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Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Selenium

(expressed on 19 October 2000)

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FOREWORD

This opinion is one in the series of opinions of the SCF on the upper levels of vitamins and minerals. The terms of reference given by the European Commission for this task, the related background and the guidelines used by the Committee to develop tolerable upper intake levels for vitamins and minerals used in this opinion, which were expressed by the SCF on 19 October 2000, are available on the Internet at the pages of the SCF, at the address: http://www.europa.eu.int/comm/food/fs/sc/scf/index_en.html.

1. NUTRITIONAL BACKGROUND

1.1. Selenium forms in foods

Foods contain a number of different selenium forms. In animal foods, there are specific selenium proteins where selenium is incorporated *via* selenide as selenocysteine, while selenomethionine, and possibly also selenocysteine to some extent, are non-specifically incorporated as analogues to methionine and cysteine in foods both of animal and plant origin. Selenomethionine, as well as the inorganic forms selenite and selenate, are the most common forms in food supplements and fodder additives. Although extensively used for research purposes, it is uncertain if the inorganic forms occur in foods. In addition to these forms a number of uncharacterised forms exist, e.g. in fish (Åkesson and Srikumar, 1994), but their contribution to total dietary selenium is unknown.

Selenium forms used in supplements are inorganic selenite and selenate and organic selenium in the form of selenomethionine, selenocystine and selenium enriched yeast. The forms of selenium found in yeast vary according to production process and the selenomethionine has been suggested to comprise 20 to 50% of the selenium and that some is bound as selenotrisulphides (SCF, 1999).

It should be noted that selenium compounds other than those nutritionally relevant, i.e. those present in food and with a tradition of use as supplements to meet nutritional requirements, are outside the scope of this document. The toxicity and biological properties of such selenium compounds (there are numerous synthetic ones) can be quite different from the nutritionally relevant selenium compounds.

1.2. Selenium intake and selenium status in European countries

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 (Gissel-Nielsen *et al.*, 1984; Frøslie, 1993). The mean intakes of non-vegetarian adults in different studies are Belgium 28-61 µg/day, Denmark 41-57 µg/day, Finland 100-110 µg/day, France 29-43 µg/day, United Kingdom 63 µg/day, The Netherlands 40-54 µg/day, Norway 28-89

µg/day, Spain 79 µg/day, and Sweden 24-35 µg/day (Alexander and Meltzer, 1995; van Dokkum, 1995; Johansson *et al*. 1997).

1.3. Metabolism of selenium

The available data indicate that selenium-containing aminoacids and probably other selenium forms, such as selenite and selenate, can be converted to selenide in mammals (Young *et al*., 1982). Selenide is a central metabolic form of selenium, which is utilised for the formation of selenocysteine, incorporated into specific selenoproteins, and in case of high exposure, into excretory products such as dimethyl selenide (which is exhaled) and trimethylselenonium ions (which are excreted into urine). Selenomethionine and selenocysteine formed by transsulfuration of selenomethionine can be non-specifically incorporated into protein as analogues to methionine and cysteine. Other forms of protein-bound selenium may also occur (Sunde, 1990; Alexander and Meltzer, 1995; Johansson *et al*., 1997).

1.4. Bioavailability of different forms of selenium

Most forms of selenium salts and organic bound selenium, i.e. selenomethionine and selenocysteine, are easily absorbed from the gastrointestinal tract. Only a few studies on the bioavailability of selenium have been performed in humans (Mutanen, 1986; Neve, 1994). Selenium in blood or serum is most effectively raised by selenium-rich wheat or yeast selenium (the latter may vary in quality), probably because of non-specific incorporation of selenomethionine into proteins. Inorganic selenium as selenate and selenite can be incorporated specifically into selenium proteins via selenide as selenocysteine and increase seleno-enzyme activity until saturation (Levander *et al*., 1983 (Alfthan *et al*., 1991). A few studies have also compared selenium bioavailability from different foods. Van der Torre *et al*. (1991) found that supplementation with selenium-rich forms of bread and meat gave similar increases in circulating selenium levels. Christensen *et al*. (1983), using a triple stable-isotope method, found that the absorption of selenium from selenite was 36% and that from intrinsically labelled poultry meat was 71%. Selenium consumed from fish had no apparent effect on the amount of selenium incorporated into functional selenoproteins and a low effect on general level of selenium in plasma (Meltzer *et al*., 1993, Åkesson and Srikumar, 1994; Svensson *et al*., 1992; Huang *et al*., 1995). Given different bioavailabilities and differences in non-specific incorporation of selenium compounds from different sources such as cereals, meat, fish and organic and inorganic supplements, the selenium concentration in whole blood will relate differently to the total intake of selenium (Alexander and Meltzer, 1995).

The total body pool of selenium has been estimated to be 5-15 mg in adults. Kinetic studies indicate that blood plasma contains at least four components with half-lives between 1 and 250 hours (Patterson *et al*., 1989).

1.5. Functional forms of selenium – selenoproteins

At least eleven selenoproteins containing the amino acid selenocysteine have been identified in mammals: cellular glutathione peroxidase (cGSHPx), extracellular glutathione peroxidase (eGSHPx), phospholipid hydroperoxide glutathione peroxidase (phGSHPx), gastrointestinal glutathione peroxidase (giGSHPx), iodothyronine deiodinase types I, II and III, prostatic epithelial selenoprotein (PES), selenoprotein P (SeP), selenoprotein W, thioredoxin reductase (Alexander and Meltzer, 1995; Johansson *et al*., 1997).

1.6. Daily requirements

The amount of dietary selenium (as DL-selenomethionine) required to saturate the selenium need of extracellular GSHPx was used as one of the approaches to define a Dietary Reference Intake for Selenium in the USA in 2000 (55 µg/day for adult men and women) (NAS; 2000). A so-called Population Reference Intake of 55 µg selenium per day for adults, but also other levels of intakes based on other criteria, were established by the Scientific Committee for Food of the European Commission (1993).

A joint FAO/IAEA/WHO Expert Consultation (WHO, 1996) gave several modes for the calculation of requirements of the individual and populations. For a 65 kg reference man the average normative requirement of individuals for selenium was estimated to be 26 µg/day, and from this value the lower limit of the need of population mean intakes was estimated to be 40 µg/day. The corresponding values for a 55 kg reference woman were 21 and 30 µg selenium/day, respectively. The latter value was estimated to increase to 39 µg/day throughout pregnancy and to attain the values of 42, 46 and 52 µg selenium/day at 0-3, 3-6 and 6-12 months of lactation, respectively. The Nordic Nutrition Recommendations (1996) have set a recommended intake of 50 µg/day for men, an average requirement of 35 µg/day and a lower limit of needed intake of 20 µg/day, the corresponding values for women being 40, 30 and 20 µg/day, respectively.

The approach to define a biochemical index for the saturation of the functional selenium requirement using a limited number of selenoproteins has given variable results (Nève, 1991). The estimations are also complicated by the fact that different forms of dietary selenium (organic *vs*. inorganic) give variable responses in different measures of selenium status (Alfthan *et al*., 1991) and the physiological relevance of the 'saturation of selenium dependent enzymes approach' can be questioned (Johansson *et al*., 1997).

1.7. Selenium deficiency and selenium in disease states

The most obvious example of a relationship between selenium status and disease is the cardiomyopathy, Keshan disease, that occurs in selenium-deficient areas of China (Xia *et al*., 1994). Prophylactic treatment with selenium supplementation dramatically decreased disease incidence. The disease is not a clear-cut selenium deficiency syndrome since it is not obligatory at very low selenium status and moreover several other factors affect its incidence.

A suspected selenium deficiency syndrome has also been demonstrated in a few patients treated with parenteral nutrition without added selenium (see Rannem *et al*., 1996). Muscular pain and muscular and cardiac dysfunction has been demonstrated in some patients, but no uniform symptomatology has been described.

In several epidemiological studies the incidence of different diseases, such as cancer and cardiovascular disease, has been related to selenium status. In addition, selenium has been related to immune function, viral infection, reproduction and mood (WHO, 1987; Flohé, 1989; Knekt *et al*., 1990; Virtamo and Huttunen, 1991; Willett *et al*., 1991; Kok *et al*., 1991; Clarke *et al*., 1996, Rayman, 2000).

2. HAZARD IDENTIFICATION AND CHARACTERISATION

2.1. Adverse and toxic effects

2.1.1. Mechanisms of toxicity

The molecular mechanisms of selenium toxicity remain unclear. Several mechanisms have been suggested: redox cycling of auto-oxidisable selenium metabolites, glutathione depletion, protein synthesis inhibition, depletion of S-adenosyl-methionine (cofactor for selenide methylation), general replacement of sulphur and reactions with critical sulphydryl groups of proteins and cofactors (Anundi *et al*., 1984; Hoffman, 1977; Martin, 1973; Stadtman, 1974; Vernie *et al*., 1974). No unifying hypothesis is possible and it is likely that several mechanisms may operate and vary among different selenium compounds. Growth reduction in experimental animals is apparently caused by selective selenium accumulation and toxicity to growth hormone producing cells in the anterior pituitary gland (Thorlacius-Ussing, 1990).

2.1.2. Acute toxicity

Selenite, selenate and selenomethionine are among the most acutely toxic selenium compounds (Högberg and Alexander, 1986). Intake of 250 mg selenium as a single dose or multiple doses of 27-31 mg resulted in acute toxicity with nausea, vomiting, nail changes, dryness of hair, hair loss, tenderness and swelling of fingertips, fatigue, irritability and garlicky breath (Jensen *et al*., 1984; WHO, 1987).

In Sweden, several cases of toxicity in children occur each year due to accidental overconsumption of selenium tablets. Acute symptoms such as vomiting have been observed, but so far no serious cases of toxicity have been recorded (Johansson *et al*., 1997).

2.2. Chronic toxicity

2.2.1. Animal toxicity data

Animals show growth reduction, liver changes, anaemia, pancreatic enlargement and some domestic animals also exhibit neurotoxicity following selenium exposure above 0.03-0.4 mg/kg bw (Alexander and Meltzer, 1995).

2.2.1.1.Cancer studies

Several early studies showed tumours following selenium exposure (Nelson *et al*., 1943; Volgarev and Tscherkes, 1967; Innes *et al*., 1969; Schroeder and Mitchener, 1971a; Schroeder and Mitchener, 1972; Schrauzer and Ishmael, 1974; IARC, 1975; US EPA, 1980). These studies have been evaluated on several occasions and, in general, the data have been considered inconclusive due to many problems with the studies (Diplock, 1984). Nelson gave low-protein diets supplemented with seleniferous wheat or 10 mg selenium salts/kg bw. A number of rats died before 18 months, but none with tumours. In surviving rats hepatic tumours were found in animals with liver cirrhosis. It has also been questioned whether identified tumours were actually regeneration nodules. The study of Volgarev and Tscherkes (1967) lacked adequate controls. Also the study by Schroeder and Mitchener (1971) lacked adequate controls, and the colony suffered from severe infections.

Synthetic selenium compounds that have shown effects indicative of carcinogenicity are as follows. Selenium diethyldithiocarbamate given to mice (10 mg/kg by gavage daily for three weeks) was found to increase the incidence of hepatomas, lymphomas and pulmonary tumours (Innes *et al.*, 1969). Seifter *et al*. (1946) gave 0.05% of bis-amino-phenyl selenium dihydroxide in the diet to rats and found an increased incidence of adenomatous hepatic hyperplasia and thyroid adenomas. Selenium sulphide in large oral doses (3 and 15 mg/kg bw/day to rats and 20 and 100 mg/kg bw/day to mice) was found to be carcinogenic to rats and mice (NCI, 1980a). In a separate study in mice, selenium sulphide was applied to the skin and there was no increased incidence of tumours attributable to selenium treatment (NCI, 1980b); under most conditions the systemic uptake of topically selenium sulphide might be insignificant (Cummins and Kimura, 1971). Carcinogenicity of selenium compounds seems primarily to be associated with the nature of the compound than with the element itself. The selenium compounds mentioned above are not used as sources of selenium in food, nor as nutrients.

2.2.1.2.Reproductive effects

It is well established that several selenium compounds such as selenate, selenite, selenocysteine and in particular selenomethionine are teratogens in avian species and in fish (Franke *et al*., 1936; Moxon and Rhian, 1943; Halverson *et al*., 1965; Palmer *et al*., 1973; Dostal *et al*., 1979; Birge *et al*., 1983; Heinz *et al*., 1987; Woock *et al*., 1987; Hoffman *et al*., 1988; Pyron and Beitinger, 1989). Both inorganic and organic forms of selenium cross the placenta in humans and experimental animals (Willhite *et al*., 1990). Terata have also been produced in sheep (Holmberg and Ferm, 1969) and pigs (Wahlström and Olson, 1959). Effects of selenium compounds on reproduction and offspring in rodents have usually been associated with overt maternal poisoning and nutritional deprivation (Schroeder and Mitchener, 1971b; Berschneider *et al*., 1977; Nobunaga *et al*., 1979; Ferm *et al*., 1990). Recent studies on macaques fed selenomethionine (25, 150 and 300 µg/kg bw/day) during organogenesis showed no signs of terata (Tarantal *et al*., 1991). A dose-dependent maternal toxicity was observed in this study. Whereas no signs of treatment related toxicity in the dams were observed at the dose of 25 µg/kg bw/day (NOAEL), maternal toxicity as indicated by poor appetite and emesis was observed in the mid- and high-dose groups. No treatment-related changes in the teeth, skin or nails were found. No indication of teratogenicity of selenium has been shown in humans even in the areas of high selenium intake in China (Yang *et al*., 1989b).

2.2.1.3.Genotoxic effects

A moderate genotoxic activity of selenium compounds (i.e., selenite, selenate, selenide, selenocysteine and selenosulphide) has been found in several *in vitro* systems (Löfroth and Ames, 1978; Ray and Altenburg, 1978; Noda *et al*., 1979; Whiting *et al*., 1980; Ray, 1984; Tennant *et al*., 1987; Kramer and Ames, 1988). There is one *in vivo* study showing chromosomal aberrations and increased SCE in hamster bone marrow cells after selenite treatment (Norppa *et al*., 1980). This occurred only at doses of 3, 4, and 6 mg Se/kg bw i.p. that were associated with severe systemic toxicity, including lethality, some hours after dosing. The numbers of aberrations in these groups were 13-55%, compared to 0.9-1% in the controls. Doses of 0.3, 0.6, 1 and 2 mg Se/kg bw did not cause any clastogenic effects. A non-pregnant macaque dosed with 600 µg selenomethionine for 15 days (lethal dose) showed in comparison with the control animal a sevenfold increase in bone marrow micronuclei (Choy *et al*., 1989). In pregnant macaques receiving 0, 150 or 300 µg selenomethionine/kg bw and showing signs

of selenosis, foetal bone marrow smears did not show any increase in the number of micronuclei (Choy *et al.*, 1993).

In vitro studies indicate that the mutagenic effects of selenium salts are associated with production of reactive oxygen radicals and that glutathione promotes these reactions (Kramer and Ames, 1988). It is well known that auto-oxidisable selenium metabolites, such as hydrogen selenide, can undergo redox cycling producing oxygen radicals and cause DNA strand breaks (Anundi *et al*., 1984; Nuttall and Allen, 1984; Garberg *et al*., 1988). Detoxification of selenide by methylation is saturable depending on the supply of methyl donors. *In vivo*, only toxic amounts were shown to be active, keeping in mind the central role of hydrogen selenide in the metabolism of most selenium compound it is likely that overproduction of this and other auto-oxidisable selenium metabolites could promote the formation of DNA reactive oxygen radicals. It is possible that glutathione might play a central role as well. It follows, given such a mechanism, that expression of selenium dependent genotoxic activity is likely to be concentration- and threshold-dependent, but this remains to be shown (Högberg and Alexander, 1986).

2.2.2. Human toxicity data

2.2.2.1.Exposure to supplements

Two individuals took selenium-containing yeast at doses of 200 and 400 µg selenium daily for 18 months. Together with dietary intake they received about 350 and 600 µg/day. Marginal haematological changes and a borderline increase in serum ALAT (alanine amino transferase) were seen (Schrauzer and White, 1978).

A small group of patients with rheumatoid arthritis receiving 250 µg Se as organic selenium in addition to selenium from food for 6 months had decreased levels of somatomedin C in serum in comparison with a group receiving placebo (Thorlacius-Ussing *et al.*, 1988). A similar effect was not observed when graded doses of 100, 200 and 300 µg selenium as selenium wheat was given to healthy, Norwegian volunteers for a six week period (Meltzer *et al.*, 1993), nor was the effect observed in North Americans with a natural selenium intake range of 68-724 µg/day (Salbe *et al.*, 1993).

In a study by van Dokkum *et al*. (1992) two groups of 6 male volunteers were given 8 slices of bread per day for six weeks. The bread was made with selenium-rich and -poor wheat. In the treatment group the bread provided 200 µg selenium/day per subject. In a study by Longnecker *et al*. (1993), groups of 4 healthy male volunteers were fed bread containing 32.4, 206 or 388 µg selenium/day. Prior to the study the intake was 80 µg/day. In both studies no adverse effects were reported, although such information was not specifically sought.

In a supplementation study where 400 µg/day of selenium as selenite or selenomethionine (total dose 450-500 µg/day) were given for 3 months to 32 healthy women, half of them experienced symptoms of depression and extreme tiredness during the month following the termination of the study (Meltzer, 1995).

In a randomised, double blind, placebo-controlled study, the effect of selenium supplementation on prevention of skin cancer was investigated (Clark *et al*., 1996). A total of 1312 patients (mean age 63, range 18-80) with a history of basal cell or squamous cell

carcinoma were treated with 200 µg selenium/day in the form of high-selenium brewer's yeast tablet (Nutrition 21, La Jolla, Calif.) or placebo for up to ten years (mean 4.5 years). The percentage of males in the control and treatment groups was 75.6 and 73.8, respectively. Mean plasma selenium concentration at the start of the study was 114 µg/l (1.44 µmol/l), which was in the lower end of the range of normal plasma levels reported in the US (in most European countries, however, the mean serum levels are lower (Alexander and Meltzer, 1995)). Plasma selenium levels remained constant throughout the study in the placebo group, while plasma selenium rose to 190 µg/l (2.4 µmol/l) in the treatment group within 6-9 months from the beginning. The safety endpoints investigated included known signs of frank selenosis (see below), including garlic breath, pathological nail changes and brittle hair. Patients were assessed every 6 months and the authors observed no dermatological or other signs of selenium toxicity. A total of 35 patients upset, 21 in the selenium group and 14 in the control group, complained about adverse effects, mostly gastrointestinal, which resulted in withdrawal from the study.

Although it is difficult to assess the intake based on serum values, as these might vary according to the source of selenium, an estimate can be that a mean intake of approximately 90 µg selenium/day would correspond to a serum value of 114 µg/l (1.44 µmol/l) (Alexander and Meltzer, 1995). Hence, the total intake after supplementation would be approximately 290 µg selenium/day.

2.2.2.2.Long term exposure, epidemiological studies

Health effects of high dietary intakes of selenium have been investigated in selenium-rich areas of South Dakota, USA (Smith and Westfall, 1937). The most common symptoms were gastrointestinal disturbances, icteroid discoloration of the skin, and decayed and bad teeth. It is difficult to evaluate the exposure levels and validity of these findings (WHO, 1987). Children living in a seleniferous area in Venezuela have been compared to children living in Caracas (Jaffe, 1976). The level of selenium in blood averaged 813 µg/l (10.3 µmol/l) in the seleniferous area, and in one child reached 1,800 µg/l (22.8 µmol/l). Using the Chinese data on blood/intake relationships (Yang *et al.*, 1989a), a level of 813 µg/l (10.3 µmol/l) corresponds to a daily intake of about 10 µg Se/kg bw. It was found that pathological nail changes, loss of hair and dermatitis were more common in the seleniferous area. However, whether these differences were due to selenium toxicity was not entirely clear, as the groups differed in several other nutritional aspects.

Clinical symptoms associated with selenium poisoning such as those described above are usually referred to as selenosis. A more detailed description of symptoms is given below.

In China, endemic selenium intoxications due to high selenium in soil have been studied by Yang and colleagues (Yang *et al.*, 1983). Morbidity was 49% among 248 inhabitants of five villages with a daily intake of about 5,000 µg selenium. The main symptoms were brittle hair with intact follicles, new hair with no pigment, and thickened nails as well as brittle nails with spots and longitudinal streaks on the surface. In more severe cases fluid effused from around the nail bed. Another common finding was lesions of the skin, mainly on the backs of hands and feet, the outer side of the legs, the forearms, and the neck. Affected skin became red and swollen, followed by the appearance of blisters and the occurrence of eruptions. Symptoms of neurological disturbances were observed in 18 of the 22 inhabitants of one heavily affected village alone. Patients complained of peripheral anaesthesia, acroparaesthesia, pain, and

hyperreflexia. At a later stage numbness, convulsions, paralysis, and motor disturbances developed. The daily intake among those with clinical signs of selenosis was estimated to range from 3,200 to 6,690 µg, with an average of 4,990 µg selenium. The mean blood level was 3,200 µg/l (40.5 µmol/l), and the mean urine level was 2,680 µg/l (33.9 µmol/l). Livestock were also affected in these areas. The residents recovered as soon as the diets were changed. In high selenium areas without occurrence of selenosis the daily intake of selenium was calculated to range from 240 to 1510 µg, with a mean intake of 750 µg. The corresponding blood levels were 440 (350-580) µg/l (5.6 (4.4-7.3) µmol/l) (mean and SD). The chemical forms of selenium were determined in Chinese rice and maize and the major form was selenomethionine (Beilstein *et al*., 1991).

In a follow up to their earlier work, Yang *et al*. (1989a, 1989b) studied a population of about 400 individuals which was evaluated for clinical and biochemical signs of selenium toxicity. A detailed study of selenium intake *via* various food items as well as measurements of selenium in tissues, i.e., whole blood, urine, hair and finger- and toe-nails, allowed more accurate estimation of the dose-response relationships observed for selenium toxicity.

The average daily intakes based on lifetime exposures were 70, 195 and 1438 µg, and 62, 198 and 1288 µg for adult males and females, respectively, in the low-, medium- and high-selenium areas. Clinical signs of selenosis (i.e., hair or nail loss, nail abnormalities, mottled teeth, skin lesions and changes in peripheral nerves) were examined among 349 adult residents and were observed among subjects in the high selenium area. Subjects with clinical signs of selenosis were classified as ++ or + (mainly finger-nail disease/changes alone and with severe hair loss/skin changes). No clinical signs were observed among those with a blood selenium concentration below 1000 µg/l (12.7 µmol/l) (intake according to regression equation, figure 1 Yang *et al*. 1989a: 853 µg Se/day). The prevalences of subjects with selenosis ++ varied between 3-7% in the groups with blood concentrations 1000-1250, 1250-1500 and 1500-2000 µg/l (12.7-15.8, 15.8-19.0, 19.0-25.3 µmol/l). The prevalences of subjects with selenosis + varied from 10-35% in the same groups. No dose response relationships were seen. The prevalence of subjects with selenosis + was 45% in subjects with a blood concentration above 2000 µg/l (25.3 µmol/l). (All prevalence figures were taken from figure 4 of Yang *et al*., 1989b.) Blood selenium concentrations in five subjects with long persistent clinical signs ranged from 1054 µg/l to 1854 µg/l (13.3 to 23.5 µmol/l) with a mean of 1346 µg/l (17.0 µmol/l), corresponding to a daily intake of 1260 µg Se (range: 913-1907 µg Se) (intake calculated from the regression equation). Prolonged bleeding time was observed clinically upon blood collection in the high selenium areas. Prolonged prothrombin time (>14 sec.) was observed among 1 of 20 subjects with a blood selenium of 200 to 990 µg/l (2.53-12.5 µmol/l) and among 45% of those subjects with a blood selenium above 1000 µg/l (12.7 µmol/l) (corresponding to an intake of about 850 µg). The mean prothrombin time only increased marginally, but ranges were not given in the publication. A strong reduction in the plasma-Se/red cell-Se was seen at blood concentrations exceeding 900 µg Se/l (11.4 µmol/l), corresponding to an intake of 750 µg/day. A decreased concentration of glutathione in blood was observed at dietary intakes exceeding 850 µg/day. The prevalence of mottled enamel teeth of school children of 7-14 years of age were 0, 49 and 95% in groups with low (130 ± 20 µg/l (1.65 ± 0.25 µmol/l)), medium (370 ± 320 µg/l (4.68 ± 4.05 µmol/l)) and high (1570 ± 440 µg/l (19.9 ± 5.57 µmol/l)) blood selenium concentrations, respectively.

The five patients showing overt signs of selenosis were followed up in a later study (Yang and Zhou, 1994). The symptoms disappeared after a change in the diet. Their blood levels decreased from 1346 ± 366 to 968 ± 115 µg Se/l (17.0 ± 4.63 to 12.3 ± 1.46 µmol/l), corresponding to an intake of 1270 ± 450 to 819 ± 126 µg Se/day (calculated from the regression equation) (819 µg corresponds to about 15 µg/kg bw, according to the authors). The latter mean value had a lower 95% confidence limit of 567 µg per day. The range was 654 to 952 µg Se/day.

In a study (Longnecker *et al.*, 1991), 142 subjects from geographical areas with high dietary selenium intakes in USA were followed over a 2-year period with respect to adverse health effects. The daily dietary intake was assessed by several 48 h duplicate-plate food collections for selenium determination from one person in the household and by diet questionnaires. The subjects were followed for one year, completed health questionnaires, and underwent physical examinations and clinical chemistry tests. Selenium in whole blood, serum, urine and toenails were also determined.

The average selenium intake was 239 µg/day, varying from 68 to 724 µg/day; half of them had an intake above 200 µg/day and 12 individuals above 400 µg/day. There was no variation in the prothrombin time with selenium intake. However, an association of selenium intake with alanine aminotransferase (ALAT) in serum was observed. The values were within the reference range and considered clinically insignificant. Increased prevalence of lethargy was also seen with increased selenium values. Nail abnormalities were not related to selenium intake, neither were any other symptoms or physical findings. In contrast to a study from Denmark (Thorlacius-Ussing *et al.*, 1988) (see above), no relationship between plasma somatomedin C and any of the selenium indices was observed in this study (Longnecker *et al.*, 1991; Salbe *et al.*, 1993).

Brätter and Negreti de Brätter (1996) studied the influence of high dietary selenium intake on the thyroid hormone levels in serum of 125 lactating mothers 20-24 days post partum from three regions with different selenium intake in Venezuela. The serum concentration of FT_3 (free, unbound T_3) (T_3 is formed from T_4 in the liver) was lower in the group having the highest mean intake, 552 (range: 250-980) µg/day, but all values were found to be within the reference range. The two other regions had mean intakes of 274 (range 170-500) and 205 (range: 90-350) µg Se/day, respectively. None of the investigated individuals had signs of selenosis.

3. DOSE RESPONSE ASSESSMENT

Acute and chronic selenium toxicity have been demonstrated in a wide variety of animals and human. Soluble selenium salts and selenomethionine show approximately similar toxicity and there is no substantial variation between animal species. Soluble selenium salts (in cases of supplementation and selenium from drinking water) may be acutely more toxic than organic bound selenium from food. On the other hand, organic forms may be more toxic during long-term consumption due to incorporation into proteins rather than excretion.

Except for some selenium compounds not used in food, i.e. selenium sulphide, selenium diethyldithiocarbamate, bis-amino-phenyl selenium dihydroxide, experimental data do not

indicate that inorganic selenium salts or organic selenium compounds relevant in food and nutrition are carcinogenic. Adequate human data do not exist.

Genotoxicity has been seen in a number of *in vitro* systems and also *in vivo* at toxic doses. It is likely, however, that these effects may be related to the generation of reactive oxygen radicals, being dose dependent and showing a threshold *in vivo* and not occurring at nutritionally adequate intakes.

There is no evidence for teratogenicity neither in humans nor in macaques fed selenomethionine.

Except for the early studies of the population in seleniferous areas in USA (Smith and Westfall, 1937), the more recent Chinese studies of endemic selenium toxicity in humans (Yang *et al*., 1983; Yang *et al*., 1989a; Yang *et al*., 1989b; Yang and Zhou, 1994) and the 1991 American study (Longnecker *et al*., 1991), there are only anecdotal reports on chronic selenium toxicity in humans.

Based on the Chinese studies (Yang *et al*., 1989a; Yang *et al*., 1989b; Yang and Zhou, 1994), the minimum daily dietary intake sufficient to cause symptoms of selenosis (i.e., hair or nail loss, nail abnormalities, mottled teeth, skin lesions and changes in peripheral nerves) is about 1200 µg Se (range: 913-1907 µg Se). The LOAEL for clinical symptoms of selenosis is about 900-1000 µg Se/day. No clinical signs of selenosis were recorded in individuals with blood selenium below 1000 µg/l, corresponding to an intake of about 850 µg/day, which could be taken as a NOAEL for clinical selenosis. Symptoms were also observed in a man taking 913 µg Se/day as selenite (Yang *et al*., 1983). In the follow up study (Yang and Zhou, 1994) of 5 patients (from the study of 349 individuals) recovered from selenosis when their mean intake was reduced to 819 µg Se/day, the 95% lower confidence limit of the mean intake was 567 µg/day).

Symptoms from the liver, which is also affected in animal studies, manifested in increased prothrombin time due to impaired synthesis of coagulation factors in the liver, became statistically significant at dietary intakes at and above 850 µg Se/day (Yang *et al*., 1989b). In the American study (Longnecker *et al.*, 1991) no signs of toxicity were seen in a population consuming on average 239 µg Se/day from food. The liver enzyme ALAT in serum, although within the reference range, showed a correlation with selenium intake (68 to 724 µg/day), but this was not considered to be clinically significant. No effect on prothrombin time was seen in the latter study. However, the American population studied covered a lower range of selenium intake than the Chinese study and, considering mean body weights, it is also likely that the intake per kg bw was greater in the Chinese study, in comparison with the American one.

Taken together, increased prothrombin time (in the Chinese study) and slight ALAT increase (in the American study) are both signs of subclinical/biochemical liver effects. The clinical relevance is uncertain.

4. DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

Taking all this information into account, the Committee decided to derive the UL using the NOAEL of 850 µg/day for clinical selenosis in the study on 349 subjects of Yang *et al*.

(1989b). In support of this NOAEL is the follow up study of Yang and Zhou (1994) of the 5 individuals who recovered from selenosis when their intake had been reduced to a mean of 819 µg Se/day. The NOAEL used was derived from a study on a large number of subjects and is expected to include sensitive individuals. It was decided to use an uncertainty factor of 3 to allow for the remaining uncertainties of the studies used in deriving an upper level.

An UL of 300 µg Se/day was derived for adults. This value covers selenium intake from all sources of food, including supplements.

The supplementation study by Clark *et al*. (1996), who did not observe any signs of selenosis in the supplemented group (selenium enriched yeast), having an estimated mean total intake of about 300 µg selenium/day, the American study (Longnecker *et al*., 1991) and the study of lactating women from Venezuela (Brätter and Negreti de Brätter, 1996) further support this UL.

No data are available to suggest that other life-stage groups have increased susceptibilityto adverse effects of high selenium intake. Therefore the UL of 300 `µg per day should be considered to apply also to pregnant and lactating women.

There are no reports of adverse effects on infants born from mothers with high intakes of selenium or adverse effects on lactating women with dietary selenium intakes below the UL for adults. Therefore, the UL for pregnant and lactating women is the same as for non pregnant and non-lactating women.

There are no data to support a derivation of an UL for children. The data on mottled enamel do not allow a NOAEL to be set. On the other hand, there are no reports indicating that children are more susceptible to adverse effects from selenium. Hence, it seems appropriate to extrapolate the UL from adults to children on a body weight basis. The reference weights derived by the Scientific Committee on Food (SCF, 1993) are used as a basis for the calculations.

Age (years)	**UL (µg selenium/day)**
1-3	60
4-6	90
7-10	130
11-14	200
15-17	250

Some selenium compounds were reviewed in the context of “Substances for nutritional purposes which have been proposed for use in the manufacture of foods for particular nutritional purposes ” by the SCF (SCF, 1999). The Committee found sodium selenate, sodium selenite and sodium hydrogen selenite acceptable for use in food for particular nutritional uses, but did not find other forms of selenium acceptable on the basis of current data. Therefore, the UL of this report relate only to the selenium compounds found acceptable and, in addition, to selenium naturally present in food.

5. CHARACTERISATION OF RISK

Based on the information on selenium toxicity, there are areas in the world where there is a human intake of selenium with no or only very small safety margins to levels where toxicity may occur. However, in most European countries the mean intake levels are much lower, in the lower range of 30-90 µg Se/day, except for Norway, that has a somewhat higher mean intake (60 µg Se/day) due to import of wheat rich in selenium. Finland has an intake of 100-110 µg Se/day because of selenium fertilisation. The margin between the present mean intake, excluding supplements, in the European population and an UL (adult) of 300 µg Se/day would be between 2.7 to 10. The 97.5 percentile intake was 81 and 90 µg Se/day in Italy and The Netherlands, respectively, giving a margin to the UL of about 2.7.

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