European Food Safety Authority

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SCIENTIFIC OPINION

L-selenomethionine as a source of selenium added for nutritional purposes to food supplements [1]

Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food

(Question No EFSA-Q-2005-103, EFSA-Q-2006-195, EFSA-Q-2006-196, EFSA-Q-2006-304)

Adopted on 14 May 2009

PANEL MEMBERS

F. Aguilar, U.R. Charrondiere, B. Dusemund, P. Galtier, J. Gilbert, D.M. Gott, S. Grilli, R. Guertler, G.E.N. Kass, J. Koenig, C. Lambré, J-C. Larsen, J-C. Leblanc, A. Mortensen, D. Parent-Massin, I. Pratt, I.M.C.M. Rietjens, I. Stankovic, P. Tobback, T. Verguieva, R. Woutersen.

SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion on the safety of L-selenomethionine as a source of selenium in food supplements and on the bioavailability of selenium from this source. The safety of selenium itself, in terms of the amounts that may be consumed, is outside the remit of this Panel.

L-selenomethionine is a selenoamino acid in which selenium replaces the sulphur of the methionine molecule. It is a natural component of the diet and has been estimated to account for at least half of all dietary selenium. Like other forms of selenium salts and organoselenium compounds, L-selenomethionine is readily absorbed from the gastrointestinal tract. In a number of studies in humans and animals, in particular those having selenium-

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deficient diets, the bioavailability of selenium from L-selenomethionine has been shown to be approximately 1.5 to 2-fold higher than that of inorganic forms of selenium.

Following absorption, L-selenomethionine is metabolised to other forms of selenium such as hydrogen selenide, which is also the key metabolite derived from the inorganic forms of selenium, selenite or selenate, and/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, selenium from L-selenomethionine is incorporated into a long term body pool. 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 selenium from L-selenomethionine is extensively utilised and re-utilised. Time to steady-state is reached after approximately 4.5 half-lives or about one year for selenite and about three years for selenomethionine.

Selenium compounds are acutely and chronically toxic. The Panel considered that the toxicity of L-selenomethionine is comparable to other forms of selenium, in terms of equivalent amounts of bioavailable selenium. A dose level of 1000 µg L-selenomethionine/kg bw/day (equivalent to 400 µg selenium/kg bw/day) was reported to be a No-Observed-Adverse-Effect-Level (NOAEL) in a 13-week study in rats, while a NOAEL of 300 µg L-selenomethionine/kg bw/day (equivalent to 120 µg selenium/kg bw/day) was derived in a similar study in Beagle dogs. The Panel has not been able to verify the findings in these studies, due to the non-availability of the study reports. In a study in female Macaque monkeys, dose levels of 300-600µg L-selenomethionine/kg bw/day, equivalent to 120-240 µg selenium/kg bw/day produced life-threatening anorexia, gastrointestinal distress and death of some animals, and the highest tolerated dose of L-selenomethionine was estimated to be equivalent to 60 µg selenium/kg bw/day. In a subsequent study in pregnant female Macaques administered 25, 150 or 300 µg selenium/kg bw/day as L-selenomethionine, a dose level of 25 µg selenium/kg bw/day produced no signs of selenium toxicity in the dams, according to the authors.

The Panel noted that L-selenomethionine has effects on the immune system and a possible endocrine disrupting effect. These effects occurred at relatively high dose levels and were considered by the Panel to be manifestations of selenium toxicity, although L-selenomethionine appears to be at least equipotent as inorganic forms of selenium in producing such effects, which is likely to be due in part to its greater bioavailability. Although L-selenomethionine did not produce developmental toxicity in a study in female monkeys, a single oral gavage dose given to pregnant Syrian hamsters at dose levels producing overt maternal toxicity (17 to 20.8 mg/kg bw) induced encephalocoele in the foetuses. Comparable doses of inorganic selenium compounds induced more severe malformations such as exencephalopathies, in addition to encephalocoele. Teratogenicity was seen also at a single dose of 4.5 mg/kg bw L-selenomethionine, a dose level that was not associated with maternal toxicity. This single dose of 4.5 mg/kg bw L-selenomethionine was therefore a Lowest-Observed-Adverse-Effect-Level (LOAEL) for developmental toxicity.

No specific genotoxicity or carcinogenicity studies have been carried out on L-selenomethionine, although a selenium-enriched yeast, containing selenium predominantly in the form of selenomethionine, has been tested with negative results in two *in vitro* genotoxicity tests (bacterial mutagenicity in bacteria and chromosomal aberrations in human lymphocytes) and in an *in vivo* mouse micronucleus test.

Selenosis has been reported in humans and in food animals in seleniferous areas. In humans, selenium intakes in the range of 3200-6990 µg/day (mean 4990 µg/day) are associated with chronic selenosis, with no selenosis being observed in the intake range of 240-1510 µg/day (mean 750 µg/day).

The recent Selenium and Vitamin E Cancer Prevention Trial (SELECT), in which L-selenomethionine was given as a supplement providing 200 µg selenium/day to a large male study population for a period of up to seven years has provided evidence of a slight increase in the incidence of alopecia and dermatitis compared with placebo and also a not-statistically significant increase in diabetes mellitus associated with selenium supplementation. The clinical/toxicological significance of this latter finding is not yet clear. The Panel noted however, that the American and Canadian subjects in the SELECT study had higher baseline plasma selenium levels (mean 135 ng/mL) than comparable European subjects. Clinical studies carried out with selenium-enriched yeast, in which the predominant selenium species is selenomethionine, have shown that selenium intakes up to 343 µg/day are not associated with toxicity, even after prolonged periods of exposure.

The Scientific Committee on Food (SCF) has previously given an opinion on the Tolerable Upper Intake Level (thereafter referred to as Upper Level, UL) for selenium, defining proportionately lower intake levels for children, based on body weight differences compared to adults. On the basis of the findings in humans, the SCF noted that a selenium intake of about 850 µg/day could be taken as a NOAEL for clinical selenosis. Using an uncertainty factor of 3 to allow for the remaining uncertainties of the studies, the SCF derived a UL for selenium of 300 µg/day.

The Panel notes that the SCF UL of 300 µg/day is the current basis for assessing the safety of inorganic selenium compounds, for example selenious acid, and that EFSA's Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) considered that this UL should also apply to organic selenium compounds from selenium-enriched yeasts. The NOAEL in humans on which the SCF based its UL was derived from findings in Chinese subjects and other populations exposed on a lifetime basis to selenium in their diet and drinking water. Since L-selenomethionine accounts for 50 to over 80 % of total selenium in plants such as cereals and soybean grown on selenium-rich soil, such as the areas from which the Chinese data were derived, L-selenomethionine was likely to have been one of the selenium compounds to which the populations in question were exposed. The Panel therefore considers that these human data, and the UL of 300 µg/day for selenium derived using these data, could also provide the primary basis for assessing the safety of L-selenomethionine as a source of supplemental selenium.

The Panel notes however that the findings of the recent SELECT study indicate that, in humans, selenium-related effects might be observed at a supplemental level of 200 µg selenium/day, in individuals also having a high dietary intake of selenium. The Panel considers that, because of these data and given the greater bioavailability of selenium from L-selenomethionine compared to a number of other sources of selenium, the safety of L-selenomethionine can only be ascertained at use levels up to 250 µg/day in food supplements (corresponding to up to 100 µg selenium/day). In the opinion of the Panel, assessment of the safety of L-selenomethionine at intakes corresponding to between 100 µg selenium/day and the current UL requires a detailed evaluation of the known dose-response data together with detailed information on the toxicokinetics of L-selenomethionine and other selenium compounds.

According to the petitioners L-selenomethionine is intended to be used in food supplements to provide an intake of 100-400 µg selenium/day, typically 200 µg /day.

Selenium is a natural component of the diet, and is present in plant foods such as cereals and nuts but also in meat and seafood. According to the SCF, the average intake of selenium by the European population lies in the range 24-89 µg/day. The high percentile of European intakes of 108 µg selenium/day is reported for the Finnish population, where selenium is added to agricultural fertilisers. The Panel noted that these estimates include higher intake figures from selenium-rich areas, or from selenium-rich foods where selenium is coming from addition of selenium to fertilisers or to animal feed.

Based on the information provided by the petitioners, and assuming for adults in Europe a mean dietary selenium intake in the range of 24-89 µg/day and a high anticipated dietary exposure of 108 µg selenium/day, the Panel estimated that consumption of a supplement containing 100 µg selenium (lowest proposed use level) in the form of L-selenomethionine by adults would provide a total anticipated exposure of between 124 and 189 µg selenium/day at the average level of dietary selenium intake and a total anticipated exposure of 208 µg selenium/day at the high percentile dietary selenium intake. These intakes will be below the UL of 300 µg/day for selenium in adults established by the SCF in 2000.

Assuming a mean dietary selenium intake for children (aged between 2 and 17 years) in Europe in the range of 23-42 µg/day, and a high percentile intake range of 32-77 µg/day, the Panel estimated that daily consumption of an additional food supplement containing 100 µg selenium (lowest proposed use level) in the form of L-selenomethionine by children would provide a total anticipated exposure of between 123 and 142 µg selenium /day at the average level of dietary selenium intake and a total anticipated exposure of between 132 and 177 µg selenium /day at the high percentile dietary selenium intake. These intakes will exceed the ULs of 60, 90, and 130 µg selenium/day for children at the ages of 1-3, 4-6 and 7-10 years respectively. The ULs of 200 µg selenium/day for children aged between 11 and 14 years and 250 µg selenium/day for children aged between 15 and 17 years will not be exceeded.

On the basis of the data provided by the petitioners and information in the literature on the bioavailability, metabolism and toxicity of L-selenomethionine, from dietary sources and in the form of dietary supplements, the Panel concludes that the use of L-selenomethionine as a source of selenium for nutritional purposes in food supplements would not be of safety concern in adults at use levels up to 250 µg/day (corresponding to up to 100 µg selenium/day). At this use level the combined intake from diet and supplement use will be below the SCF's UL, even for individuals also having a high dietary intake of selenium (i.e. greater than 100 µg selenium/day from the diet).

In relation to the use of supplements containing L-selenomethionine by children, when dietary intake of selenium by children is also taken into account, the ULs defined by the SCF for children are likely to be exceeded, except in the case of children aged between 11 and 17 years and consuming supplements containing 100 µg/day selenium (lowest proposed use level) or less.

The Panel considers, however, that the current database on selenium compounds and the new data provided by the recent SELECT study indicate the need for an integrated reconsideration of the UL for selenium from all sources, and recommends that this also considers systemic selenium levels rather than external dose in characterising dose-response relationships, in

order to allow a comparison of selenium-containing compounds with different bioavailabilities.

Key words:

L-selenomethionine, selenomethionine, selenium, CAS Registry Number 3211-76-5, 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 requests for the evaluation of L-selenomethionine added for nutritional purposes to food supplements. The relevant Community legislative measure is:

- 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 supplements[2].

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 L-selenomethionine as a source of selenium added for nutritional purposes in food supplements.

ACKNOWLEDGEMENTS

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The ANS Panel would like to thank E. Konings (Seconded National Expert of the DATEX Unit) for his contribution to the preparation of this opinion.

[2] OJ L 183, 12.7.2002, p.51.

ASSESSMENT

1. Introduction

The present opinion deals only with the safety of L-selenomethionine as a source of selenium in food supplements and with the bioavailability of selenium from this source. The safety of selenium (Se) itself, in terms of amounts that may be consumed, is outside the remit of this Panel.

This opinion is based on the information on L-selenomethionine provided by four petitioners.

2. Technical data

2.1. Chemistry

L-selenomethionine, the naturally occurring form of selenomethionine that is also produced by a manufacturing process, is a white to off-white, water-soluble crystalline solid. L-selenomethionine is a molecule in which the sulphur atom of methionine is replaced by a selenium atom.

Chemical Name: (S)-2-Amino-4-(methylselanyl)butanoic acid

Synonyms:

(S)-2-amino-4-(methylselano)butanoic acid

L-2-amino-4-(methylselenyl)butyric acid

L-selenomethionine

L-seleno-methionine

Seleno-L-methionine

Selenomethionine

Selenium methionine

L-SeMet

CAS Registry Number: 3211-76-5

Molecular weight: 196.11 g/mol

Molecular and structural formula: $C_5H_{11}NO_2Se$

Figure 1 shows the structure of (S)-2-amino-4-(methylselanyl)butanoic acid, hereafter referred to as L-selenomethionine.

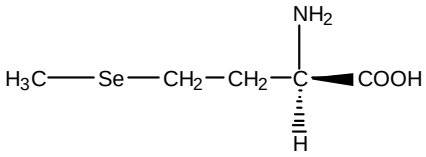

Figure 1. **Structure of L-selenomethionine**

2.2. Specifications

According to information from some of the petitioners, L-selenomethionine is typically 98-99 % pure. Impurities include: D-selenomethionine (typically < 0.5 %), heavy metals < 20 mg/kg, lead < 10 mg/kg, cadmium < 1 mg/kg, mercury < 1 mg/kg, arsenic < 3 mg/kg. The specifications provided by all petitioners were similar. The selenium content is 39-41 % (40.6 % cited by one petitioner).

The Panel notes that according to Commission Regulation (EC) No 629/2008 the maximum levels of lead, mercury and cadmium in food supplements as sold should be 3 mg/kg, 0.1 mg/kg and 1 mg/kg, respectively (EC, 2008).

Some petitioners provided analytical data for L-selenomethionine, which indicated that levels of heavy metals were below those specified in Commission Regulation (EC, 2008).

2.3. Manufacturing process

The manufacturing process has been adequately described by all petitioners, involving L-methionine as starting material and dimethylselenide as selenium source.

2.4. Methods of analysis in food

Comprehensive methods for the analysis of L-selenomethionine have been provided by several petitioners. Quantitative determination of selenium in food supplements is performed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

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

L-selenomethionine is reported by all petitioners to be stable, with a shelf-life of three years in supplements. Degradation products have not been identified and effects of processing are not anticipated although limited information on reaction and fate in foods was provided.

2.6. Case of need and proposed uses

All petitioners state that L-selenomethionine is intended to be used in food supplements, e.g. in capsules, tablets, ampoules or powders at proposed levels of 100-400 µg selenium/day, typically 200 µg selenium/day.

2.7. Existing authorisations and evaluations

L-selenomethionine has been approved by the US Food and Drug Administration (FDA) as a dietary supplement in many formulations and is listed in the United States Pharmacopoeia. The inorganic selenium sources, sodium selenate, sodium hydrogen selenite and sodium selenite have already been included in Annex II of Commission Directive 2002/46/EC (EC, 2002).

The SCF, in 1999, considered L-selenomethionine and selenium-enriched yeasts for use in Foods for Particular Nutritional Purposes (PARNUTS), and restated their earlier concerns (when considering the essential requirements for infant formulae and follow-on formulae; SCF, 1993a, 1994) about the way in which the body handles organic selenium compounds compared with inorganic forms (SCF, 1999).

EFSA's Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) has recently given a scientific opinion on the safety and bioavailability of selenium-enriched yeast as a source for selenium when added for nutritional purposes to foods for particular nutritional uses and foods (including food supplements) for the general population (EFSA, 2008). L-selenomethionine is the predominant selenium-containing compound in selenium-enriched yeast (EFSA, 2008).

A Population Reference Intake (PRI) of 55 µg selenium per day for adults was established by the SCF in 1993 (SCF, 1993b). The SCF has also established a UL for selenium of 300 µg/day (SCF, 2000), while the UK Expert Committee on Vitamins and Minerals (EVM) from the UK derived a Safe Upper Level (SUL) of 450 µg/day for total selenium (EVM, 2003). The US Food and Nutrition Board (FNB) estimated a UL of 400 µg /day (FNB, 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 a UL for children, they noted that there are no reports indicating that children are more susceptible than adults 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 and 90 µg/day for children aged 4-6 years, to 250 µg/day for ages 15-17 years. Specific legislative provisions on nutrient sources apply to foods manufactured for infants and young children.

2.8. Exposure

Selenium is a natural component of the diet, and is present in fish (0.32 mg/kg), offal (0.42 mg/kg), brazil nuts (0.25 mg/kg), eggs (0.16 mg/kg) and cereals (0.02 mg/kg). In foods, selenium is generally present as the amino acid derivates selenomethionine, Se-methylselenocysteine and selenocysteine, with lesser amounts of inorganic forms such as selenite and selenate (EVM, 2003). L-selenomethionine accounts for 50 to over 80 % of total selenium in plants such as cereals and legumes including soybean grown on selenium-rich soil (Rayman *et al.*, 2008, based on the work of several authors). Plants initially absorb

selenium from the soil and convert it to L-selenomethionine and other selenium-containing amino acids such as selenocysteine. L-selenomethionine from plant crops has been estimated to account for at least half of the dietary selenium (FNB, 2000). Selenium-enriched yeast, a dietary supplement source of selenium for humans which is also used as a feed additive for food animals, also contains high levels of L-selenomethionine (EFSA, 2008). The amount of selenium available in the soil for plant growth and corresponding variations in the intake of selenium by humans varies considerably among regions and countries (SCF, 2000).

Table 1 summarises information on selenium intake from food in European countries, anticipated exposures to selenium by using L-selenomethionine supplements as proposed by the petitioner, and ULs.

According to the petitioners, L-selenomethionine is intended to be used in food supplements to provide an intake of 100-400 µg selenium/day, typically 200 µg selenium/day. The latter intake is equivalent to 496 µg L-selenomethionine/day.

Assuming a mean dietary selenium intake for adults in Europe in the range of 24–89 µg/day and a high percentile intake of 108 µg/day, the Panel estimated that consumption of an additional food supplement containing 400 µg selenium/day (highest proposed use level) would result in a total anticipated exposure between 424 and 489 µg selenium/day in an adult at the average level of dietary exposure, and a total anticipated exposure of 508 µg selenium/day at the high percentile dietary selenium intake. Consumption of a supplement containing 100 µg selenium (lowest proposed use level) by adults would provide a total anticipated exposure of between 124 and 189 µg selenium/day at the average level of dietary selenium intake and a total anticipated exposure of 208 µg selenium/day at the high percentile dietary selenium intake, while consumption of a supplement containing 200 µg selenium (typical proposed use level) would provide a total anticipated dietary exposure of between 224 and 289 µg selenium/day at the average level of dietary selenium intake and a total anticipated exposure of 308 µg selenium/day at the high percentile dietary selenium intake.

Assuming a mean dietary selenium intake for children in Europe in the range of 23-42 µg/day and a high percentile intake range of 32-77 µg/day, the Panel estimated that daily consumption of an additional food supplement containing 400 µg selenium (highest proposed use level) would result in a total anticipated exposure between 423 and 442 µg/day at the average level of dietary exposure and a total anticipated exposure between 432 and 477 µg selenium/day at the high percentile dietary selenium intake. Consumption of a supplement containing 100 µg selenium (lowest proposed use level), for children would provide a total anticipated exposure of between 123 and 142 µg selenium /day at the average level of dietary selenium intake and a total anticipated exposure between 132 and 177 µg selenium /day at the high percentile dietary selenium intake, while consumption of a supplement containing 200 µg selenium (typical proposed use level) would provide a total anticipated dietary exposure of between 223 and 242 µg selenium/day at the average level of dietary selenium intake and a total anticipated exposure between 232 and 277 µg selenium/day at the high percentile dietary selenium intake.

Table 1. **Summary information on selenium intake and anticipated exposure to selenium from L-selenomethionine supplements.**

Nutrient: Selenium	**Intake (µg/day)**		**References**
Recommended daily intake for adults Range of recommended daily intakes for all age groups and all population groups (all ages)	55 (8 - 70)		SCF, 1993b
UL for adults	300		SCF, 2000
UL for children (1-3 years/15-17 years old)	60 - 250		SCF, 2000
Nutrient: Selenium	**Average intake (µg/day)**	**High intake (95th or 97.5th, µg/day)**	**References**
Intake range from food in Europe for adults	24 - 89	108	SCF, 2000; Paturi *et al.*, 2008
Intake range from food in Europe for children (2-17 years old)	23 - 42	32 - 77	Lyhne *et al.*, 2005; Ocké *et al.*, 2008; Enghardt Barbieri *et al.*, 2006; AFSSA, 2009
Typical amount (range) of selenium added to supplements by L-selenomethionine as indicated by the petitioners	200 (100-400)		Technical dossiers
Total anticipated exposure to selenium from supplement and food intake[1] for adults - Supplement of 100 µg/day - Supplement of 200 µg/day - Supplement of 400 µg/day	 124-189 224-289 424-489	 208 308 508	Calculation by the Panel
Total anticipated exposure to selenium from supplement and food intake[2] for children (2-17 years old). - Supplement of 100 µg/day - Supplement of 200 µg/day - Supplement of 400 µg/day	 123-142 223-242 423-442	 132-177 232-277 432-477	Calculation by the Panel

[1]calculation based on proposed use levels of 100, 200 or 400 µg/day plus average dietary intake of 24-89 µg/day and high dietary intake of 108 µg/day for adults.

[2]calculation based on proposed use levels of 100, 200 or 400 µg/day plus average dietary intake of 23-42 µg/day and high dietary intake of 32-77 µg/day for children.

The Panel notes that these estimates include higher intakes figures from selenium-rich foods or where selenium is coming from addition of selenium to fertilisers e.g. Finland (SCF, 2000; Rayman, 2004) or to animal feed (EFSA, 2006a,b).

3. Biological and toxicological data

3.1. Bioavailability of selenium from selenomethionine

The Panel noted that the available information does not distinguish between the bioavailability of selenium from different selenium compounds/species in the body. References in this opinion to the bioavailability of selenium from selenomethionine should therefore be regarded as applying to selenium irrespective of form or speciation.

3.1.1. Human studies

Ingested selenomethionine 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). In a number of studies in humans, the bioavailability of selenium from L-selenomethionine has been shown to be higher than that from other, inorganic, selenium compounds and has been estimated to be greater than 90 percent. In a study on four women, designed to examine the fate of an oral dose of [^{75}Se]-selenomethionine, intestinal absorption was 96-97 % of the administered dose (Griffiths *et al*., 1976).

The enzyme glutathione peroxidase (GPx) contains selenium-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. In a selenium-deficient population in China in which five groups of 10 men each were given 0.5 g/day DL-methionine, 150 µg selenium/day as sodium selenite with or without methionine, or 150 µg selenium/day as L-selenomethionine with or without methionine for eight weeks, selenium as selenomethionine was reported to have nearly twice the bioavailability of selenium as sodium selenite (Luo et al., 1987). Plasma levels of selenium reached approximately 60 ng/mL plasma at the end of the 8-week period in the group given sodium selenite, compared with approximately 100 ng/mL in the group given L-selenomethionine, although levels of GPx were comparable. The greater bioavailability of selenium from L-selenomethionine compared with selenite, as evidenced by higher plasma selenium levels, has been confirmed more recently in a further, more extensive, supplementation trial in a selenium-deficient population in China using 120 subjects administered sodium selenite or L-selenomethionine at levels ranging from 13 to 66 µg selenium/day for 20 weeks (Xia *et al.*, 2005).

In a study examining the effects of selenium supplementation on plasma selenium biomarkers and urinary selenium excretion in selenium-replete subjects, moderate (approximately 200 µg selenium/day) to high levels (approximately 600 µg selenium/day) of selenium supplements in the form of sodium selenite, high-selenium-enriched yeast or L-selenomethionine were administered (Burk *et al*., 2006). Plasma biomarkers (selenium concentration, selenoprotein P concentration, and GPx activity) were determined before supplementation and then every four weeks for 16 weeks, and urinary selenium excretion was determined at 16 weeks. Supplementation with L-selenomethionine and high-selenium-enriched yeast raised the plasma selenium concentration in a dose-dependent manner, while sodium selenite did not. Urinary selenium excretion was greater after L-selenomethionine administration than after selenite administration. The authors concluded that selenium in the form of L-selenomethionine was better absorbed than selenium in the form of sodium selenite (Burk *et al*., 2006).

In a study designed to examine differences in the effects on indicators of selenium status of different chemical forms proposed for selenium supplementation in healthy subjects, organic selenium forms (selenium-enriched yeast, selenomethionine and food selenium) were found to increase blood selenium levels more rapidly and to a greater extent than inorganic forms (selenite and selenate) (Nève, 1995). However, no significant differences in the response of both plasma and erythrocyte GPx activity to supplementation with the different sources of selenium could be observed.

In a study with a small number of female subjects (n=3), involving supplementation for 11 weeks at 100 μg selenium/day, L-selenomethionine caused an increase in plasma selenium from 0.08 to 0.18 mg/mL (Robinson *et al.*, 1978). The equivalent dose of sodium selenite increased blood selenium more slowly until a plateau was reached after 7–8 weeks at 0.11 mg/ml (Robinson *et al.*, 1978). In a further study by the same group, using prolonged selenium supplementation in 12 female subjects with sodium selenite or L-selenomethionine (100 μg selenium/day), increases in selenium concentrations in whole blood, erythrocytes, and plasma were greater after L-selenomethionine administration than after selenite administration. Selenium concentrations tended to plateau after selenite administration while after L-selenomethionine administration they continued to rise as long as dosing continued (Thomson *et al*., 1982).

3.1.2. Animal studies

The majority of studies on the bioavailability of selenium from selenomethionine in rats have been carried out using selenium-enriched yeast rather than L-selenomethionine. A study on the effects of various dietary levels of selenium as selenite or selenomethionine on tissue selenium levels and GPx activity in rats indicated that selenium from L-selenomethionine is more bioavailable than selenium from inorganic sources of selenium such as selenite, as found in humans (Whanger and Butler, 1988). In a study in weanling Sprague-Dawley rats fed a basal selenium-deficient diet containing 2 mg/kg selenium as D,L-selenomethionine, selenite or selenocystine, selenium content in testis, muscle, pancreas, heart, spleen, whole blood, erythrocytes and plasma was significantly higher in rats fed D,L-selenomethionine than in those fed either selenite or selenocystine. The greatest increase due to D,L-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).

In a study comparing the toxicity of nanoparticulate elemental selenium (Nano-Se) with selenomethionine (enantiomer not specified) in Kunming mice (Wang *et al.*, 2007), both forms of selenium possessed equal efficacy in increasing the activities of GPx and thioredoxin reductase. The bioavailability of selenium from selenomethionine was much higher than that of the Nano-Se, as evidenced by selenium blood and tissue levels.

A large number of studies in farm animals, examining dietary sources of selenium and selenium-enriched diets, have again principally focussed on selenium-enriched yeast. These studies have shown that selenium from selenomethionine and selenocysteine is more bioavailable (approximately two-fold) than selenium from inorganic selenium compounds (EFSA, 2006a,b). The findings of these studies, and also those examining comparative uptake of selenium from inorganic and organic selenium and from foodstuffs known to be rich in selenomethionine, support the findings on bioavailability in humans, that selenium salts and organoselenium compounds are all readily absorbed from the gastrointestinal tract but that

selenium from selenomethionine and selenocysteine is more bioavailable than from inorganic sources of the element (EFSA, 2006a, 2006b, 2008).

3.2. Metabolic fate and biological distribution

L-selenomethionine is a selenoamino acid in which selenium replaces the sulphur of the methionine molecule. Data from human and animal studies show that the metabolism of L-selenomethionine shares common pathways with other organic and inorganic forms of selenium, as shown in Figure 2 (adapted from Rayman, 2004 and Combs, 2001). Following absorption, L-selenomethionine is ultimately metabolised (via selenocysteine or methylselenol) to hydrogen selenide, which is also the key metabolite derived from the inorganic forms of selenium, selenite or selenate. In addition to conversion to selenophosphate ($HSePO_3$) followed by incorporation into essential selenoproteins (Berry *et al.,* 1991, 1993), hydrogen selenide can be (i) methylated and excreted, or converted into selenosugars and excreted, or (ii) oxidised to selenium dioxide, a pathway associated with toxicity, due to the production of superoxide and other reactive oxygen species (Rayman *et al*., 2008). L-selenomethionine may also be incorporated non-specifically into normal body proteins in place of methionine where it is stored (non-functionally) but may later be released by catabolism, at which time it can be converted to hydrogen selenide and thence to other products as shown in Figure 2.

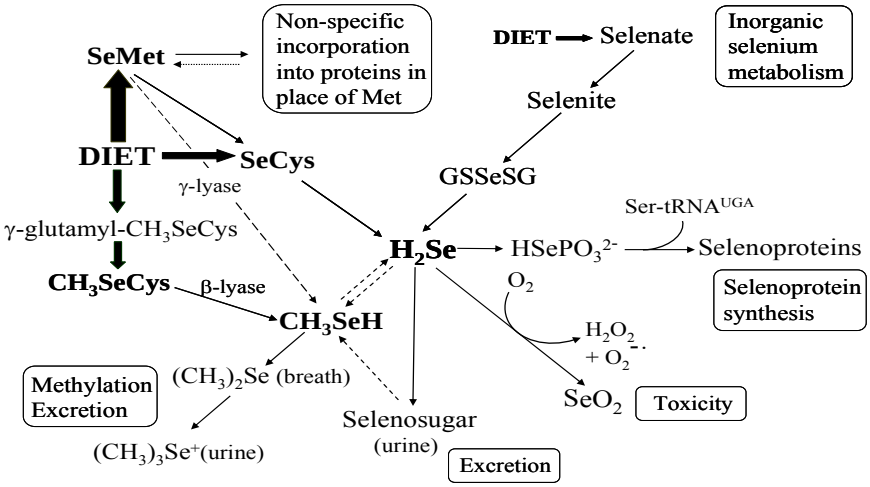

Figure 2. **Metabolic fate of selenomethionine and other dietary selenium compounds (adapted from Rayman (2004) and Combs (2001)).**

Following a single oral dose of ^{74}Se labelled selenomethionine, given to six human volunteers, after initial uptake from the intestine 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 were 1.6 to 3.1 days, while in peripheral tissues showing the slowest turnover they ranged from 61 to 86 days. When compared with selenite, the whole-body turnover of selenomethionine was slower which was attributed to reutilisation of selenomethionine, indicating that selenomethionine is incorporated into a long-term body pool.

In a study with two women the intestinal absorption of an oral dose of 1000 or 100 µg L-selenomethionine was compared with the equivalent dose of sodium selenite. Twenty-four hours after L-selenomethionine dosing, 5-22 % was eliminated in the urine and 3.5 % in the faeces. During the same period, 41-85 % of the equivalent dose of selenite was excreted in the urine and 11-13 % with the faeces (Thomson *et al*., 1978). A lower proportion of the total dose was excreted in the case of the lower dose of 100 µg of either selenite or L-selenomethionine, compared with the higher dose of 1000 µg (a mean 66 % of a dose of 1000 µg selenite and a mean 38% of a dose of 100 µg selenite, compared with 18 % of a dose of 1000 µg L-selenomethionine (one subject only) and a mean 8 % of a dose of 100 µg L-selenomethionine).

The total body pool of selenium has been estimated to be 5-15 mg in adults (SCF, 2000). Most of the selenium (50 %) is present in skeletal muscle, which thus appears to act as a selenium storage organ (Oster *et al*., 1988). Kinetic studies indicate that blood plasma contains at least four components with half-lives between 1 and 250 hours (SCF, 2000). The whole body half-life of L-selenomethionine (252 days) is longer than that of inorganic selenite (102 days), indicating that once absorbed, selenium from L-selenomethionine is incorporated into a long term body pool. Time to steady-state is reached after approximately 4.5 half-lives or about one year for selenium from selenite and about three years for selenium from selenomethionine (NCI, 1993). 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). L-selenomethionine is incorporated into tissue proteins in place of L-methionine, especially in the skeletal muscles, the liver and the testes (Schrauzer, 2003).

The AFC Panel noted that there was a discrepancy between the reported whole body half-lives of L-selenomethionine and selenite and the reported time taken to reach steady-state in blood (EFSA, 2008). 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 AFC Panel considered 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 (EFSA, 2008). The Panel concurs with the views of the AFC Panel.

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 showed an increase in muscle selenium concentration with increasing dose of supplemental selenium in the range of 62.5 to 262.5 µg selenium/day (EFSA, 2008). The relative increase in the muscle selenium was lower than the relative increase in selenium dose, and the results indicated that muscle selenium levels did not depend on duration of supplementation (1-24 years). These results indicated that supplementation with selenium does not lead to the continuous storage of the element but rather to a situation of steady-state with the excretion of surplus selenium (EFSA, 2008).

3.3. Toxicological data

3.3.1. Acute toxicity

The median lethal dose (LD_{50}) of L-selenomethionine in rats given an intraperitoneal injection was determined to be 4.25 mg selenium/kg bw, comparable to that of selenite or selenate (Schrauzer, 2000).

3.3.2. Subacute, subchronic and chronic toxicity in animals

A review of the nutritional significance, metabolism and toxicology of L-selenomethionine, reports that oral administration at 0.5 and 1 mg/kg bw/day (equivalent to 200 or 400 µg selenium/kg bw/day respectively) to groups of 10 male and 10 female rats per group in a 13-week study did not produce toxicity (Schrauzer, 2003). The Panel noted that the NCI (1993) reference cited by Schrauzer (2003) relates to the Proceedings of a Workshop on Selenium Compounds in Cancer ChemoPrevention Trials organized by the US National Institutes of Health, and that the openly available report of the meeting does not include any mention of this study, nor is it available from the National Cancer Institute (NCI) website. The Panel has not been able to verify the findings of the NCI Study described by Schrauzer (2003). As described by Schrauzer, in this study a dose level of 2, 3 or 4.5 mg/kg bw/day L-selenomethionine (equivalent to 800, 1200 or 1800 µg selenium/kg bw/day) was reported to result in weight loss, decreased food consumption and severe liver damage in rats. Females were reported to be more sensitive than males; all females at 1200 or 1800 µg selenium/kg bw/day were dead at the end of the study while only one male receiving 1200 µg selenium/kg bw/day died (Schrauzer, 2003). The Panel thus concluded that a dose level of 400 µg selenium/kg bw/day appeared to be a NOAEL in this 90-day study in the rat, based on the description of the author, although it was unable to examine a report of the study to confirm this.

Schrauzer (2003) also reports that Beagle dogs orally administered 0.1 and 0.3 mg L-selenomethionine/kg bw/day (equivalent to 40 or 120 µg selenium/kg bw/day) showed no evidence of toxicity. Administration of 1 mg L-selenomethionine/kg bw/day (equivalent to 400 µg selenium/kg bw/day) was reported to result in elevated activities of aspartate aminotransferase (AST), glutamate pyruvate aminotransferase (GPT) and alanine aminotransferase (ALT), in addition to inflammation, telangiectasis, vacuolar changes, brown pigmentation in hepatocytes, thymic atrophy, lymphocyte depletion in tonsils and intestine and gastrointestinal haemorrhage (Schrauzer, 2003). Again, the reference provided in the Schrauzer review relates to the Proceedings of a Workshop on Selenium Compounds in Cancer ChemoPrevention Trials organized by the US National Institutes of Health (NCI, 1993). The Panel concluded that a dose level of 120 µg selenium/kg bw/day appeared to be a NOAEL in this 90-day study in the dog, based on the description of the authors, although it was unable to confirm this since it was not possible to examine a report of the study.

Groups of five adult female macaques (*Macaca fascicularis*) received 0, 150, 300 or 600 µg L-selenomethionine/kg bw/day (equivalent to 0, 60, 120 or 240 µg selenium/kg bw/day) by nasogastric intubation for up to 30 days (Cukierski *et al.*, 1989). Due to signs of acute toxicity developing at 600 µg L-selenomethionine/kg bw/day in the 2nd week of the study, resulting in the death of two animals, the dose administered to the animals in the 300 or 600 µg L-selenomethionine/kg bw/day groups was reduced to give final time-weighted average doses

over the study of 0, 25, 62-117, 150, 188-203 or 300-600 µg L-selenomethionine/kg bw/day, equivalent to 0, 10, 25-47, 60, 75-81 or 120-240 µg selenium/kg bw/day.

In addition to morbidity and lethality seen in two of the five animals receiving 600 µg L-selenomethionine/kg bw/day and severe toxicity in one animal receiving 300 µg L-selenomethionine/kg bw/day, necessitating removal from treatment on day 19 of the study, treated animals showed dose-related anorexia, gastrointestinal disturbances, dermatological changes, reduced body temperature and body weight loss. Histopathology of animals dying or killed during the study showed evidence of liver and kidney damage. At dose levels above 150 µg L-selenomethionine/kg bw/day, equivalent to 60 µg selenium/kg bw/day, menstrual function was impaired, associated with decreases in serum progesterone and urinary estrogen excretion and increased intermenstrual intervals. The authors suggested that this was indicative of an effect on the function of the corpus luteum. No consistent treatment-related changes in clinical chemistry or urinalysis were seen during the study. Evidence of cutaneous lesions ranging from mild xerosis and desquamation to severe dermatitis and hyperkeratosis was seen at all dose levels down to 25 µg L-selenomethionine/kg bw/day.

The authors concluded that 150 µg L-selenomethionine/kg bw/day (equivalent to 60 µg selenium/kg bw/day) was a Maximum Tolerated Dose (MTD) in this study, meaning that animals given up to this dose level did not show marked signs of selenium-induced toxicity and survived for the duration of the study, although dose-dependent changes in body weight and dermatological changes were evident (Cukierski *et al.*, 1989). In contrast, animals receiving 300-600 µg L-selenomethionine/kg bw/day, equivalent to 120-240 µg selenium/kg bw/day showed life-threatening anorexia, gastrointestinal distress and death of some animals. The authors also considered that a No-Observed-Effect-Level (NOEL) was not established in the study, given the dermatological changes observed in animals receiving a time-weighted average dose of 25 µg L-selenomethionine/kg bw/day over the period of the study (equivalent to 10 µg selenium/kg bw/day) (Cukierski *et al.*, 1989).

Groups of 10 pregnant female macaques (*Macaca fascicularis*) received 0, 25, 150 or 300 µg selenium/kg bw/day as L-selenomethionine by nasogastric intubation for up to 30 days (Hawkes *et al.*, 1992). All treated groups showed a decrease in mean body weight, although the decrease was significantly different from controls only in the group receiving 300 µg selenium/kg bw/day. The mean percentage body weight loss in this group was 3-times greater than in the group receiving 150 µg selenium/kg bw/day. Other signs of toxicity in the animals receiving 300 µg selenium/kg bw/day were anorexia and vomiting. Selenium status was assessed by measurement of erythrocyte, plasma selenium and hair selenium, and levels of > 2.3 mg/L, > 2.8 mg/L and > 27 µg/g, respectively were associated with increased weight loss due to selenium toxicity (Hawkes *et al.*, 1992).

A number of toxicological studies have compared the toxicity of L-selenomethionine with other forms of selenium, principally the inorganic forms selenite and selenate. Overall these studies indicate that L-selenomethionine has a comparable toxicity with the other forms of selenium. Groups of male weanling rats (n=8) were fed diets containing 2.5, 5 or 10 mg selenium/kg diet 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 selenium/kg (1000 µg selenium/kg bw/day), irrespective of the form in which the selenium was administered. At the lower dietary level of 5 mg selenium/kg diet (500 µg selenium/kg bw/day), L- and D-selenomethionine were less toxic than selenite, as evidenced by fewer mortalities (5/10 deaths

in animals receiving selenite, 3/10 deaths in animals receiving L-selenomethionine, 1/10 deaths in animals receiving D-selenomethionine and 1/10 deaths in animals receiving selenate) and a less marked effect on body weight gain. A level of 2.5 mg selenium/kg diet (250 µg selenium/kg bw/day), over a 6-week period produced no evidence of depressed growth or diminished survival, other than for two animals receiving selenate.

At all dose levels, selenium retention in tissues (plasma, red blood cells, liver, heart, skeletal muscle) was greater for rats fed D- or L-selenomethionine compared with selenite or selenate. The authors concluded on the basis of this study and results of earlier studies in their laboratory on the acute toxicity or nutritional bioavailability of D-selenomethionine and L-selenomethionine in the rat that there was little difference between the effect of the two enantiomers in the rat (McAdam and Levander, 1987).

In contrast, in a study in which female weanling rats were administered diets containing 10 or 16 mg selenium/kg diet as L-selenomethionine or D-selenomethionine (1000 or 1600 µg selenium/kg bw/day) or 16 mg selenium/kg diet as sodium selenite (1600 µg selenium/kg bw/day) for eight weeks, the toxicity of L-selenomethionine was similar to that of selenite and appeared to be greater than that of D-selenomethionine (Hermann *et al*., 1991).

Mu and co-workers showed that the toxicity of selenite to weanling Wistar rats was greater than that of selenomethionine (enantiomer not specified) when fed at dietary levels of 0, 3, 6, 10 mg selenium/kg diet for 12 weeks, and that female rats were more sensitive than males (Mu *et al.*, 2004). Body weight loss was recorded in animals receiving 6 mg selenium/kg diet, and histopathological evidence of liver damage was evident at all dose levels, although it was less marked in the selenomethionine-treated rats. The authors concluded that 3 mg selenium/kg diet (equivalent to 300 µg selenium/kg bw/day) may represent a level at which selenium toxicity becomes apparent in the rat.

Methionine-deficient or methionine-replete rats were fed a *Torula* yeast-based diet containing 0.1, 0.5, or 2.5 mg selenium/kg diet (10, 50 or 250 µg selenium/kg bw/day diet) as L-selenomethionine or sodium selenate. Methionine-deficient rats gained significantly less weight than methionine-replete rats, despite being fed the diet for one week longer. Toxicity was most marked in methionine-deficient rats fed 2.5 mg selenium/kg as sodium selenate whilst selenium retention in tissues was greater for those fed L-selenomethionine and more so for methionine-deficient rats (Salbe and Levander, 1989).

The Panel noted the results of a recent study comparing the toxicity of nanoparticulate elemental selenium (Nano-Se) with selenomethionine (enantiomer not specified) in Kunming mice (Wang *et al.*, 2007), in which Nano-Se had much lower toxicity as indicated by median lethal dose, acute liver injury, and short-term toxicity, although the bioavailability of selenium from selenomethionine was much higher than that of the Nano-Se, as evidenced by selenium blood and tissue levels. The Panel considered that since the chemical structures of the compounds are different, selenomethionine being an organic selenium compound while the nanoparticulate selenium is an inorganic compound, this study did not provide useful information regarding the relative toxicity of the two forms.

The Panel also noted that the toxicological findings in repeat dose studies with L-selenomethionine in laboratory animals as described above have also been described in studies in larger animal species (e.g. pigs) (Herigstad *et al*., 1973).

3.3.3. Reproductive and developmental toxicity

Selenium compounds have been reported to have effects on reproduction and offspring in rodents, usually associated with maternal toxicity (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). Selenomethionine has been reported to have a greater teratogenic potential and to be generally more embryotoxic than sodium selenite in Mallard ducks, possibly due to a higher uptake of selenomethionine (Hoffman and Heinz, 1988).

A single day oral gavage treatment of pregnant Syrian hamsters with L-selenomethionine induced encephalocele at all doses without a clear indication for a dose-effect relationship (2/59(3 %), 13/52(25 %), 4/56(7 %) foetuses examined, respectively in 2/5, 4/59($p<0.05$) and 3/5 litters from the groups treated with 14.7, 17.3 or 19.6 mg/kg bw; Ferm *et al.,* 1990). No malformations were seen in the foetuses of mothers from the untreated control and gavaged control, respectively (0/74 and 0/63 examined foetuses). The observation of maternal toxicity, as evidenced by marked body weight loss, general nutritional deprivation and lack of food in the gastrointestinal tract seen at necropsy, led the authors to suggest that the teratogenic effect was associated with maternal toxicity (Ferm *et al.,* 1990). However a few foetuses with encephalocoele were also seen at the lowest dose of L-selenomethionine tested, of 4.5 mg/kg bw, which was without maternal toxicity.

A significant reduction in maternal body weight was also observed when a total dosage of either 14.7 or 19.6 mg/kg bw was divided over gestation days (GD) 5, 6, 7 and 8; however no fetal malformations were seen and no significant effect on either the average fetal body weight or crown-rump length was found. These results concur with the knowledge that a single high dose of a teratogen given on the day of major CNS organogenesis (as GD 8 is for the Syrian hamster) can induce a serious brain abnormality like encephalocoele. On the other hand, resorptions are most likely to occur when treatment takes place during the early embryonic development.

The parallel experiments described in the same paper, also in pregnant Syrian hamsters, with single oral gavage of the two inorganic selenium compounds sodium selenite and sodium selenate demonstrated teratogenicity at all the administered dose levels, with a dose-related increase both for the frequency and severity of effect.

In a study in pregnant female Macaques (*Macaca fascicularis*) administered L-selenomethionine (at a dose level providing 25, 150 and 300 µg selenium/kg bw/day) via nasogastric intubation 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; Hawkes *et al*., 1994). The dose of 25 µg selenium/kg bw/day administered as L-selenomethionine was considered by the authors to produce no signs of selenium toxicity in the dams (a NOEL for maternal toxicity). Despite elevated selenium concentrations in fetal tissues, neonatal blood and milk, no deleterious effects on neonates were observed. The authors suggested, based on these results, that primate foetuses are well protected against selenium toxicity arising from high maternal L-selenomethionine intakes (Hawkes *et al*., 1994).

3.3.4. Genotoxicity

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 the production of reactive oxygen radicals (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).

In contrast, a selenium-enriched yeast strain, containing 2000 mg/kg 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) in accordance with OECD TG 471 (OECD, 1997a); ii) chromosomal aberrations in human lymphocytes *in vitro* in accordance with OECD TG 473 (OECD, 1997b); iii) *in vivo* mouse micronucleus test in bone marrow in accordance with OECD TG 474 (OECD, 1997c) (Griffiths *et al.,* 2006).

3.3.5. Carcinogenicity

No specific carcinogenicity studies have been carried out on L-selenomethionine.

3.3.6. Effects on the immune system

Both high selenium exposure and selenium deficiency are known to produce impairment of the immune system (Kiremidjian-Schumacher and Stotzky, 1987). In a study in C57BL/6N female mice fed with low (20 µg/kg/diet), sufficient (200 µg/kg/diet), or excess L-selenomethionine (2000 µg/kg/diet) in the diet for 50 days, excess L-selenomethionine intake increased splenocyte proliferation and reduced B cell numbers in comparison to controls, without affecting oxidative stress markers (Vega *et al.*, 2007). IL-4 and IL-12 secretion from mitogen-stimulated splenic cell cultures from L-selenomethionine-treated mice was also reduced compared with controls. These data suggest that excess L-selenomethionine in the diet may have an adverse effect on immune function.

3.3.7. Human studies

Selenium is chronically toxic and selenosis has been reported in humans 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. The available literature suggests that 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, 1989a,b). Signs of selenosis are hair loss, brittle, thickened and stratified nails, garlic breath and skin lesions (Whanger *et al.,* 1996). Investigations on the health effects of high dietary intakes of selenium in populations living in the seleniferous areas of South Dakota (Longnecker *et al.,* 1991, 1993), Venezuela (Brätter *et al.*, 1991) and China (Yang *et al.*, 1983, 1989a,b) have indicated that the highest long-term daily dose of selenium that can be taken without the development of toxicity in most

individuals is approximately 800 µg, while prolonged intakes (e.g. lifetime exposure) of daily selenium doses of 1 mg or greater may cause adverse reactions.

The SCF report of 2000 indicates little evidence of toxicity in humans taking selenium-containing supplements at levels up to 400 µg daily over a prolonged period (from three months up to 10 years) (SCF, 2000).

More recently, a major clinical study, the Selenium and Vitamin E Cancer Prevention Trial (SELECT), has assessed L-selenomethionine and vitamin E for their efficacy in the prevention of prostate cancer (Lippman *et al*., 2009). The study was a phase 3 randomised double-blind placebo-controlled trial of either selenium (200 µg/day from L-selenomethionine) or vitamin E (400 IU/day of all *rac*-α-tocopheryl acetate), or both together, compared to placebo. A total of 35533 men were recruited and randomly assigned to one of the four groups over a period between August 2001, and June 2004. At the start of the study, each study population contained approximately 8700 American, Canadian and Puerto Rican men, predominantly in the age range of 55-75 years old. The subjects enrolled in SELECT had higher initial plasma levels of selenium (mean 135 ng/mL) than participants in an earlier clinical trial, the Nutritional Prevention of Cancer (NPC) trial, in which mean initial plasma levels of selenium were 113 ng/mL (Hatfield and Gladyshev, 2009). In the NPC trial, 200 µg selenium/day from selenium-enriched yeast was reported to reduce the incidence of human prostate, lung and colon cancers (Clark *et al.*, 1996).

The SELECT study commenced in July 2001, with an intended duration of five years for each surviving subject, a planned follow-up period for each subject of a minimum of seven years and a maximum of 12 years. The study was however ended prematurely in October, 2008 on the basis that the alternative hypothesis of no evidence of benefit from either study agent was convincingly demonstrated ($P<0.0001$) (Lippman *et al.,* 2009). L-Selenomethionine or vitamin E, alone or in combination, did not prevent prostate cancer in this population.

The trial showed a non-significant increase in diabetes mellitus associated with selenium alone supplementation, Relative Risk (RR): 1.07 (99 % CI 0.94 – 1.22) compared with placebo, which was not seen in the vitamin E + selenium test group (Lippman *et al.*, 2009). A potential association of selenium with an increased risk for type 2 diabetes has previously been reported, although prior studies provide mixed data (Bleys *et al.*, 2007; Rajpathak *et al.*, 2005; Stranges *et al.,* 2007). The study also showed a non-significant increase in prostate cancer in the vitamin E-alone group. Known, clinically less significant adverse effects of the study agents (alopecia, dermatitis, halitosis, nail changes, fatigue, and nausea) were monitored during the study. The only statistically significant differences ($P<0.01$) were for selenium *vs* placebo for alopecia (RR:1.28; 99 % CI 1.01 – 1.62) and grades 1-2 dermatitis (RR:1.17; 99 % CI 1.00 – 1.35). Small increases in RR were also evident in the vitamin E alone and selenium + vitamin E groups for both alopecia (1.06 and 1.15 respectively) and grades 1-2 dermatitis (1.14 and 1.07 respectively), but these were not statistically significant. Incidence of grades 3-4 dermatitis was also increased in all three test groups compared with placebo (RR 1.49 for vitamin E alone, 1.74 for selenium alone and 2.0 for selenium + vitamin E), but these increases were not statistically significant due to the small numbers of affected subjects in each group showing these changes. A trend towards increased halitosis in all three groups compared with placebo was also not statistically significant. Nail changes, fatigue and nausea showed no convincing evidence of a treatment-related trend.

The Panel noted that the American and Canadian subjects in this study had higher baseline plasma selenium levels than comparable European subjects. This reflects the fact that the American continent has high levels of selenium in soil and rock, and hence the American population in general has higher dietary intakes of selenium than those in Europe. Serum or plasma selenium levels in European populations without selenium supplementation are in the range of 50-90 ng/mL (Rayman, 2002). In the UK, measurements on approximately 1 000 subjects, from the National Diet and Nutrition Surveys of the elderly and of adults and children, showed mean plasma selenium levels of 71 ng/mL (in 1994-5) and 68 ng/mL (in 1997) respectively (Gregory *et al*., 2000; Bates *et al*., 2002). These levels may be compared with the mean initial plasma levels of selenium (135 ng/mL) of participants in SELECT, and in the US NPC trial, in which mean initial plasma levels of selenium were 113 ng/mL.

In a supplementation study in 24 healthy women to which 400 µg selenium was administered daily in the form of selenomethionine for 15 weeks in a placebo controlled study, no effect was observed on serum somatotropin and IGF-1 concentrations, nor was there any effect on Insulin-like Growth Factor (IGF)-binding proteins 1 and 3 (Meltzer and Haug, 1995). The study had been undertaken on the basis that the administration of large doses of selenium to rats had been reported to lead to reduced serum levels of somatotropin (growth hormone) and IGF-1, followed by growth retardation (Grønbaek *et al.*, 1995). No other adverse effects were reported. In the treatment group, serum selenium concentrations increased by more than 100 %. Recent clinical trials carried out in Denmark and the UK with selenium-enriched yeast, in which the predominant selenium-containing species is selenomethionine, have indicated that intakes of up to 343 µg selenium/day are not associated with toxicity, even after prolonged periods (years) of exposure (EFSA, 2008).

4. Discussion

L-selenomethionine is a selenoamino acid in which selenium replaces the sulphur of the methionine molecule. In assessing the safety of L-selenomethionine, the Panel considers that exposure to selenium in the form of selenium compounds such as hydrogen selenide, methyl selenol and selenium dioxide, all of which are also key metabolites of the inorganic forms selenite and selenate, must be taken into account.

Like other forms of selenium salts and organoselenium compounds, L-selenomethionine is readily absorbed from the gastrointestinal tract. In a number of studies in humans, in particular individuals having selenium-deficient diets, the bioavailability of selenium from L-selenomethionine has been shown to be higher than that from inorganic forms of selenium. Selenium from L-selenomethionine has been reported to have nearly twice the bioavailability of selenium from sodium selenite, and has been estimated to be greater than 90 % bioavailable. Supplementation studies with L-selenomethionine, either short-term or long-term, have demonstrated increases in plasma biomarkers of selenium (selenium concentration, selenoprotein P concentration, and GPx activity).

A number of toxicological studies have compared the toxicity of L-selenomethionine with other forms of selenium, principally the inorganic forms selenite and selenate. Overall these studies indicate that L-selenomethionine has a comparable toxicity with the other forms of selenium. The Panel has noted the concerns expressed by the SCF in 1999, when the Committee initially considered L-selenomethionine and selenium-enriched yeasts for use in foods, namely that there was uncertainty about the way in which the body handles organic

selenium compared with inorganic forms. The view of the AFC Panel in its 2008 opinion on selenium-enriched yeasts was that these concerns have been addressed via additional data provided to EFSA at that time. The ANS Panel agrees with the view of the AFC Panel on this issue.

A dose level of 1000 µg L-selenomethionine/kg bw/day (equivalent to 400 µg selenium/kg bw/day) was reported to be a NOAEL in a 13-week study in rats, while a NOAEL of 300 µg L-selenomethionine/kg bw/day (equivalent to 120 µg selenium/kg bw/day) was derived in a similar study in Beagle dogs. The Panel has not been able to verify the findings in these studies, due to the non-availability of the study reports. In the 30-day study of Cukierski *et al.* (1989) in female Macaques, dose levels of 300-600µg L-selenomethionine/kg bw/day, equivalent to 120-240 µg selenium/kg bw/day produced life-threatening anorexia, gastrointestinal distress and death of some animals, and the highest tolerated dose of selenomethionine was estimated to be equivalent to 60 µg selenium/kg bw/day. At dose levels above 60 µg selenium/kg bw/day, menstrual function was impaired, associated with decreases in serum progesterone and urinary estrogen. A NOEL was not established in this study, given the dermatological changes observed in animals receiving a time-weighted average dose of 25 µg L-selenomethionine/kg bw/day over the study (equivalent to 10 µg selenium/kg bw/day). In a subsequent study of Hawkes *et al.* (1994) in pregnant female Macaques administered 25, 150 or 300 µg selenium/kg bw/day as L-selenomethionine, a dose level of 25 µg selenium/kg bw/day produced no signs of selenium toxicity in the dams, according to the authors.

Selenium compounds have been reported to have effects on reproduction and offspring in a number of animal species in the presence of maternal toxicity and nutritional deprivation. L-selenomethionine has been reported to have a greater teratogenic potential and to be generally more embryotoxic than sodium selenite in avian species. Although L-selenomethionine did not produce developmental toxicity in a study in female Macaques at levels of up to 300 µg selenium/kg bw/day, effects on sex hormones in Macaques may be indicative of a possible endocrine disrupting effect. These effects were considered by the Panel to be manifestations of selenium toxicity, although the Panel considered that L-selenomethionine appears to be at least equipotent as inorganic forms of selenium in producing such effects, which is likely to be due in part to its greater bioavailability.

The Panel noted that L-selenomethionine given as a single oral gavage dose to pregnant Syrian hamsters at dose levels producing overt maternal toxicity (17.3, 19.6 or 21.6 mg/kg bw) induced encephalocoele in the foetuses. Comparable doses of inorganic selenium compounds induced in addition to encephalocoele more severe malformations such as exencephalopathy. Teratogenicity was seen also at a single dose of 4.5 mg/kg bw L-selenomethionine, a dose level that was not associated with maternal toxicity. This single dose of 4.5 mg/kg bw L-selenomethionine, was therefore a LOAEL for developmental toxicity.

No specific genotoxicity or carcinogenicity studies have been carried out on L-selenomethionine, however an extract of a selenium-enriched yeast strain, containing 2000 mg/kg of selenium, predominantly (more than 98 %) in the form of selenomethionine, has been tested with negative results in two *in vitro* genotoxicity tests (bacterial mutagenicity in bacteria and chromosomal aberrations in human lymphocytes) and in an *in vivo* mouse micronucleus test.

Data obtained from studies in several species indicate that exposure to L-selenomethionine may have an adverse effect on immune function.

Overall the Panel considers that the toxicity of L-selenomethionine is comparable to other forms of selenium, in terms of equivalent amounts of bioavailable selenium. As with other selenium compounds, the results of toxicological studies with L-selenomethionine in animals are indicative of a steep dose-response curve, with a threshold for onset of toxicity in the range of 100–400 µg selenium/kg bw/day, dependent on the species.

In humans, selenium intakes in the range of 3200-6990 µg/day (mean 4990 µg/day) are associated with chronic selenosis, with no selenosis being observed in the intake range of 240-1510 µg/day (mean 750 µg/day). The recent SELECT study, in which L-selenomethionine was given as a supplement providing 200 µg selenium/day to a large male study population for a period of up to seven years has provided no evidence of efficacy in preventing prostate cancer. It did however show a slight, but statistically significant, increase in the incidence of alopecia and dermatitis compared with placebo, and a not-statistically significant increase in diabetes mellitus associated with selenium supplementation. The clinical/toxicological significance of this latter finding is not yet clear. The Panel noted however that a potential association of selenium with an increased risk for type 2 diabetes has previously been reported, although prior studies provide mixed data.

Also, the Panel noted that the American and Canadian subjects in the SELECT study had higher baseline plasma selenium levels (mean 135 ng/mL) than comparable European subjects. Serum or plasma selenium levels in European populations without selenium supplementation are in the range of 50-90 ng/mL. Recent clinical studies carried out in Denmark and the UK with selenium-enriched yeast, in which the predominant selenium species is selenomethionine, show that selenium intakes up to 343 µg/day in these studies were not associated with toxicity, even after prolonged periods of exposure.

On the basis of the findings of Yang *et al.* (1983, 1989a,b) in Chinese subjects living in seleniferous areas, the SCF noted that a selenium intake of about 850 µg/day "could be taken as a NOAEL for clinical selenosis." The various expert bodies including the SCF that have derived tolerable daily intakes for selenium have all agreed that the human data provided by the Yang *et al*. (1983, 1989a,b) and other epidemiological studies, based on lifetime exposures to selenium, are an appropriate basis for standard-setting, and that the NOAEL of approximately 850 µg/day could be used as a departure point. The SCF decided to use an uncertainty factor of 3 to allow for the remaining uncertainties of the studies used in deriving an upper level in arriving at a UL for selenium of 300 µg/day.

The Panel notes that the SCF's UL of 300 µg/day is the current basis for assessing the safety of inorganic selenium compounds, for example selenious acid (EFSA, 2009) and that the EFSA AFC Panel considered that this UL should also apply to organic selenium compounds from selenium-enriched yeasts. The NOAEL in humans on which the SCF based their UL was derived from findings in Chinese subjects and other populations exposed on a lifetime basis to selenium in their diet and drinking water. Since L-selenomethionine accounts for 50 to over 80 % of total selenium in plants such as cereals and soybean grown on selenium-rich soil, such as the areas from which the Chinese data were derived, L-selenomethionine was likely to have been one of the selenium compounds to which the populations in question were exposed. The Panel therefore considered that these human data, and the UL of 300 µg/day for

selenium derived using these data, could also provide the primary basis for assessing the safety of L-selenomethionine.

The Panel notes however that the findings of the recent SELECT study indicate that in humans, selenium-related effects might be observed at a supplemental level of 200 µg selenium/day, in individuals also having a high dietary intake of selenium. The Panel considers that, because of these data and given the greater bioavailability of selenium from L-selenomethionine, the safety of L-selenomethionine can only be ascertained at use levels up to 250 µg/day (corresponding to up to 100 µg selenium/day) in food supplements.

The Panel notes that according to the petitioners, L-selenomethionine is intended to be used in food supplements to provide an intake of 100-400 µg selenium/day, typically 200 µg/day.

According to the SCF, the average intake of selenium by the European population lies in the range 24-89 µg/day. The high percentile of European intakes of 108 µg selenium/day is reported for the Finnish population. The Panel notes that these estimates include higher intake figures from selenium-rich areas, or where selenium is coming from addition of selenium to fertilisers e.g. in Finland or where selenium is added to animal feed, resulting in selenium-rich foods.

Assuming a mean dietary selenium intake for adults in Europe in the range of 24–89 µg/day and a high percentile intake of 108 µg/day, the Panel estimated that consumption of an additional food supplement containing 100 µg selenium (lowest proposed use level) in the form of L-selenomethionine by adults would provide a total anticipated exposure of between 124 and 189 µg selenium/day at the average level of dietary selenium intake and a total anticipated exposure of 208 µg selenium/day at the high percentile dietary selenium intake. This intake will be below the UL of 300 µg/day for selenium in adults established by the SCF in 2000.

Assuming a mean dietary selenium intake for children (aged between 2 and 17 years) in Europe in the range of 23-42 µg/day, and a high percentile intake range of 32-77 µg/day, the Panel estimated that daily consumption of an additional food supplement containing 100 µg selenium (lowest proposed use level) in the form of L-selenomethionine by children would provide a total anticipated exposure of between 123 and 142 µg selenium /day at the average level of dietary selenium intake and a total anticipated exposure of between 132 and 177 µg selenium /day at the high percentile dietary selenium intake. These intakes will exceed the ULs of 60, 90, and 130 µg selenium/day for children at the ages of 1-3, 4-6 and 7-10 years respectively. The ULs of 200 µg selenium/day for children aged between 11 and 14 years and 250 µg selenium/day for children between aged 15 and 17 years will not be exceeded.

CONCLUSIONS

The present opinion deals only with the safety of L-selenomethionine as a source of selenium and with the bioavailability of selenium from this source, intended to be used in food supplements. The safety of selenium itself, in terms of amounts that may be consumed, is outside the remit of this Panel.

The selenium source is a selenoamino acid in which selenium replaces the sulphur of the methionine molecule. L-selenomethionine is bioavailable, and the Panel concludes that the bioavailability of selenium from L-selenomethionine is higher than that from inorganic

sources of selenium such as selenite and selenate. Data on the toxicity of L-selenomethionine indicate that it is comparable to other forms of selenium, in terms of equivalent amounts of bioavailable selenium.

The Panel notes that the SCF's UL of 300 µg/day is the current basis for assessing the safety of inorganic selenium compounds, for example selenious acid, and that the EFSA AFC Panel considered that this UL should also apply to organic selenium compounds from selenium-enriched yeasts.

The Panel also notes however that the findings of the recent SELECT study using L-selenomethionine indicate that in humans, selenium-related effects might be observed at a supplemental level of 200 µg selenium/day, in individuals also having a high dietary intake of selenium. The Panel considers that, because of these data and given the greater bioavailability of selenium from L-selenomethionine, the safety of L-selenomethionine can only be ascertained at use levels of up to 250 µg/day in food supplements (corresponding to up to 100 µg selenium/day). In the opinion of the Panel, assessment of the safety of L-selenomethionine at intakes corresponding to between 100 µg selenium/day and the current UL requires an evaluation of the dose-response data together with detailed information on the toxicokinetics of L-selenomethionine and other selenium compounds.

The Panel concludes that the use of L-selenomethionine as a source for selenium in food supplements would not be of safety concern in adults at use levels up to 250 µg /day (corresponding to up to 100 µg selenium/day). At this use level the combined intake from diet and supplement use will be below the SCF's UL, even for individuals also having a high dietary intake of selenium (i.e. greater than 100 µg selenium/day from the diet).

The Panel considers, however, that the current database on selenium compounds and the new data provided by the recent SELECT study indicate the need for an integrated reconsideration of the UL for selenium from all sources, and recommends that this also considers systemic selenium levels rather than external dose in characterising dose-response relationships, in order to allow a comparison of selenium-containing compounds with different bioavailabilities.

DOCUMENTATION PROVIDED TO EFSA

1. Technical dossier for the use of L-selenomethionine in foods for particular nutritional uses (PARNUTS) and supplements. June, 2005. Submitted by the UK Food Standards Agency, on behalf of the petitioner, Sabinsa Corporation, USA.

2. Technical dossier on L-selenomethionine for use in the manufacture of foods supplements. June, 2005. Submitted by PRO MEDICO- PEWA MED Handels gmbH, Austria.

3. Technical dossier on L-selenomethionine for adding to Directive 2002/46/EC Annex II. December, 2005. Submitted by Zentiva, a.s., Czech Republic.

4. Technical dossier for safety evaluation of selenomethionine for use in the manufacture of foods supplements. April, 2005. Submitted by Pharmaforte, Ltd., Hungary.

ADDITIONAL INFORMATION PROVIDED TO EFSA

a. Technical dossier on selenomethionine. November, 2008. Submitted by the Netherlands Ministry of Public Health, Welfare and Sport, Food, Health Protection and Prevention Directorate.

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GLOSSARY AND ABBREVIATIONS

AFC	Scientific Panel on Food Additives, Flavourings, Processing aids and Materials in contact with food
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
CAS	Chemical Abstract Service
CI	Confidence Interval
EC	European Commission
EFSA	European Food Safety Authority
EVM	UK Expert Group on Vitamins and Minerals
FDA	Food and Drug Administration
FNB	Food and Nutrition Board
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
GPx	Glutathione Peroxidase
IGF	Insulin-like Growth Factor
IU	International Unit
LD	Lethal Dose
LOAEL	Lowest-Observed-Adverse-Effect-Levels
NCI	National Cancer Institute
NOAEL	No-Observed -Adverse –Effect-Level
NOEL	No-Observed-Effect-Level
NPC	Nutritional Prevention of Cancer
OECD	Organisation for Economic Co-operation and Development
PARNUTS	Foods for Particular Nutritional Purposes
PRI	Population Reference Intake
RR	Relative Risk
SCF	Scientific Committee on Food

SELECT	Selenium and Vitamin E Cancer Prevention Trial
SUL	Safe Upper Level
UL	Tolerable Upper Intake Level