993
Switzerland	70	Kumpulainen, 1993
UK	29-39	Ministry of Agriculture, Fisheries and Food, 1997
Australia	57-87	Fardy <i>et al.</i> , 1989
Canada	98-224	Gisse-Nielsen, 1998 (cited by Combs, 2001)
China	7-4990	Combs, 2001
Japan	104-199	Miyazaki <i>et al.</i> , 2001
New Zealand	55-80	Vannoort <i>et al.</i> , 2000
USA	106	Food and Nutrition Board, 2000
Venezuela	200-350	Combs & Combs, 1986 (cited by Combs, 2001)

* For detailed citations see Rayman (2004)

Assuming a mean dietary selenium intake in the range of 30 – 70 µg/day (rounded from the estimated average intake of the European population, of 27 – 70 µg/day), consumption of an additional food supplement containing 100 µg Se/day would result in a total daily Se intake of between 130 and 170 µg Se/day in an adult at the average level of dietary exposure, with higher intakes being anticipated in individuals from selenium-rich areas, or where consumption of selenium-rich cereals may contribute to a higher intake, e.g. in Finland. In the case of a food supplement containing 200 µg Se, the total daily intake would be between 230 and 270 µg Se/day in an adult exposed at the average level of dietary exposure, with higher intakes being anticipated in the above-mentioned higher intake individuals.

Assuming a use level of selenium-enriched yeast in foods for particular nutritional uses and for fortification purposes that would provide an intake of less than 50 µg selenium/day, the total daily selenium intake of an adult exposed at the average level of dietary exposure from these sources plus dietary sources would lie in the range of 80-120 µg/day.

2.9. Information on existing authorisations and evaluations

2.9.1. Use in food supplements

Selenium-enriched yeast has been authorised in Denmark, since 1981 (Danish Food and Veterinary Administration acceptances: L 283-1, L 28-208, 552.1702-0342), including several of the products that are the subject of this opinion. It has similarly been authorised in France

since 1999 for use in foods supplements. In France the authorisation is limited to a maximum selenium daily intake of 75µg (Note of information from the Ministry of Economy, DGCCRF N° 1999-218). In Hungary, selenium-enriched yeast has been authorised as a 'paramedicament for human use'. In UK, selenium-enriched yeast is freely available for use in foods and food supplements. Selenium-enriched yeast is also understood to be sold in food supplements within a number of other EU Member States not specifically mentioned above. The product is also authorised and/or sold in a number of countries outside the European Union, including the US, China, Japan, Korea and a number of Middle-Eastern countries. The inorganic forms of selenium, sodium selenite, sodium selenate and sodium hydrogen selenite are permitted in PARNUTS (see Directive 2001/15/EC) and fortified foods.

2.9.2. Use in foods for particular nutritional uses (PARNUTS)

In the EU, sodium selenite, sodium selenate and sodium hydrogen selenite are authorised for use in foods for particular nutritional purposes (PARNUTS), via Directive 2001/15/EC. Selenium-enriched yeast is authorised in PARNUTS in Germany for overweight persons (Allgemeinverfügung n° 1996-031-00) provided the daily intake of selenium does not exceed 100 µg.

2.9.3. Evaluations

A Population Reference Intake of 55 µg selenium per day for adults was established by the SCF in 1993 (SCF, 1993). The SCF also provided an opinion on selenium in relation to the essential requirements for infant formulae and follow-on formulae (SCF, 1993), which indicated that where the selenium content would naturally be below 1µg/100 kcal, selenium may be added in the form of sodium selenate, sodium selenite, selenomethionine or selenium-enriched yeast provided the maximum content did not exceed 3 µg/100 kcal. In addition, in 1994, the SCF considered the acceptability of selenomethionine and of selenium-enriched yeasts as sources of selenium to be used in the manufacture of infant formulae (SCF, 1994), and recommended that organic selenium compounds should not be used in the manufacture of infant formulae until their acceptability has been established. In 1999, SCF issued a further opinion on the use of organic selenium compounds for nutritional purposes which have been proposed for use in the manufacture of foods for particular nutritional purposes (PARNUTS) (SCF, 1999). The SCF indicated that in view of the uncertainty about the range of selenium-enriched yeast preparations which could be used and the range of selenium compounds they may contain, and differences in the way in which the body handles organic compared with inorganic forms, the Committee's earlier reservations about the use of organic selenium compounds still applied. The EFSA Panel on additives and products or substances used in animal feed (FEEDAP) has provided an opinion on the safety and efficacy of the products selenium-enriched yeast and SelPlex 2000 as feed additives for all species in accordance with Regulation (EC) No 1831/2003.

A safe and adequate range for selenium intake of between 50 and 200 µg/day has been determined by the US Food and Nutrition Board (NAS, 2000) and a Recommended Daily Allowance (RDA) of 55 µg/day selenium for adult men and women has been established by the Board (NAS, 2000). The US has also established Dietary Reference Intakes for selenium for children, of 20 µg/day for ages 1-3 years, 30 µg/day for ages 4-8 years and 40 µg/day for ages 9-13 years.

The Scientific Committee on Food (SCF) has also established a Tolerable Upper Intake Level for selenium of 300 µg/day (SCF, 2000), while the UK Expert Committee on Vitamins and Minerals from the UK derived a safe upper level of 450 µg/day for total selenium (EVM, 2003). The US Food and Nutrition Board (FNB) estimated a Tolerable Upper Intake Level of 400 µg/day (NAS, 2000). Both the SCF UL and that of the FNB also apply to pregnant and lactating women, and while the SCF commented that there were no specific data to support a derivation of an UL for children, they noted that there are no reports indicating that children are more susceptible to adverse effects from selenium. Hence, they concluded that it was appropriate to extrapolate the UL from adults to children on a body weight basis (SCF, 2000). This provided ULs ranging from 60 µg/day for children aged 1-3 years to 250 µg/day for ages 15-17 years.

3. Biological and toxicological data

3.1. Biological data

Following ingestion of selenium-enriched yeast, disruption of the yeast cells and proteolytic digestion of the yeast proteins releases cellular constituents (amino acids and peptides, sugars/carbohydrates, vitamins and minerals, all of which are also anticipated to be endogenous in the human body. Digestion of the yeast proteins liberates selenomethionine and other minor selenium-containing compounds including selenocysteine and selenopeptides, as well as traces of inorganic selenium. The released selenomethionine, like other forms of selenium salts and organoselenium compounds, is readily absorbed from the gastrointestinal tract. In the studies described below, information has been provided both on the bioavailability of selenium from selenium-enriched yeast and from selenomethionine, as the principal selenium-containing component of the enriched yeast.

3.1.1. Pharmacokinetic studies in humans

Absorption of selenium from a ⁷⁷Se-labelled selenium-enriched yeast in 12 male subjects was determined to be approximately 90% (Sloth *et al.*, 2003), similar to the reported absorption of selenium from food sources (Reilly, 1996). In a study in four women, designed to examine the long-term fate of an oral dose of [⁷⁵Se] selenomethionine, intestinal absorption was 95.5-97.3% of the administered dose (Griffiths *et al.*, 1976).

Bügel *et al.* (2004) studied the pharmacokinetics of a single dose of selenium-enriched yeast containing 327 µg ⁷⁷Se/day in 12 healthy men (as part of the same study reported by Sloth *et al.*, 2003). The subjects had been supplied with a stable daily intake of 300 µg Se as selenium-enriched yeast over the previous 10 weeks. Urine and plasma were collected for 3 days and faeces for 5 days. The maximal plasma concentration (C_{max}) was 9.8 ± 1.5 µg ⁷⁷Se/l at a maximal time (T_{max}) of 9.16 h and 89 ± 4% of the selenium isotope was absorbed, with 74 ± 6% retained after 5 days.

Forsberg (1999) studied the effect of 200 µg Se/day as a selenium-enriched yeast, selenomethionine, or selenocystine for 12 weeks on serum selenium concentrations in 27 healthy subjects (7 males and 10 females). Serum selenium increased from 81 to 114 µg Se/l (41%) in the selenocystine group, from 83 to 149 µg Se/l (80%) in the selenium-enriched yeast group, and from 72 to 156 µg Se/l (116%) in the selenomethionine group. Serum selenium

reached a plateau after 4 weeks in the selenocystine group and after 8 weeks in the selenium-enriched yeast and selenomethionine group.

Kvicala *et al.* (2003) found that serum selenium concentrations in 46 subjects from a low selenium region supplemented with 50 or 100 µg Se/day as a selenium-enriched yeast for 41 days increased from 48 µg Se/l at baseline to 86 and 115 µg Se/l, respectively. At the end of the study selenium levels were still rising in serum, though this is not unexpected considering the relatively low initial level and the relatively short period of supplementation.

Three groups of ten Finnish men of low selenium status were given 200 µg selenium/day as selenium-rich wheat, selenium-rich yeast, or sodium selenate for 11 weeks, with twenty unsupplemented control subjects. Plasma selenium levels rose in the wheat and yeast groups for the duration of the supplementation period without plateauing, reaching a level of approximately 170 ng/ml, whereas in the selenate group, plasma selenium had essentially levelled off at a level of 100-110 ng/ml after 4 weeks (Levander *et al.*, 1983).

Nève reviewed the available studies that had examined differences in the effects on indicators of selenium status of different chemical forms proposed for selenium supplementation in healthy subjects (Nève, 1995). Overall these studies showed that organic selenium forms (selenium yeast, selenomethionine and food selenium) increased blood selenium more rapidly and to a greater extent than inorganic forms (selenite and selenate). On average plasma selenium levels were approximately two-three folds greater in healthy subjects taking selenium-enriched yeast supplements compared with levels achieved in supplements given selenite or selenate (Nève, 1995). Burk and co-workers carried out a study to examine the effects of selenium supplementation on plasma selenium biomarkers and urinary selenium excretion in selenium-replete subjects, involving administration of moderate (approximately 200 µg/day) to large (approximately 600 µg/day) amounts of selenium in the form of sodium selenite, selenium-enriched yeast or selenomethionine (Burk *et al.*, 2006). Supplementation with selenomethionine and yeast raised the plasma selenium concentration in a dose-dependent manner, while selenite did not. The authors concluded that selenium in the form of selenomethionine was better absorbed than selenite (Burk *et al.*, 2006). Similar results have been reported in a number of other human studies (e.g. Clausen and Nielsen, 1988; Thomson *et al.*, 1993; Kumpulainen *et al.*, 1985).

In contrast, in a study in thirty-five male volunteers carried out by Fox and co-workers, the absorption of selenium when given as selenium-enriched yeast was approximately 54%, around 60% of that of fish selenium and 58% of that of selenate (Fox *et al.*, 2004). However the yeast used in this study was not typical of manufactured selenium-enriched yeasts, and had a selenium concentration of 145 µg/g dry yeast, compared with typical levels of 1400 µg selenium/g for enriched yeast. One petitioner argues that the results in this study are unlikely to be representative of selenium yeasts on the market.

Xia *et al.* (1992) gave 200 µg Se/day as selenium-enriched yeast to 10 selenium-depleted Chinese men for 1 year. Plasma selenium concentration increased from approximately 20 µg/l to plateau at approximately 150 µg/l after 10 months, while erythrocyte selenium increased from approximately 0.1 ng Se/mg haemoglobin (Hb) to plateau at approximately 0.8 ng Se/mg Hb after 6 months. The authors concluded that supplementation with a selenium-enriched yeast effectively corrected selenium deficiency without any indication of accumulation to a level associated with adverse effects.

Clark *et al.* (1996) gave 200 µg Se/day as selenium-enriched yeast or placebo to 1,312 patients with a history of carcinoma of the skin for a mean period of 4.5 years (with standard deviation (SD) of 2.8 years), with some subjects receiving selenium for up to 10 years. Mean plasma selenium concentration at the time of randomisation was 114 µg/l (SD 23 µg/l). Plasma

selenium concentration in the selenium group increased by approximately 67 % to 190 µg/l within 6-9 months, after which it was essentially constant (trend for a small decline with increasing duration of supplementation). Plasma selenium concentration in the placebo group was constant throughout the trial. The authors concluded that these data indicated that in response to an increase in dietary selenium intake, plasma selenium concentration increases to a new steady-state level that is maintained for many years, if selenium intake is unchanged, in agreement with other studies with selenium-enriched yeast.

Hercberg et al. (2004) gave a preparation containing 100 µg Se as selenium-enriched yeast (plus 120 mg ascorbic acid, 30 mg vitamin E, 6 mg beta carotene, and 20 mg zinc) or placebo to 13,017 subjects in a randomised, double-blind trial with a mean follow-up of 7.5 years. Plasma selenium concentrations were measured in a fraction of randomly selected subjects at 2 and 7 years. Supplementation with 100 µg Se as selenium-enriched yeast resulted in a doubling of plasma selenium concentration, with the change being statistically significant in men and women. A slight (approximately 10 µg/l) increase in plasma selenium was seen between year 2 and year 7 in the placebo group. When this increase was subtracted from the 7-year values for the selenium group, plasma selenium concentration was essentially unchanged in women and slightly increased in men between year 2 and year 7. These data are in agreement with the study of Clark et al. (1986) and other studies with selenium-enriched yeast.

Data are also available on bioavailability and distribution between mother and child in lactating women and on the efficacy of selenium-rich yeast in achieving adequate selenium status in young infants. This is of relevance in considering the transfer of selenium to nursing infants in the milk of mothers consuming selenium supplements and to the use of selenium-enriched yeast in PARNUTS.

McGuire and co-workers studied the effect of providing selenomethionine or selenium-enriched yeast supplements on the selenium status of lactating and non-lactating women (McGuire et al., 1993). Plasma selenium declined in unsupplemented lactating women but not in non-lactating women. Selenomethionine increased plasma selenium in both lactating and non-lactating women, whereas selenium-enriched yeast increased plasma selenium only in non-lactating women. Plasma glutathione peroxidase (GPx) activity decreased with duration of lactation in unsupplemented women and selenomethionine or selenium-enriched yeast supplementation prevented the decline. Glutathione peroxidase contains Se-amino acid residues, and activity of this enzyme has been used as a biological response parameter to determine relative bioavailability of selenium from different sources. Milk selenium declined markedly for 20 weeks after parturition in unsupplemented women. Selenomethionine significantly increased milk selenium concentrations whereas selenium-enriched yeast prevented a decline. Kumpulainen demonstrated that 100 µg/day of selenium from selenium-enriched yeast was more effective than the same dose of selenite in increasing maternal serum selenium (Kumpulainen et al., 1985). The selenium levels reached a plateau within six months of initiation of the yeast supplementation. In contrast, a Polish study showed no significant difference between the selenium intake of breast-fed infants whose mothers were supplemented with 200 µg/day of selenium-enriched yeast or sodium selenite for three months (Trafikowska et al., 1998).

The bioavailability of selenium-enriched yeast in pre-term infants living in a low selenium area (Hungary) was higher than that of other selenium compounds used for pre-term infants. The authors concluded that selenium-enriched yeast was a safe and effective form of short-term enteral selenium supplementation for pre-term infants (Bogye et al., 1998).

Metabolic fate and turnover of selenomethionine

As already indicated, selenium from selenium-enriched yeast is largely present as selenomethionine with smaller amounts of other organic selenium components, the metabolic fate of which is shown in Figure 1 (adapted from Combs, 2001).

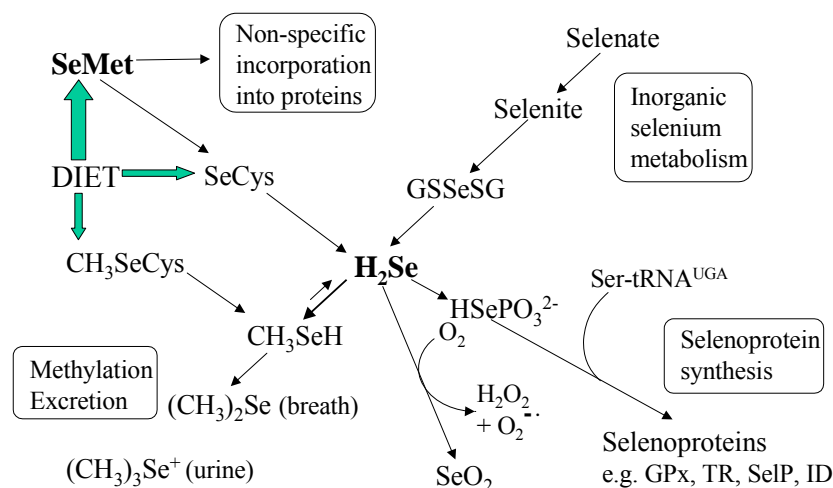


Figure 1. Metabolic fate of selenomethionine and other organic selenium compounds from the diet (adapted from Combs, 2001).

Ingested selenomethionine from selenium-enriched yeast is absorbed in the small intestine (Schrauzer, 2003) via a single, Na^+ -dependent, carrier-mediated process which is also the carrier for methionine (Wolffram *et al.*, 1989). Selenomethionine metabolism is closely linked to protein turnover (Schrauzer, 2000). Selenomethionine from selenium-enriched 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 selenocysteine 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 incorporated in selenoproteins (Berry *et al.*, 1991, 1993).

Following a single oral dose of ^{74}Se labelled selenomethionine, given to six human volunteers, 46% of the dose was found to re-enter the intestine (Swanson *et al.*, 1991). Average turnover time of plasma components varied from 0.01 to 1.1 days. Turnover times in the liver, pancreas and the peripheral tissues were 1.6 to 3.1 days and 61 to 86 days, respectively. When compared with selenite, the whole-body turnover of selenomethionine was slower which was attributed to reutilization of selenomethionine, indicating that selenomethionine is incorporated into a long-term body pool.

The total body pool of selenium has been estimated to be 5-15 mg in adults (SCF, 2000). It is incorporated into tissue proteins such as skeletal muscle, liver, erythrocytes and plasma albumin from which it can subsequently be released by catabolism to maintain increased selenium status for a time. Kinetic studies indicate that blood plasma contains at least four

components with half-lives between 1 and 250 hours (SCF, 2000). The reported average whole-body half-lives of selenomethionine and selenite in humans are 252 and 102 days, respectively, indicating that selenomethionine is extensively utilized and re-utilized (Patterson *et al.*, 1989; Swanson *et al.*, 1991). The “recycling” of selenomethionine probably accounts for its longer half-life, given that although it is incorporated into protein it is less available for immediate selenoprotein synthesis than inorganic selenium (Sunde, 1990). It has a more rapid turnover in plasma, liver, pancreas and peripheral tissues than selenite, and these compartments also show a slower rate of renewal (SCF, 1999). However, its half-life is approximately 5 times longer in the body as a whole than in the compartments with the lowest rate of renewal, implying a substantial re-use of the selenium from organic forms (SCF, 1999).

On repeated supplementation/exposure to a selenium-rich source such as yeast it can be anticipated that levels of selenium will reach a steady-state. Luo *et al.* demonstrated that in methionine-adequate human subjects, supplemental selenomethionine caused tissue selenium levels to increase in proportion to dosage until steady-state was reached. Erythrocyte selenium (representative of tissue selenium) began to plateau after six weeks of supplementation (Luo *et al.*, 1987). In contrast, in the study of Levander and co-workers in men of low selenium status given 200 µg selenium/day as selenium-rich yeast, plasma selenium levels rose for the duration of the supplementation period of 11 weeks without plateauing (Levander *et al.*, 1983). Ten weeks after the supplements were discontinued, platelet levels of the enzyme glutathione peroxidase (GPx) was higher in the group supplemented with selenium-enriched yeast than in a parallel group supplemented with selenium-enriched yeast selenate group, indicating a higher retention of selenium from organic sources than inorganic sources (Levander *et al.*, 1983). However, in a study of similar design, Alfthan and co-workers showed that selenium levels reached a plateau after 11 weeks rather than increasing indefinitely (Alfthan *et al.*, 1991). In a study carried out by Schrauzer and White, involving supplementation of human subjects with 150 µg/day of selenium yeast, a steady-state of selenium in whole blood was reached in approximately about two months (Schrauzer and White, 1978).

The Panel noted that there was a discrepancy between the reported whole body half-lives of selenomethionine and selenite and the reported time taken to reach steady-state in blood. Taking account of the SCF observations that there was extensive recycling of selenium and the methodology used to determine the whole body half-lives, the Panel considers that the consistency in the steady-state measurements across several studies suggests that the plasma elimination half-life is considerably shorter than the reported whole body half-lives.

One petitioner provided the results of a muscle biopsy study in male volunteers who had taken selenium supplements in the form of selenium-enriched yeast and/or L-selenomethionine for a period of 1 to 24 years (Behne, 2005). The study showed an increase in muscle selenium concentration with increasing dose of supplemental selenium in the range of 62.5 to 262.5 µg selenium per day. The relative increase in the muscle selenium was lower than the increase in selenium dose, and the results indicated that muscle selenium level did not depend on duration of supplementation (1–24 years). The petitioner argues that supplementation with selenium-enriched yeast does not lead to the continuous storage of selenium, but rather to a situation of steady-state with the excretion of surplus selenium, and that these data effectively rule out the likelihood of an increase in tissue selenium concentrations to toxic levels during long-term supplementation with selenium-enriched yeast.

Excess/catabolised selenium is metabolised by successive methylation of hydrogen selenide (Figure 1), yielding methyl selenol (CH₃SeH), dimethyl selenide [(CH₃)₂Se] and trimethyl selenonium ion [(CH₃)₃Se⁺], the latter two of which are excreted in breath and urine, respectively. CH₃SeCys, present to a small extent in selenium-enriched yeast and in some foods is acted upon by another β-lyase to give CH₃SeH directly (Combs, 2001).

3.1.2. Pharmacokinetic studies in animals

The bioavailability of selenium in selenium-deficient rats fed either high selenium-enriched yeast or sodium selenite has been assessed by measuring tissue selenium accumulation and glutathione peroxidase (GPx) activity (Yoshida et al., 1999). At lower administered selenium levels (0.04 and 0.08 mg/kg diet), selenite produced higher selenium deposition and higher GPx activities than selenium-enriched yeast, but at a higher selenium level (0.32 mg/kg diet), selenium-enriched yeast showed higher absorption and deposition. The bioavailability of selenium in selenium-enriched yeast compared to selenite was 135%-165% (approximately 1.5-fold increase) in terms of tissue selenium content and 105% - 197% (up to 2-fold increase) in terms of GPx activity.

Female rats were fed a basal diet or a diet containing 0.015 µg Se/g as selenite or selenomethionine throughout mating, pregnancy and lactation (Lane et al., 1990). GPx activity was measured in a range of tissues from fetuses and 7 or 14 day old pups. After 7 days GPx activity was higher in the tissues of pups born to selenomethionine treated dams with the exception of the heart, where there was a small elevation in the offspring of the selenite treated group compared to the selenomethionine group. GPx activity in all tissues increased further by day 14, and was highest in the pups born to dams in the selenomethionine dose group. In a second experiment, the dams were fed with a basal diet and the pups weaned onto a diet containing 0.1 or 0.2 µg Se/g in the form of selenite, selenomethionine or selenocysteine. After both 14 and 21 days of repletion, the highest hepatic GPx activity was found in the selenite treated group, followed by the selenomethionine treated group and the lowest in the basal diet group. The highest tissue selenium concentration was found in the selenocysteine treated animals. It was suggested that the low levels of GPx activity in the selenocysteine treated group could be due to lower bioavailability of this compound or to reduced selenocysteine lyase activity in young rats.

In a study in weanling Sprague-Dawley rats fed a basal selenium-deficient diet containing 2 mg/kg selenium as selenomethionine, selenite or selenocystine, selenium content in testis, muscle, pancreas, heart, spleen, whole blood, erythrocytes and plasma was significantly higher in rats fed selenomethionine than in those fed either selenite or selenocystine. The greatest increase due to selenomethionine compared with the selenite and selenocystine treatments was about 10-fold in the muscle compared with 1.3- to 3.6-fold for the other tissues (Deagen *et al.*, 1987).

A 28-day dietary study was carried out in Wistar rats (5 males and 5 females per group), to evaluate the short-term (28-day) toxicity of a selenium-enriched yeast in comparison with an inorganic selenium/yeast supplement (a mixture of sodium selenite and yeast) and to determine the selenium and selenomethionine accumulation in blood and selected tissues (Institut Rosell, 2004). Dose levels were selected to provide 0, 50, 250 or 1000 µg/kg bw/day selenium, as selenium yeast or sodium selenite. The study showed a significant increase in total selenium content in the liver and blood samples in rats receiving 1000 µg Se/kg bw/day compared with controls, whether supplementation was with selenium-enriched yeast or sodium selenite. At the end of the 28-day dosing period, blood serum selenium levels were 1.36 ± 0.45 µg/ml in the case of rats receiving the organic form of selenium and 1.44 ± 0.78 µg/ml in the case of rats receiving the inorganic form, while liver levels were 1.12 ± 0.99 µg/g and 0.82 ± 0.34 µg/g, respectively. There was no significant difference between selenium levels found in the organic or inorganic treatment groups. There was no increase in selenium in liver or blood tissues in animals receiving 50 or 250 µg/kg bw/day selenium, either in the organic or the inorganic form, and no selenium was detected in muscle tissues.

In addition to determination of total selenium in liver and blood, the study also determined levels of selenomethionine in liver and muscle from animals receiving 1000 µg Se/kg bw/day. Both organic and inorganic supplementation resulted in a significant increase in selenomethionine levels in liver, compared with control diet, the levels being 0.057 ± 0.024 µg/g selenomethionine in the case of animals supplemented with organic selenium and 0.077 ± 0.026 µg/g in the case of animals supplemented with inorganic selenium. In the case of muscle, these levels were 0.10 ± 0.05 µg/g and 0.16 ± 0.07 µg/g, respectively.

A large number of studies have been carried out in farm animals, examining dietary sources of selenium and selenium-enriched diets, including supplementation with selenium-enriched yeast. These consistently show that selenium salts and organoselenium compounds are all readily absorbed from the gastrointestinal tract but that selenomethionine and selenocysteine are more bioavailable (approximately two-fold) than inorganic sources of the element (EFSA, 2006a, 2006b). When dairy cows were supplemented with selenium as sodium selenite, selenate or selenium-enriched yeast, selenium concentration rose to a plateau in plasma from 50 µg/l (control) to 75 µg/l (selenite), 80 µg/l (selenate) and 90 µg/l (selenium-enriched yeast) at four weeks (Ortman and Pehrson, 1999). 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 selenium-enriched yeast groups, respectively showing that selenium-enriched yeast was much more effective in raising the concentration in milk.

Kim & Mahan (2001) examined the accumulation and toxicity of selenium-enriched yeast or sodium selenite at 5, 10, 15, or 20 mg Se/kg diet for 12 weeks in pigs. In pigs fed 5 mg Se/kg, erythrocyte selenium concentration reached a steady-state of ~ 3.1 mg/kg after 6 weeks' intake of selenite, while extrapolation of the data for selenium-enriched yeast suggest a steady-state of ~ 3.7 mg/kg would be reached by week 15 (3.59 mg/kg at week 12). Selenium from selenium-enriched yeast was accumulated more efficiently than that from selenite and the difference between the two sources was greatest in the loin (striated muscle), heart, and hoof. Although the retention of selenium was greater when fed as selenium-enriched yeast, selenosis was more severe and occurred sooner when inorganic rather than organic selenium was fed. Signs of selenosis were first observed at diets containing > 5 mg Se/kg, which is a level 60–125-times the contribution of 100–200 µg supplemental selenium to a typical human diet.

3.1.3. Conclusion on bioavailability

In studies in humans and animals, the bioavailability of selenium from selenium-enriched yeast appears to be similar to that of selenomethionine, either as the pure substance in supplement form or as selenomethionine present in selenium-rich food such as wheat. Bioavailability is higher than that of inorganic forms of selenium (approximately two-fold) and has been estimated to be greater than 90 percent in humans. Following absorption, selenium from selenomethionine is incorporated into tissue proteins such as skeletal muscle, liver, erythrocytes and plasma albumin. The reported average whole-body half-lives of selenomethionine and selenite in humans are 252 and 102 days, respectively, indicating that selenomethionine is extensively utilized and re-utilized.

On repeated supplementation/exposure to a selenium-rich source such as yeast the available evidence indicates that levels of selenium reach a steady-state in blood within a maximum of 6–12 months, rather than increasing indefinitely. Several studies have reported that steady-state is achieved more rapidly than this, within a period of 2–4 months. Excess/catabolised selenium is metabolised by successive methylation of hydrogen selenide (s. Figure 1), yielding

methyl selenol (CH_3SeH), dimethyl selenide $[(\text{CH}_3)_2\text{Se}]$ and trimethyl selenonium ion $[(\text{CH}_3)_3\text{Se}^+]$, the latter two of which are excreted in breath and urine, respectively.

3.2. Toxicological data

3.2.1. Acute toxicity

Acute toxicity studies in rats have been carried out with selenium-enriched yeast and sodium selenite (Vinson & Bose, 1987). Six groups ($n = 10$) of male weanling Sprague-Dawley rats (average weight 50 g) were given a single dose of selenium as selenium-enriched yeast or sodium selenite by gavage. The LD_{50} of selenium-enriched yeast was 37.3 mg/kg compared with 12.7 mg/kg for sodium selenite, indicating that the acute toxicity of organic selenium in the form of yeast is less toxic than inorganic selenium in the form of sodium selenite. The median lethal dose (LD_{50}) of selenomethionine in rats given an intraperitoneal injection was determined to be 4.25 mg Se/kg body weight (bw) and thus is comparable to that of selenite or selenate (Schrauzer, 2000). In mice, the LD_{50} of D-selenomethionine was 8.8 Se/kg bw after intravenous injection.

3.2.2. Subacute and subchronic toxicity

Selenium is known to be chronically toxic and selenosis has been reported in humans and in food animals in seleniferous areas. A number of expert bodies (e.g. SCF, 2000; FNB, 2000; EVM, 2003) have reviewed the subacute and subchronic toxicity of selenium, and the safety/toxicity of selenium itself is outside the remit of the Panel. The present opinion is therefore restricted to an assessment of available subacute and subchronic studies on selenium-enriched yeast, in particular those that have compared the toxicity of selenium yeasts and other organic forms of selenium compared with inorganic selenium.

A 28-day dietary study was carried out in Wistar rats (5 males and 5 females per group), to evaluate the short-term (28-day) toxicity of a selenium-enriched yeast in comparison with an inorganic selenium/yeast supplement (a mixture of sodium selenite and yeast) and to determine the selenium and selenomethionine accumulation in blood and selected tissues (Institut Rosell, 2004). Dose levels were selected to provide 0, 50, 250 or 1000 $\mu\text{g}/\text{kg}$ bw/day selenium, as selenium yeast or sodium selenite. Body weight gain and food consumption were reduced at the highest dose of selenium (1000 $\mu\text{g}/\text{kg}$ bw/day) whether given as selenium-enriched yeast or sodium selenite. The reduction in body weight gain was however significantly greater ($p < 0.01$) with inorganic selenium supplement; in both sexes, body weights were approximately 25% lower compared with controls in rats given 1000 μg Se/kg bw/day in the form of selenium-enriched yeast and approximately 50% lower when the selenium was given in the form of sodium selenite. There was no effect on body weight at lower levels of selenium.

Haematological, histopathological and clinical chemistry changes were indicative of hepatotoxicity following 1000 $\mu\text{g}/\text{kg}$ bw/day selenium supplementation. In general the increase in parameters such as levels of liver enzymes was greater in rats receiving sodium selenite compared with those receiving selenium-enriched yeast. Overall, no effect was seen on these haematological, histopathological and clinical chemistry parameters in animals receiving 50 or 250 $\mu\text{g}/\text{kg}$ bw/day selenium, either in the organic or the inorganic form showed no significant increases. Decreases in weights of several organs were evident in the animals receiving sodium selenite at a dose level of 1000 μg Se/kg bw/day, organs affected included thymus, liver,

kidney, heart and testes. No effect was seen on organ weights in animals receiving selenium-enriched yeast, at a dose level of 1000 µg Se/kg bw/day, nor in animals receiving 50 or 250 µg/kg bw/day selenium, either in the organic or the inorganic form. Evidence of hepatotoxicity was seen in all treatment groups, given either organic or inorganic selenium, including vacuolization and necrosis of hepatocytes, evidence of mitotic activity, increased apoptosis and acute inflammation. Hepatotoxicity was however consistently less severe in the treatment groups receiving the selenium yeast.

In this study, only the highest dose of selenium (1000 µg/kg bw selenium/day) caused significant toxicity, as evidenced by decreases in body weight gain and food consumption, and changes in haematological, histopathological and clinical chemistry parameters indicative of hepatotoxicity. Haematological and clinical chemistry parameters in the dose groups receiving 50 or 250 µg/kg bw/day selenium showed little evidence of a treatment-related effect, although histopathological evidence of liver damage was reported in all three treatment groups, without significant dose-related trend, whether the rats were supplemented with selenium-enriched yeast or sodium selenite. The degree of change in the parameters investigated was less in animals supplemented with the organic selenium source (yeast) compared with those supplemented with sodium selenite, indicating that selenium-enriched yeast supplements are of lower toxicity than inorganic selenium supplements.

A study comparing the toxicity of a selenite and a selenium-enriched yeast diet in rats similarly showed that Se-yeast was less toxic than selenite (Spallholz & Raftery, 1987). Severe hepatotoxicity, cardiotoxicity and splenomegaly were observed in rats fed selenite at levels of 16 mg/kg 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.

Groups of male weanling rats (n=8) were fed diets containing 2.5, 5 or 10 mg Se/kg added as L-selenomethionine, D- selenomethionine, sodium selenite or sodium selenate (McAdam and Levander, 1987). Severe growth depression and mortality occurred within 29 days when rats were fed diets containing 10 mg Se/kg (800 µg Se/kg bw/day), irrespective of the form in which the selenium was administered. However, selenomethionine was consistently less toxic than the inorganic forms of selenite or selenate. A dietary level of 2.5 mg Se/kg (200 µg Se/kg bw/day), over a 6-week period produced no evidence of depressed growth or diminished survival.

In a 30-day study with long-tailed pregnant female Macaques (*Macaca fascicularis*), the highest tolerated dose of selenomethionine was estimated to be 150 µg Se/kg bw/day (Hawkes *et al.*, 1992). Levels of erythrocyte, plasma selenium and hair selenium associated with increased weight loss due to selenium toxicity were > 2.3 mg/l, > 2.8 mg/l and > 27 µg/g, respectively.

3.2.3. Reproductive and developmental toxicity

Selenium compounds have been reported to have effects on reproduction and offspring in rodents, however these are largely associated with overt maternal poisoning and nutritional deprivation (SCF, 2000). Selenium compounds including selenate, selenite, selenocysteine and selenomethionine are also reported to be developmental toxicants in avian species and fish, at toxic levels (SCF, 2000).

Mahan & Kim (1996) gave 0.1 or 0.3 mg Se/kg as selenium-enriched yeast or sodium selenite from ~ 60 days before breeding and throughout pregnancy to female pigs. The number of pigs

born (total, live, stillborn) and individual pig birth weights were not affected by the type or level of selenium fed. Litter birth weights were higher with the addition of 0.3 mg Se/kg diet, though the difference was not statistically significant. Litter size, number of pig deaths from 0 – 7 days postpartum, and pig and litter weight at 7 and 21 days postpartum were not affected by the type or level of selenium fed.

Groups of up to 10 pregnant Syrian hamsters were treated with selenite, selenate or selenomethionine during the critical stages of embryogenesis (Ferm *et al.*, 1990). The dosing regimes used were oral, intravenous and osmotic mini-pump infusion. The doses used were: sodium selenite - oral 3.98-19 mg/kg; intravenous 1.9-3.98 mg/kg; sodium selenate- oral 17-20.8 mg/kg; intra venous 4.35-14.2 mg/kg; selenomethionine oral - 14-7-19.6 mg/kg; osmotic mini-pump 49.4 mg/kg over the seven day lifetime of the pump. An additional group was treated with an oral dose of 14.7 or 19 mg/kg selenomethionine which was divided over four days. The animals were killed and examined on day 13 of gestation. Malformations, mainly encephaloceles were observed following treatment with oral or intravenous selenite or selenate but this was at doses where maternal toxicity was apparent (50 and 30% mortality, respectively in the top dose groups during the 5 day observation period). The maternal animals were lethargic and at necropsy there was evidence of inanition. No gross pathological or neurological effects were noted in the dams. The hamsters treated with selenate were less lethargic than the selenite treated ones. A dose-related increase in resorptions was apparent at the top dose levels. Fetal body weights and lengths were reduced in a dose-dependent manner following treatment with the inorganic forms of selenium. Single oral doses of >14.7 mg/kg selenomethionine induced the same type of malformations but this did not occur when the dose was given over four days or when administered by the mini-pump over several days; although significant maternal weight loss occurred, this did not result in any decreases in fetal body weight or length. However, fetal body weights and lengths were reduced in a dose-dependent manner following treatment with single oral doses of selenomethionine. At the higher doses of selenomethionine, maternal toxicity was pronounced; lethargy, inanition, weight loss and severely reduced food consumption were observed but no mortality occurred in the maternal animals. The authors concluded that it was not possible to assign a specific teratogenic effect to selenium since it was confounded by maternal toxicity.

In a study in female Macaques (*Macaca fascicularis*) fed selenomethionine (25, 150 and 300 µg/kg bw/day) during organogenesis, dose-dependent maternal toxicity as indicated by poor appetite and emesis was observed in the mid- and high-dose groups (Tarantal *et al.*, 1991). There was no evidence of developmental toxicity in this study, and the dose of 25µg/kg bw/day was considered to be a NOAEL.

3.2.4. Genotoxicity

An extract of a selenium-enriched yeast strain, containing 2000 ppm of selenium, predominantly (more than 98 %) in the form of selenomethionine, has been tested with negative results in the following battery of genotoxicity assays: i) reversion in bacteria (Ames test); ii) chromosomal aberrations in human lymphocytes *in vitro*; iii) micronucleus test in mouse bone marrow (Griffiths *et al.*, 2006).

In contrast, studies with selenium compounds (selenite, selenate, selenide, selenocysteine and selenosulphide) have given positive results in several *in vitro* systems (SCF, 2000). Mainly negative or equivocal results were obtained in micronucleus and chromosomal aberration studies *in vivo* in rodents (Norppa *et al.*, 1980; Moore *et al.*, 1996) and primates (Choy *et al.*, 1989; 1993). The mutagenic effects of selenium salts *in vitro* are considered to be associated

with production of reactive oxygen radicals and glutathione is known to promote these reactions (Kramer and Ames, 1988). Auto-oxidisable selenium metabolites, such as hydrogen selenide, are known to undergo redox cycling producing oxygen radicals and cause DNA strand breaks (Anundi *et al.*, 1984; Garberg *et al.*, 1988).

It is proposed that the greater ability of selenomethionine to be incorporated non-specifically in cellular proteins acts as a sink, and determines the lower toxicity and lack of genotoxicity of selenomethionine compared to other selenium compounds (Griffiths *et al.*, 2006).

3.2.5. Carcinogenicity

No specific carcinogenicity studies have been carried out on selenium-enriched yeast or on selenomethionine.

3.2.6. Human studies

Selenium is known to be chronically toxic and adverse health effects have been reported in humans and in food animals in seleniferous areas. Intakes of selenium in the range of 3200-6990 µg/day (mean 4990 µg/day) by humans are associated with chronic selenosis, with no selenosis observed in the intake range of 240-1510 µg/day (mean 750 µg/day) (Yang *et al.*, 1983). Signs of selenosis are hair loss, brittle, thickened and stratified nails, garlic breath and skin lesions (Whanger *et al.*, 1996).

The SCF report of 2000 provides information on the toxicity in humans of selenium-containing supplements or dietary selenium taken over a prolonged period (up to 6 months) (SCF, 2000). It has been suggested that organic forms of selenium may be more toxic during long-term consumption as they are incorporated into proteins rather than excreted. However, in the rat studies described above (Institut Rosell, 2004; Spallholz and Raftery, 1987), selenium-enriched yeast had a lower toxicity (4-8 weeks of feeding) than selenite or selenate.

Long-term supplementation studies with selenium-enriched yeast have been carried out by three research groups:

- Arizona Cancer Centre, University of Arizona (Nutritional Prevention of Cancer Trial)
- Cancer Institute, Chinese Academy of Medical Sciences, Beijing
- The UK PRECISE study group at the University of Surrey in collaboration with the Institute of Cancer Research
- The Danish PRECISE study group at Odense University Hospital

In the Nutritional Prevention of Cancer (NPC) Trial (Clark *et al.*, 1996), a total of 1312 subjects whose baseline intake was around 90 µg Se/day were randomly allocated to receive supplements of 200 µg of selenium as selenium-enriched yeast or placebo yeast for a mean of 4.5 years. A total of 35 subjects complained about adverse effects, most of which involved gastrointestinal upset, resulting in their withdrawing 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 provided by one petitioner showed that administration of 200 µg/day of selenium as selenium-enriched yeast gave a rapid increase in plasma selenium up to about three months, after which the increase levelled off, reaching a clear plateau at around 190 µg/l by one year. This plasma selenium

level is well below the level associated with toxicity which was reported to amount to 1054-1854 µg/l in whole blood, corresponding to a value in plasma that is generally 23% higher (Yang *et al.*, 1989).

In a clinical trial in which men with localised prostate disease and a life expectancy of < 10 years receive 200 µg or 800 µg selenium/day as selenium-enriched yeast, there have been no safety concerns even after supplementation with 800 µg selenium-enriched yeast/day for three years or more (Stratton *et al.*, 2003). In an arm of this trial that was discontinued in 2001, eight subjects received a dose of 1600 µg Se-yeast/day and 16 subjects a dose of 3200 µg/day (Reid *et al.*, 2004). Subjects were on these doses for average periods of almost 12 months. Mean plasma selenium levels achieved were 492±188 and 640±491 µg/l, respectively. While the 3200 µg/day group reported more selenium-related side effects such as garlic breath, brittle nails and hair, stomach upset and dizziness than the 1600 µg/day 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).

In the study carried out by the Cancer Institute, Chinese Academy of Medical Studies, Beijing, two hundred and twenty six Hepatitis Surface-Antigen Positive subjects were provided 200µg selenium-enriched yeast or placebo yeast daily for four years. No side effects were found (Yu *et al.*, 1997).

A pilot study for the UK PRECISE (PREvention of Cancer by Intervention with SEelenium) trial was initiated in October 1999 (Rayman, 2002). In this double-blind placebo-controlled pilot study, 500 male and female were given either placebo or 100, 200 or 300 µg/day of selenium as selenium-enriched yeast. 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. Neither the distribution nor the severity of side effects correlated with the intake of selenium. None of the subjects in the 300 µg group developed hair or nail problems, known signs of selenium toxicity. 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 bioavailability of the Se-yeast, the plasma level of 233 µg/l attained in the 300 µg/day treatment group was well within safe limits.

A pilot study for the Danish PRECISE (PREvention of Cancer by Intervention with SEelenium) Trial initiated in December 1998 had a similar design and provided similar results to those of the UK trial reported by Rayman (Rayman, 2002). Mean whole-blood selenium concentration in 13 subjects from the 300 µg/day 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. From this study there is no evidence that selenium intakes up to 343 µg/day (300 µg/day from the Se-yeast supplement and approximately 43 µg/day 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 (Larsen *et al.*, 2004).

Patients with rheumatoid arthritis were supplemented with 600 µg/day "selenomethionine-containing yeast" (presumably selenium-enriched yeast) enriched with vitamin E for eight months in a double-blind manner. Though a significant alleviation of articular pain and morning stiffness were observed, no adverse effects were reported (Aaseth *et al.*, 1998). Plasma selenium plateaued at 500 µg/l at four months.

Information relevant to chronic exposure to high levels of selenium as selenium-enriched yeast can also be gleaned by analogy to natural exposure to high levels of dietary selenium, which is

largely in the form of selenomethionine, the major component of selenium-enriched yeast. Data exist on populations in seleniferous geographic areas of the world, such as the northern Great Plains of the United States, parts of Venezuela and Colombia, Greenland and one county in China. From published data, no health or toxicity problems have been observed at intakes of up to 819 µg/day in China (Yang and Zhou, 1994) or 724 µg/day in the USA (Longnecker *et al.*, 1991, 1993).

3.2.7. Selenium-enriched yeast and lactation

The European Commission SCF Additives Working Group noted, in relation to a PARNUTS application for selenium-enriched yeast from IDACE that:

- *Selenium-enriched Yeast, when taken by lactating women, can increase milk selenium contents to levels above the adequate selenium intake of infants. It has not been investigated if undesirably high milk selenium concentrations can be reached, when Selenium-enriched Yeast intake is prolonged and is higher than the reported 100 to 200 µg selenium/day.*

Two petitioners addressed this concern as follows:

a. Supplementation studies with selenium-enriched yeast

Though a number of studies have investigated the effect of supplementation of lactating women with selenium-enriched yeast (Se-yeast), none has used a level above 200 µg/day. Data on supplementation with 200 µg/day Se-yeast are summarised below and give some indication of likely effects at higher levels of Se-yeast supplementation.

- (i) US women supplemented with 200 µg/day selenium as selenium-enriched yeast from 4 to 8 weeks post-partum showed no increase in the selenium intake of their infants after four weeks of supplementation compared to infants of unsupplemented lactating women viz. 11.2 vs. 11.1 µg/day (McGuire *et al.*, 1993). 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. This value of 83 µg/l is more than an order of magnitude below levels associated with toxicity (3200 µg/l in whole blood (Yang *et al.*, 1983) equivalent to approximately 2560-2880 µg/l in plasma). Additionally, supplemental Se-yeast was beneficial in preventing the longitudinal decline in milk selenium content that occurred with advancing lactation (McGuire *et al.*, 1993).
- (ii) A Polish study supplemented lactating mothers for three months with 200 µg/day selenium as Se-yeast or sodium selenite (Trafikowska *et al.*, 1998). Supplementation with Se-yeast resulted in an increase in intake compared with levels in the basal diet, from 6.1 µg/day to a level of 11.3-12.8 µg/day 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/day as sodium selenite. 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. The latter concentration may be compared with mean whole-blood values of 440-968 µg/l that are associated with safe intakes of selenium (Yang *et al.*, 1983, Yang and Zhou, 1994).

Both these studies show that at a maternal Se-yeast supplementation level of 200 µg/day of selenium from selenium-enriched yeast, infant Se intakes of 11.2-12.8 µg/day achieved are four- to five-fold lower than the Upper Limit of 45-60 µg/day recommended for infants of 0-12 months (Food and Nutrition Board, 2000).

b. Extrapolation from studies on natural exposure to high levels of selenium

As there are no studies that have investigated the effect of higher intakes of selenium as selenium-enriched yeast than 200 µg/day, the likely effects of such intakes can be inferred from analogous studies of the effect of higher intakes from natural sources. This can be justified on the grounds that such sources largely contain selenomethionine, the main component of Se-yeast (Schrauzer, 2000).

There are no reported cases of selenosis in breast-fed infants even in seleniferous areas (Dorea, 2002). No adverse effects were detected in infants of Venezuelan mothers from seleniferous areas who consumed a mean of 554 µg selenium /day (Dorea, 2002). Similarly, in China, a much higher maternal daily intake of selenium that raised breast-milk selenium to 283 µg/l (Keshan Disease Research Group, 1979) and therefore infant intake to 184-221 µg/day (assuming a breast-milk intake of 650-780 ml/day) seems to be without adverse effects on the breast-fed infant (Dorea, 2002). Chinese data also show that a maternal selenium intake of 1238 µg/day in seleniferous areas is associated with a milk concentration of 121 µg/l (Yang *et al.*, 1989), equating to a daily intake of around 79-94 µg/day by the infant. 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 (Dorea, 2002). This is consistent with the observation that nursing pigs are capable of buffering against excess of milk selenium (organic) by storing more selenium in body tissues (Kim & Mahan, 2001).

Although there is no evidence for selenosis among breast-fed infants of women with a high selenium intake, concern has been expressed that such an intake may adversely reduce the zinc (Zn) status of breast-fed infants resulting in growth inhibition (Brätter *et al.*, 1991a, Brätter and Negretti de Brätter, 1996). While a significant inverse association was found between selenium and zinc in breast milk in seleniferous areas of Venezuela, only 11% of the decline in zinc content of the milk was explained by the increase in selenium content ($r = 0.3382$) (Brätter and Negretti de Brätter, 1996). In addition, the authors of the study point out that all the families selected for the study belonged to low socio-economic groups (strata IV and V) typified by a low calorie intake and late and inadequate weaning. Zinc intake is therefore likely to be low or inadequate in these population groups in any case, as demonstrated by the slower rate of growth in these children well beyond the age of weaning (Brätter *et al.*, 1991a).

3.2.8. Safety of the yeast source in selenium-enriched yeast

The quantity of yeast ingested as a result of the use of supplements containing selenium-enriched yeast will be small (up to 200 mg daily). Digestion of the yeast cells can be anticipated to release cellular constituents (amino acids and peptides, sugars/carbohydrates, vitamins and minerals), all of which are anticipated to be endogenous in the human body. Thus consideration of the safety of the source can be restricted to the assessment of the selenium-containing compounds released from the cell, as detailed above.

Some individuals may be sensitive to yeast protein, as shown by skin prick tests and radioallergosorbent testing (RAST), particularly those who have had a yeast infection such as *Candida*, or people who may be exposed to yeasts by inhalation or people with atopic dermatitis. However, serious allergic responses to yeast including anaphylaxis are reported to be extremely rare, despite the fact that yeast has been used as a food ingredient since records began. The Panel concludes that the quantity of yeast ingested as a result of the use of supplements containing selenium-enriched yeast is unlikely to present an allergenic risk, and that individuals with yeast sensitivity will be alerted to the presence of yeast protein via the labelling of these products.

4. Discussion

The information considered by the Panel on the bioavailability of selenium from selenium-enriched yeast, when primarily in the form of organo-selenium compounds, mainly selenomethionine, shows that selenium is readily bioavailable from this source. In a number of studies in humans and animals, in particular those on selenium-deficient diets, the bioavailability of selenium from selenium-enriched yeasts and the bioavailability of selenomethionine have been shown to be higher than that of inorganic forms of selenium. It is more than 90 percent bioavailable and has been reported to have nearly twice the bioavailability of selenium as sodium selenite. Supplementation studies with selenium-enriched yeasts and selenomethionine, either short-term or long-term, have demonstrated bioavailability as evidenced by increases in plasma biomarkers of selenium (selenium concentration, selenoprotein P concentration, and glutathione peroxidase activity).

Following absorption, selenomethionine from selenium-enriched yeast is either metabolised to other functional forms of selenium (e.g. selenocysteine) or diverted into pathways of methionine metabolism and stored as selenoproteins. The half-life of L-selenomethionine (252 days) is longer than that of inorganic selenite (102 days), indicating that once absorbed, selenomethionine is incorporated into a long term body pool in which it reaches a steady-state. The steady-state is reached after a maximum of 6-12 months of supplementation with selenium in the form of selenomethionine or selenium-enriched yeast, as shown by measurement of plasma selenium and in muscle biopsy samples from human volunteers. The selenium is incorporated into tissue proteins such as skeletal muscle, liver, erythrocytes and plasma albumin from which it can subsequently be released by catabolism to maintain increased selenium status, indicating that selenomethionine is extensively utilized and re-utilized. The Panel noted that there was a discrepancy between the reported whole body half-lives of selenomethionine and selenite and the reported time taken to reach steady-state in blood. Taking account of the SCF observations that there is extensive recycling of selenium and the methodology used to determine the whole body half-lives, the Panel considers that the consistency in the steady-state measurements across several studies suggests that the plasma elimination half-life is considerably shorter than the reported whole body half-lives.

Recent data presented by the petitioners show that in response to an increase in dietary selenium intake from sources such as selenium-enriched yeast, plasma selenium concentration increases to a new steady-state level that is maintained for many years if the level of selenium intake is unchanged.

Selenium is known to be chronically toxic and selenosis has been reported in humans and in food animals in seleniferous areas. Intakes in the range of 3200-6990 µg/day (mean 4990 µg/day) are associated with chronic selenosis, with no selenosis observed in the intake range of 240-1510 µg/day (mean 750 µg/day) (Yang *et al.*, 1983). Investigations into the health effects

of high dietary intakes of selenium in populations living in the seleniferous areas of South Dakota, Venezuela and China have indicated that the highest long-term daily intake that can be ingested without the development of toxicity in most individuals is approximately 800 µg while prolonged intakes of daily selenium doses of 1000 µg or greater may cause adverse reactions. The toxicity of organic forms of selenium including selenium-enriched yeast and selenomethionine has however been shown to be lower than that of inorganic selenite or selenate. In addition, recent clinical studies carried out with selenium-enriched yeast show that selenium intakes up to 343 µg/day (300 µg/day from the Se-yeast supplement and approximately 43 µg/day from diet) over a maximum period of 4 years and 8 months, and whole-blood levels of 441 selenium µg/l, are not associated with toxicity, even after prolonged periods of exposure.

From all the above, the Panel considers that the reservations expressed by the SCF in 1999, when the Committee initially considered selenium-enriched yeasts for use in PARNUTS, namely that there was uncertainty about the range of selenium-enriched yeast preparations which could be used and the range of selenium compounds they may contain, and differences in the way in which the body handles organic compared with inorganic forms, have been addressed via the additional data considered in this opinion on selenium-enriched yeasts.

Having considered the general product characteristics of the selenium-enriched yeast products covered by this opinion, and the additional data that have become available on the identity and speciation of the organo-selenium compounds contained in the yeast, the Panel considers that five of the selenium-enriched yeasts included in this opinion are sufficiently well characterised in terms of the selenium content and the nature of the selenium compounds contained therein, enabling the broad product characteristics for these five products to be defined.

For the two remaining products: biotransformed yeast produced using selenium dioxide as a source and selenium-enriched yeast produced using selenium-aminoate, the Panel considered that insufficient information was provided on the selenium species likely to be present in these products. The Panel also noted that in the case of these two products, the selenium source used in the manufacturing process was not sodium selenite. The Panel considered therefore that it was not possible to conclude that the profile of the selenium species in these two selenium-enriched yeast products is likely to be similar to those reported for the other five products, with selenomethionine accounting for approximately 60 - 85% of the total selenium. Thus, due to deficiencies in the bioavailability and safety data provided on the selenium species likely to be present in these products, the Panel was unable to evaluate their safety.

Average dietary intake of selenium by the European population has been estimated to lie in the range of 27-70 µg/day. Assuming a mean dietary selenium intake in the range of 30-70 µg/day, consumption of an additional food supplement containing 100 µg selenium would result in a total daily selenium intake of 130-170 µg/day in an adult at an average level of dietary exposure. Intake from the proposed use of supplements containing selenium-enriched yeast would thus be approximately 2-3 times the average dietary intake from food. The total intake (from dietary sources and from supplementation with selenium-enriched yeast) can be compared with the Tolerable Upper Intake Level established by the SCF in 2000 for selenium of 300 µg/day or that of 400 µg/day established by the US Food and Nutrition Board. In the case of a food supplement containing 200 µg Se, the total daily Se intake would be between 230-270 µg/day in an adult at an average level of dietary exposure, with higher intakes being anticipated in individuals from selenium-rich areas where consumption of selenium-rich cereals may contribute to a higher intake. For these individuals, the Tolerable Upper Intake Level for selenium established by the SCF in 2000 could be exceeded.

Assuming a use level of selenium-enriched yeast in foods for particular nutritional uses and for fortification purposes that would provide an intake of less than 50 µg selenium/day, the total

daily selenium intake of an adult exposed at the average level of dietary exposure from these sources plus dietary sources would lie in the range of 80-120 µg/day.

The Panel noted the results of a series of studies carried out to investigate the possibility that selenium-enriched yeast, when taken by lactating women, could increase milk selenium contents to levels above the adequate selenium intake of infants. These included a study showing that at a maternal selenium-enriched yeast supplementation level of 200 µg/day of selenium from selenium-enriched yeast, infant selenium intakes of 11.2-12.8 µg/day achieved are four- to five-fold lower than the Upper Limit of 45-60 µg/day recommended for infants of 0-12 months (Food and Nutrition Board, 2000).

In the absence of studies that have investigated the effect of higher intakes of selenium than 200 µg/day, as selenium-enriched yeast, in lactating women, the Panel noted that the likely effects of such intakes can be inferred from analogous studies of the effect of higher intakes from natural sources. The petitioners reported that there have been no reported cases of selenosis in breast-fed infants even in seleniferous areas, and no adverse effects were detected in infants of Venezuelan mothers from seleniferous areas who consumed a mean of 554 µg Se/day. Chinese data also show that a maternal selenium intake of 1238 µg/day in seleniferous areas is associated with a milk concentration of 121 µg/l, equating to a daily intake of around 79-94 µg/day by the infant. It is notable that at this high level, infant intake is lower than maternal intake by a factor of around 14 whereas at a more typical maternal intake of around 86 µg/day, infant intake, at around 11 µg/day, is lower by a factor of 8 (Levander *et al.*, 1987). This suggests that at high intakes, the mother acts as a buffer, protecting the infant against excess. The Venezuelan data also show that at a maternal selenium intake of 300 µg/day - the recommended SCF Tolerable Upper Intake Level - milk selenium concentration only rises to 60 µg/l (Brätter *et al.*, 1991b) giving an infant intake of 39-47 µg/day which is below the UL of 60 µg/day for children aged 1-3 years recommended by the SCF (SCF, 2000) and the UL of 45-60 µg/day for infants aged 0 - 12 months recommended by the Food and Nutrition Board (Food and Nutrition Board, 2000).

CONCLUSIONS

The present opinion deals only with the safety and bioavailability of selenium-enriched yeast as a particular source of selenium to be used in foods for particular nutritional uses (PARNUTS) and in foods (including food supplements) for the general population. The safety of selenium itself, in terms of amounts that may be consumed, is outside the remit of this Panel.

The conclusions of the present opinion thus relate only to selenium-enriched yeasts in compliance with the following product characteristics:

Selenium-enriched yeasts produced by culture in the presence of sodium selenite as selenium source and containing, in the dried form as marketed, not more than 2.5 mg Se/g. The predominant organic selenium species present in the yeast is selenomethionine which constitutes between 60 and 85% of the total selenium in the product. The content of other organic selenium compounds including selenocysteine does not exceed 10%.

Levels of inorganic selenium in selenium-enriched yeast normally do not exceed 1%. Since inorganic Se in the form of sodium selenite, sodium selenate and sodium hydrogen selenite has been reviewed by the SCF (SCF, 1999) and has been permitted in PARNUTS (see Directive 2001/15/EC) and fortified foods, inorganic selenium from a selenium-enriched yeast source is not a safety issue.

The Panel notes that the bioavailability of selenium from selenium-enriched yeast is higher than the bioavailability from inorganic sources of selenium previously considered by the SCF in its 1999 opinion on substances which have been proposed for use in the manufacture of foods for particular nutritional purposes. The Panel also notes the view of SCF, expressed in that opinion, that *“in view of the uncertainty about the range of selenium-enriched yeast preparations which could be used and the range of selenium compounds they may contain, and differences in the way in which the body handles organic compared with inorganic forms, the Committee’s earlier reservations about the use of organic selenium compounds still applied.”*. In addressing this, the Panel has considered (i) the general product characteristics for the majority of the selenium-enriched yeast products covered by this opinion, (ii) the additional data that have become available on the identity and speciation of the organo-selenium compounds contained in the yeast, (iii) the available information indicating that on repeated supplementation/exposure to a selenium-rich source such as yeast, levels of selenium reach a steady-state within 2-4 months, rather than increasing indefinitely in plasma and in tissues such as muscle, (iv) the results of recent clinical trials with selenium enriched yeast indicating intakes of up to 343 µg/day are not associated with toxicity, even after prolonged periods (years) of exposure. The Panel thus considers that the reservations expressed by the SCF in 1999, when the Committee initially considered selenium-enriched yeasts for use in PARNUTS have been addressed via the additional data considered in this opinion.

Average dietary intake of selenium by the European population has been estimated to lie in the range of 27-70 µg/day, with higher intakes seen in Finland, where selenium is added to agricultural fertilizers. Assuming a mean dietary selenium intake in the range of 30-70 µg/day, consumption of an additional food supplement containing 100 µg Se would result in a total daily selenium intake of 130-170 µg/day in an adult at an average level of dietary exposure. Intake from the proposed use of supplements containing selenium-enriched yeast would thus be approximately 2-3 times the average dietary intake from food. In the case of a food supplement containing 200 µg selenium, the total daily selenium intake would be between 230-270 µg/day in an adult at an average level of dietary exposure, with higher intakes being anticipated in individuals from selenium-rich areas where consumption of selenium-rich cereals may contribute to a higher intake. For these individuals, the Tolerable Upper Intake Level for selenium established by the SCF in 2000 could be exceeded. Since the above exposure estimates are based on average intakes from the diet, the Tolerable Upper Intake Level for selenium established by the SCF in 2000 could also be exceeded in the case of individuals with high exposure from dietary sources. Data in lactating women supplemented with selenium or exposed to high dietary selenium levels suggest that the mother acts as a buffer, protecting the infant against excess intake of selenium. Even at a maternal selenium intake of 300 µg/day (the SCF-recommended Tolerable Upper Intake Level) milk selenium concentration only rises to 60 µg/l giving an infant intake of 39-47 µg/day, which is below the UL of 60 µg/day for children aged 1-3 years recommended by the SCF (SCF, 2000) and the UL of 45-60 µg/day for infants aged 0 – 12 months recommended by the Food and Nutrition Board (Food and Nutrition Board, 2000).

The Panel notes that the quantity of yeast ingested as a result of the use of supplements containing selenium-enriched yeast will be small (up to 200 mg daily) and that the cellular constituents of the yeast are anticipated to be endogenous in the human body. The Panel also concludes that the quantity of yeast ingested as a result of the use of supplements containing selenium-enriched yeast is unlikely to present an allergenic risk, and that individuals with yeast sensitivity will be alerted to the presence of yeast protein via the labelling of these products.

On the basis of the data provided by the petitioners and information in the literature on the bioavailability, metabolism and toxicity of selenium-enriched yeast and selenomethionine, from dietary sources and in the form of dietary supplements, the Panel concludes that the use of

selenium-enriched yeast, complying with the general product characteristics outlined above, as a source of selenium when used in food supplements or added for nutritional purposes in food does not present a safety concern at the proposed intake levels.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on Selenium-enriched Yeast Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. February 2005. Submitted by Wassen UK- Lallemand FR- Lesaffre FR.
2. Submission for Safety Evaluation of Selenium-enriched Yeast, SelenoPrecise, Pharma Nord Proposed for Use in the Manufacture of Food Supplements (2002/46/EC). January 2005. Submitted by Pharma Nord Vojens, Denmark.
3. Dossier on Bio-transformed Selenium Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. Original submission June 2005. Additional information submitted January 2008. Submitted by Higher Nature Ltd, UK.
4. Dossier for safety evaluation of selenium yeast for use in the manufacture of food supplements. April 2005. Submitted by Béres Pharmaceuticals Co. Ltd.
5. Dossier on yeast enriched with selenium. April 2005. Submitted by Vireco Ltd.
6. Submission for safety evaluation of selenium yeast (SELPLEX 2000). May 2005. Submitted by Procon.

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GLOSSARY / ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
AFC	Scientific Panel on food additives, flavourings, processing aids and materials in contact with food.
bw	body weight
CAS	Chemical Abstract Service
EU	European Union
FEEDAP	Panel on additives and products or substances used in animal feed (FEEDAP)
FNB	US Food and Nutrition Board
GMM	Genetically Modified Microorganism
GMO	Genetically Modified Organism
GPx	Glutathione Peroxidase
Hb	Haemoglobin
HPLC	High Performance Liquid Chromatography
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IDACE	Industries des Aliments Diététiques de l'Union Européenne
LD ₅₀	Lethal Dose, 50% i.e. dose that causes death among 50 % of treated animals
NOAEL	No-Observed-Adverse-Effect-Level
PARNUTS	Foods for Particular Nutritional Uses
PCR	Polymerase Chain Reaction
RAST	radioallergosorbent testing
RDA	Recommended Daily Allowance
SCF	Scientific Committee for Food
SD	Standard Deviation
Se	Selenium
tRNA	transfer RNA
UL	Upper level