EXPERT GROUP ON VITAMINS AND MINERALS

REVISED REVIEW OF SELENIUM

The following annexes are also included:

Annex 1. Tables on toxicity data for selenium

Annex 2. Intakes of selenium from food and supplements in the UK

Annex 3. Summary table of selected nutrition related information and existing guidance on intakes

Expert Group on Vitamins and Minerals Secretariat
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SELENIUM

Chemistry and Geochemistry

1. Selenium is abundant in the earth's crust at concentrations of 50 to 90 μg/kg, often in association with sulphur containing compounds. High concentrations may be found in volcanic, sedimentary and some carbonate rocks. The concentration of selenium in soil varies from 5 to 1,200,000 μg/kg. Many chemical forms of selenium such as selenite, selenate and elemental selenium occur.

2. Selenium is a group VI element and has both metallic and non metallic properties. It has an atomic weight of 78.96. It can exist in four oxidation states (-2, 1, +2, +6) and forms chemical compounds analogous to those of sulphur. The salts of selenous acid (H_2SeO_3) and selenic acid (H_2SeO_4) are selenites (Se^{4+}) and selenates (Se^{6+}) respectively.

Natural Occurrence

3. Several species of grasses and herbaceous plants are known to accumulate selenium (reviewed ATSDR, 1996). Primary accumulators are *Astragalus*, *Oonopsis*, *Stanelya*, *Xylorhiza* and *Machaeranthera* containing 100-100,000 mg Se/kg plant tissue. Secondary accumulators are *Astor*, *Gatierreaia*, *Atriplex*, *Grindelia*, *Castillaja* and *Comandra* containing 25-100 mg Se/Kg plant tissue. Non-accumulators contain less than 25 mg Se/kg plant tissue.

Occurrence in food, food supplements and medicines.

4. Selenium occurs in a number of foodstuffs. In the UK, the highest mean levels are found in nuts (0.29 mg/kg), offal (0.42 mg/kg), fish (0.39 mg/kg) eggs (0.19 mg/kg) and poultry (0.15 mg/kg). (MAFF, 1997a). Mean selenium levels in bread and miscellaneous cereals were 0.04 mg/kg and 0.03 mg/kg respectively. In the US levels in bread are higher, being 0.32 mg/kg in white bread and 0.44 in wheat bread (quoted, ATSDR, 1996). Most of the selenium in food is thought to be present as the amino acid derivatives selenomethionine or selenocysteine (COMA, 1991), however, knowledge of the other selenium compounds present in food and their absorption is incomplete (Nordic Project Group, 1995). However, in some plants, including the leaves of beets and cabbage and in garlic up to 50% of selenium present may be in the form of selenate (reviewed ATSDR, 1996). Selenium is present in a variety of dietary supplement products

Licensed medicines for oral use

5. Eight licensed medicines containing selenium as an active ingredient are available for oral use. These are multi-nutrient products which are only available under the supervision of a pharmacist. They are indicated for debilitation and poor nutrition and the maximum daily dose of selenium is 100 micrograms.

Purity Criteria

6. No concerns regarding the purity of selenium in dietary supplement products have been identified.

Intake/Exposure

7. Human exposure to selenium is mainly through food. Intakes of selenium from food and supplements in the UK are attached at annex 1. In the 1995 Total Diet Survey (TDS) (MAFF, 1997b)), the population average intake of selenium in the UK was 29-39 μg/day (the population average intake in the 1994 TDS (MAFF, 1997a) was 43 μg/day compared to an intake of 100 μg/day in the high level (97.5 percentile) consumer). In Nordic Countries, selenium intake is estimated to be 30-80 μg/day. The average intake of selenium from food in the US has been estimated to be 71 to 152 μg/day. In the UK intakes of selenium have fallen since the 1970s (based on analysis of Total Diet Studies by MAFF). This is thought to be due in part to the trend over the past 25 years towards using selenium-poor European wheat rather than the selenium-rich North American wheat for bread making. Additionally the overall consumption of cereal foods, most notably bread, has declined in the last 20 years (COMA, 1998).

8. Swanson *et al*., (1990) investigated the factors affecting selenium status in adults living in a seleniferous area of the US. Selenium intake (μg/kg bw) was strongly correlated with the selenium concentration in serum, whole blood and toenails. Smokers had lower levels of tissue selenium, which was at least partly due to a lower selenium intake; the average selenium intake was 121 and 186 μg/day in smokers and non-smokers respectively (when calculated on a body weight basis, intakes were 1.5 and 2.5 μg/kg respectively). Selenium intake was higher in males than females (213 compared to 128 μg/day respectively) due to the larger quantity of food consumed.

9. In a study conducted in the US (Longnecker *et al*., 1991), 142 subjects from areas of high selenium intake in South Dakota and Wyoming were followed for a two year period. Samples of blood, urine, toenails and duplicate-plate food collections were taken for analysis of the selenium content. The average selenium intake was estimated to be 239 μg/day (the range was 68 to 724 μg/day).

10. Selenium concentrations in water vary greatly geographically but are generally less than 10 μg/l (Nordic Project Group, 1995).

Recommended amounts

11. Deficiency symptoms have only been observed in man at intakes below 20 μg Se/day. Comparisons of dietary selenium intakes in areas of China with and without deficiencies have been conducted to estimate the minimum human requirement for selenium. In areas of endemic deficiency, intake of selenium was 7.7 and 6.6 μg/day for males and females respectively. In non-endemic areas selenium intake was 19.4 and 14.1 μg/day. Other methods used to estimate selenium requirements have included balance and depletion-repletion studies.

12. In the 1991 report Dietary Reference Values for Food Energy and Nutrients for the United Kingdom, the Committee on the Medical Aspects of Food Policy (COMA) recommended Reference Nutrient Intakes (RNIs) of 75 and 60 μg Se/day for adult males and females respectively. It was noted by COMA that the intake of selenium in the UK was sufficient to permit functional saturation of the enzyme glutathione peroxidase (ie the level at which the activity of the enzyme reaches a plateau); the RNIs were established at a level (1μg/kg) to maintain this. An increase of 15 μg is recommended for lactating women. The 1989 "Nordic Nutritional Recommendations" recommend an intake of 30-60 μg Se/day (Nordic Project Group, 1995). This was based on the actual intake of selenium in Sweden and Denmark and the absence of deficiency symptoms at these levels. In the US, the National Research Council established Recommended Dietary Allowances for selenium of 70 and 55 μg/day for males and females respectively.

13. The WHO estimated the population mean minimum intake of selenium to be 21 μg/day based on work by Yang and colleagues who determined that 19.1 μg Se/day was the mean level of selenium intake in areas where selenium deficiency was not endemic and corrected to this apply to higher body weights. The data used to derive the normative requirements was taken from another study by the same group which examined the relationship between glutathione peroxidase (GPX) activity and selenium intake in adult men. The results showed that a dietary selenium intake of 41 μg/day was sufficient to fully saturate activity of this enzyme in adult men. The level of intake needed to achieve 2/3 saturation was taken to be the normative requirements. It is noted that this two-thirds level, as opposed to the maximum level, is arbitrary but is based on the observation that abnormalities in the ability of blood cells to metabolise hydrogen peroxide becomes apparent only when GPX activity in these cells declines to a quarter of normal. The average normative requirement for the individual 65 kg reference male is therefore 26 μg/day. From this the lower safe range of the population mean intake that will meet the normative requirement is 40 μg/day. Requirements for other groups were derived by extrapolation.

Measures of tissue Selenium levels and Selenium Status

14. Selenium levels can be measured in plasma or serum, whole blood, red cells, hair and nails. Selenium status can also be assessed by measuring tissue GPX activity (Sunde, 1990). When growing animals are fed a selenium deficient diet, a rapid drop in GPX activity occurs, suggesting that selenium stores are being depleted. A level of approximately 0.1μg Se/g diet is necessary to maintain activity levels. Above this level, the activity of the enzyme tends to plateau suggesting homeostatic regulation. Although other measures have been proposed, such as the levels of plasma selenoprotein, GPX activity remains the best measure (Sunde, 1990). Both tissue selenium levels and particularly GPX activity are used to assess the bioavailability of different selenium compounds.

15. Nevé (1995) reviewed data on the effect of different forms of selenium on indicators of selenium status. It was concluded that inorganic forms of selenium increased blood selenium levels more rapidly and to a greater extent than organic forms, quickly reaching a plateau. In contrast, selenomethionine supplementation caused blood selenium levels to increase steadily without reaching a plateau; the results of supplementation with selenium-rich yeast were more variable. The response of erythrocyte selenium to changing selenium status tended to be slower than that of plasma selenium, partly due to slower kinetics but also reflecting the time taken to synthesise erythrocytes. However, no significant differences were apparent in the response of plasma or red cell GPX activity to the different forms of selenium. Platelet GPX activity was more sensitive to the chemical form of the selenium and was saturated at lower plasma selenium levels when selenite or selenate was used than when organic forms were used as supplements.

16. The ability of methylated selenium compounds to replete glutathione peroxidase in selenium deficient rats was investigated (Ip *et al*., 1990). All three compounds tested were able to fully replete the enzyme, though with a wide range of efficiency; Se-methylselenocysteine> dimethylselenoxide> trimethylselenonium, suggesting that complete demethylation to in-organic selenium is a normal process of selenium metabolism.

Function

17. Selenium is an essential trace element. It is necessary for the functioning of the enzyme glutathione peroxidase (GPX), which protects against oxidative damage to intracellular structures. The biologically active form of selenium is selenocysteine. Other selenoproteins have been isolated, these include peripheral terathiodothyronone 5'-I-deiodinase I which converts thyroxine to T3 in the thyroid and other peripheral organs. Selenium status also appears to affect terathiodothyronone 5'-II-deiodinase activity but the enzyme itself (at least in the rat) does not contain selenium. Selenoprotein P is a plasma protein containing 7.5 atoms of selenium per mole protein. It has been suggested that it may have a transport as well as an anti-oxidant function but its precise role is unclear.

Deficiency

18. In animals, the symptoms of selenium deficiency include growth retardation and reproductive failure and dysfunction. Degenerative changes are observed in several organs. Myopathies occur in a number of species particularly ruminants; cardiac myopathy occurs in pigs. Liver necrosis, haemolysis, kidney degeneration and pancreatic fibrosis have also been reported. In animals, combined selenium and vitamin E deficiency may be involved in nutritional muscular dystrophy in sheep and cattle, exudative diathesis in chickens and liver necrosis in swine and rats. In the absence of vitamin E deficiency, the level of dietary selenium needed to prevent deficiency is approximately 20 μg/kg in ruminants and 30 to 50 μg/kg in poultry.

19. In humans, selenium deficiency is thought to be involved in Keshan disease, an endemic juvenile cardiac myopathy occurring in the Keshan region of China where selenium intake is extremely low. The main clinical features are acute or chronic episodes of heart disorder characterised by cardiogenic shock and/or congestive heart failure (Ge and Yang, 1993). The disease is characterised by multiple focal areas of myocardial necrosis without changes in the coronary arteries. Ultra-structural studies have shown that membranous organelles such as mitochondria or the sarcolemma are affected first. Changes in the thyroid have also been noted in Keshan disease. However, other factors may be involved in the aetiology of this disease such as a cardiotoxic virus, mycotoxins in food or other nutrient deficiencies. Keshan disease can be organised into 4 types depending on severity - acute, sub-acute, chronic and insidious. Once the disease is established, selenium treatment is of little or no therapeutic value. The joint and muscle disease, Kashin-Beck disease, also found in the Keshan area, may possibly be connected with selenium deficiency. Advanced cases of this disease are characterised by enlargement and deformation of the joints. The principal pathological change is multiple degeneration and necrosis of the hyaline cartilage tissue. It has also been suggested that selenium is involved in the immune system, cancer and cardiovascular and other degenerative diseases. In China a low selenium intake is reported to be associated with a high incidence of hepatitis B virus infections (Yu *et al*., 1989, Yu *et al*., 1997).

20. It has been suggested that selenium deficiency is involved in the development of atherosclerotic cardiovascular disease. In a number of studies there appears to be a negative correlation between low selenium and cardiovascular disease, though this has not been confirmed in others. The Nordic Project Group concluded that it might only be possible to observe such a relationship at low levels of selenium intake.

21. The reduced activity of GPX in selenium deficient individuals is also accompanied by a reduction in the levels of GPX protein (Sunde, 1990). Conversely during selenium repletion, the increase in GPX activity paralleled the increase in GPX protein.

Overview of reported non-nutritional beneficial effects

22. It has been suggested that increased selenium intake may reduce cancer incidence but the results of epidemiological studies have been conflicting. COMA (1998) recently concluded that there is insufficient evidence for a specific link. Liver cirrhosis, kidney disease, rheumatoid arthritis, coeliac disease, cystic fibrosis and other degenerative diseases have been linked with low selenium levels. However it is uncertain whether this is because selenium is involved in the pathogenesis of the disease or whether low selenium is a result of the disease and/or disturbed protein synthesis. Selenium has also been claimed to affect immune function.

23. Supplementation with selenium and other antioxidant vitamins has been reported to improve symptoms in AIDS sufferers and may slow the progression of the disease (Schrauzer and Sacher, 1994). It has also been suggested that low selenium status may be a contributory factor in male sub-fertility. Studies have found low levels of glutathione peroxidase in patients with acne and other skin disorders. Clinical trials with selenium or selenium plus vitamin E have given positive results (Juhlin *et al*., 1982, Bruce 1986). It has also been suggested that selenium deficiency may cause mood problems and selenium supplementation was associated with a elevation of mood and decrease in anxiety (Benton and Cook, 1991). It has been reported that asthmatics have lower levels of selenium than the "normal" population. Asthmatics receiving selenium supplementation showed a significant increase in glutathione peroxidase levels and significant clinical improvement (Hasselmark *et al*., 1993).

24. In a variety of animal experiments, selenium supplementation decreased the frequency of a variety of chemically-induced or transplanted tumours. The mechanism is uncertain; selenium can alter the metabolic activation of certain compounds, inhibit mutation and provide protection in the later stages of cancer development. Ganther (1999) reviewed the potential mechanisms by which selenium is chemopreventative suggesting that monomethylated forms of selenium are the critical metabolites, acting through the induction of apoptosis of transformed cells. Manifest tumours, however, respond poorly to selenium supplementation and it should be noted that many of the animal experiments used non-physiological doses and species of selenium compounds (Nordic Project Group, 1995). Anti-mutagenic effects (*in vivo* and *in vitro*), have also been reported (reviewed Shamberger, 1987). Finley *et al.,* (2000) reported that selenium from high Se broccoli reduced the number of chemically initiated preneoplastic lesions indicative of colon cancer in rats compared to diets supplemented with selenate, selenite or broccoli alone. The authors speculated that selenium from high Se broccoli, despite being less bioavailable than selenite or selenate, may be metabolised in a manner that diverts much of the Se into the pool that provides protection against colon cancer.

Interactions

Vitamin C

25. Ascorbic acid decreases the uptake of selenite by reducing it to elemental selenium, which is biologically inert. However the reduction of selenite appears to occur only when taken on an empty stomach with no other nutrients (see below).

Iodine Metabolism

26. Selenium is an essential component of the enzyme tetrathiodothyronone 5'-deiodinase I and is thus involved in iodine metabolism. A lack of both selenium and iodine in rats results in severe hypothyroidism and goitre (quoted in Anonymous, 1992). Selenium deficiency can increase hypothyroid stress associated with iodine deficiency. Other data suggest that selenium deficiency impedes urinary iodine loss, thus supplementation with selenium alone may exacerbate the situation where there is combined iodine and selenium deficiency (Nordic Project Group, 1995).

27. The selenium status of subjects living in an area of Zaire where goitre was endemic was lower than that of subjects living in a non-endemic area (Vanderpas *et al.,* 1990) In a moderately affected village, selenium status was moderately reduced. The selenium status of schoolchildren and children suffering from cretinism in the same village was similar. Selenium supplementation for two months normalised both serum selenium levels and erythrocyte glutathione peroxidase activity. The authors considered that the data support the hypothesis that the combined iodine and selenium deficiency could be associated with the elevated frequency of myxedematous goitre observed in Central Africa.

28. The selenium supplementation also resulted in the reduction of serum thyroid hormone levels and an increase in the levels of thyroid stimulating hormone (TSH) in hypothyroid subjects (Contempre *et al.,* 1991). However, in normal subjects, serum T4 (thyroxine) levels decreased but TSH did not increase. It was uncertain whether the main effect of selenium supplementation was on thyroid or extrathyroid tissue. In experiments in rats, selenium supplementation corrected thyroid GPX deficiency and decreased serum T4 levels presumably by diminishing the availability of the peroxide substrate for T4 synthesis. The extra-thyroid model also demonstrated experimentally that selenium supplementation increases the conversion rate of T3 (tri-iodothyronine) to T4, inducing a decline in serum TSH levels. Both mechanisms lead to a decline in T4 following selenium supplementation, but the combination might explain why TSH levels in normal subjects remain stable. It is proposed that in a severe iodine deficiency area, selenium deficiency might mitigate the severity of the deficiency and protect the foetus against brain damage and neurological cretinism but that it would also favour thyroid destruction and myxoedematous cretinism. In contrast, selenium supplementation did not decompensate thyroid hormone synthesis of euthyroid female subjects with reduced thyroid iodine organification (Roti *et al.,* 1993). The subjects had experienced a previous episode of subacute or post partum thyroiditis. It was suggested that the derangement of thyroid hormones on selenium supplementation might only occur in subjects with minimal residual thyroid hormone production and very low

iodine intake. Thus the lack of effect might be because the subjects had an adequate selenium intake before supplementation and only a marginally reduced dietary iodine intake.

29. In further work by Contempre *et al*., (1995) iodine and iodine and selenium deficient female Wistar rats were treated with an acute dose of iodide. In the iodine deficient animals, necrosis was common in the thyroid and this was increased by the acute iodine dose. Before the overload dose was given, the proportion of dividing cells in the thyroid was equal in both groups. Following the iodine overload dose there were four times fewer dividing epithelial cells in the rats deficient in both elements compared to those only deficient in iodine. The authors concluded that selenium deficiency coupled to iodine deficiency increased necrosis, induced fibrosis and impeded compensatory proliferation of epithelial cells.

Metallic compounds

30. Selenium interacts with metallic compounds, generally reducing their toxicity by forming inert metal selenide complexes.

Xenobiotic agents

31. Selenium status may affect the toxicities of xenobiotic agents (Combs and Combs, 1987). Selenium deficiency increases the toxicity of agents such as paraquat and nitrofurantoin and decreases the toxicity of acetoaminophen, aflatoxin B1 and iodoipamide. Selenium supplementation of selenium adequate animals also appears to provide protection against the toxicity of xenobiotics. The mechanisms involved are uncertain but appear to depend on the metabolism of the xenobiotics concerned and hence on the different effects of selenium dependent factors.

Absorption and Bioavailability

32. Absorption and thus bioavailability can be affected by the physical or chemical form of the selenium compound or the dosing regimen. In general, the degree of selenium absorption is independent of the exposure but in some instances, absorption can be greater where selenium deficiency exists. It is thought that 55-60% of the selenium in food is absorbed following ingestion.

Human

33. Selenium compounds are readily absorbed from the human gastro-intestinal tract (reviewed ATSDR, 1996). Human volunteer studies suggest that there is greater absorption of selenate and selenomethionine than selenite. For example, in three human volunteers, (Thomson and Stewart, 1974) 44 to 70% of a 10 μg dose of ^{75}Se labelled selenite was absorbed. Absorption of 94-97% of a 1 mg dose of selenite and selenomethionine respectively has also been reported (reviewed ATSDR, 1996).

34. A group of 48 Norwegian women were given a 200 μg supplement of selenium in the form of selenite or selenium-rich pea flour (Meltzer *et al.,* 1990). The supplements were taken with either placebo or ascorbic acid in a double-blind design. Indications of an increase in blood levels of selenium were seen but these were only significant in the group taking selenite and ascorbic acid. Analysis of 72 hour urine samples indicated that approximately 50% of the supplement had been absorbed. After at least a 5 week break, 28 of the subjects entered phase 2 of the trial in which selenite or pea flour supplements were given with a placebo or a vitamin E supplement. Commercially available 200 μg supplements of selenium (consisting of selenium-rich yeast) were given to two additional groups. Of these supplements, only one (a commercial preparation of yeast selenium containing vitamins A, C and E) produced a significant increase in serum and blood selenium levels. The addition of vitamin E did not have any effect on blood selenium levels or GPX activity. Blood GPX activity was not affected by any of the form of supplementation.

35. Plasma selenium levels were lower in 14 US infants fed formula milk compared to 31 infants fed human milk (McGuire *et al*., 1993b). The precise data are not provided but the figure indicates that over weeks 4-12 of age, plasma selenium was 400- 500 nmol/l in formula fed infants and 850-900 nmol/l in infants fed human milk. A further 14 infants were fed formula supplemented with selenite (0.253 μmol Se/l); mean plasma selenium was in the range 750-850 nmol/l. Plasma GPX activity at 4 and 8 weeks was also increased by the addition of selenite. In a second study, infants were fed human milk from women supplemented with 0 or 200 μg Se as selenomethionine or selenium-enriched yeast. Over the 12 week period, plasma selenium levels in the infants (approximately 850-900, 850-1200 and 800-1100 nmol/l in the unsupplemented, selenomethionine and enriched yeast groups respectively) matched selenium intakes but GPX activity was not related to selenium intake. Urinary selenium excretion was higher in both infants fed human milk and those receiving selenium supplemented formula compared to formula fed infants. It was also higher in the group fed human milk from women supplemented with selenomethionine. The levels of Selenium in human milk declined over the 12 weeks of the study. Erythrocyte selenium levels in the infants also declined in all groups except those fed human milk from supplemented women, where the levels were stable.

Animals

36. Efficient absorption of selenium by animals is also apparent (reviewed ATSDR, 1996). In rats, 80-100% of dietary selenium given as selenite, selenate, selenomethionine or selenocysteine was absorbed. For example, absorption of 4 μg/ml selenium as selenite by male Sprague Dawley rats was nearly complete, with only 10% of the ingested Se being detected in the faeces (Janghorbani *et al.,* 1990). A similar degree of absorption was reported in mice and dogs. Absorption of selenite by rhesus monkeys is less marked that that of selenomethionine. In rats there is little difference, absorption of both being high (Butler *et al*., 1990).

37. Absorption of selenium compounds occurs in the small intestine rather than the stomach (ATSDR, 1996). In *vitro* investigations using everted hamster intestinal sacs suggest that selenomethionine is transported against a concentration gradient with the same characteristics as methionine (Spencer and Blau, 1962). Absorption does not appear to be homeostatically controlled since no differences were observed between selenium deficient and selenium sufficient rats administered moderately toxic doses of selenium (reviewed NTP, 1994).

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

Distribution

39. Absorbed selenium is rapidly distributed and does not accumulate in any specific organs although the concentration is higher in the liver and kidney. The pattern of distribution is similar for both organic and non-organic selenium. Tissue and blood levels reflect dietary intakes. Selenium from sodium selenite and selenate is found in the highest concentrations in the liver and kidneys of both humans and animals following oral exposure.

Human

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

41. Following a 10 μg dose of ^{75}Se-labelled selenite given to three human volunteers, (Thomson and Stewart, 1976) turnover in selected body tissues (liver, heart, kidney, skeletal muscle and bone) was measured by whole body counting. Turnover rates were highest in the liver, particularly within the first few days following dosing. The ^{75}Se turnover curve for the heart was similar to that for blood, indicating that it was the turnover of the cardiac blood pool that was being measured. Turnover rates in skeletal muscle and bone were lower than in liver and blood. The level of ^{75}Se in the plasma decreased rapidly, one day after dosing indicating utilization of post-hepatic selenium by the tissues or incorporation into rapidly metabolised plasma proteins. The initial phases of whole body turnover were followed by a gradual disappearance of the radioactivity, which appeared to represent metabolic turnover and excretion of the ^{75}Se label which had become incorporated into a long-term selenium pool.

42. In a study by Meltzer *et al.,* (1992) serum Se was found to increase in a dose dependent manner following the consumption of 100, 200 or 300 μg Se/day in bread for six weeks by female volunteers. Serum Se increased by 20, 37 and 53 μg/l respectively (the range at the start of the experiment was 115-130 μg/l). At the end of the study, 50% of the administered selenium was excreted in the urine compared to 67% at the start of the study. The renal clearance results and the response in blood levels were considered to compare well to data from non-replete populations. The authors concluded that it supported the hypothesis that selenomethionine from the diet (wheat and yeast are the most significant dietary sources of selenomethionine) was incorporated into a non-specific amino acid pool.

43. The tissue selenium concentration in males (Swanson *et al* 1990) did not reflect their higher intake from food. Since there is no evidence that absorption of selenium is lower in males or that excretion is higher, it was concluded that the difference in muscle mass, and hence the muscle pool of selenium, could be responsible. Age was not associated with tissue selenium content.

44. Following oral exposure, selenium can be found in human milk (ATSDR, 1996, McGuire, *et al*., 1993a). The levels of selenium in human milk declined in the 20 weeks post parturition (McGuire *et al*., 1993a). Supplementation with selenomethionine increased the levels present in the milk whereas supplementation with selenium enriched yeast arrested the decline in levels. Plasma GPX activity also decreased in supplemented women. Selenomethionine supplementation increased plasma selenium levels in both lactating and non-lactating women, but the selenium-enriched yeast increased plasma levels in non-lactating women only.

45. Finley *et al*., (1999) investigated the effects on selenium retention in individuals with low dietary selenium intakes following selenium supplementation for 6 months. 29 females and 15 males in New Zealand with background dietary intakes of 30 μg/day consumed 100 μg ^{74}Se before being supplemented with 0, 10, 20, 30 or 40 μg/Se (as selenomethionine) for 6 months. Subjects were administered a 2nd dose of 100 μg ^{74}Se during the last 3 weeks of

supplementation. The distribution of ^{74}Se in plasma (which reflects short term intake), platelets and RBCs (which both reflect long term intake) was assessed at the beginning and end of supplementation. The authors reported that Se supplementation had relatively little effect on the retention of ^{74}Se, especially by tissues that reflect long term intake. The authors speculated that this may have been because subjects had adapted to their normal relatively low Se intake, perhaps through increased retention of selenium.

46. Finley (1999) investigated the absorption and distribution of stable isotopes of selenium consumed as selenate (^{74}Se) or broccoli (^{82}Se). 27 young males consumed either a low (32.6 μg/day) or high (227 μg/day) selenium diet for 105 days. On day 85 they were fed a test meal containing 100 μg ^{82}Se and 11.5 μg ^{74}Se (^{74}Se was also added in the form of selenite but absorption and distribution was so variable it was not analysed statistically). Both absorption and urinary excretion of stable isotopes was significantly higher in subjects on the high selenium diet. Also more ^{74}Se was excreted in the urine than ^{82}Se; as a result more Se was retained when the selenium source was broccoli than selenate. However, despite the greater retention, less ^{82}Se was present in plasma than ^{74}Se, indicating that the tissue distribution of selenium from the two sources differs.

Animal

47. Selenium from selenomethionine tends to be retained in the tissues at higher concentrations and for longer periods of time than selenium from inorganic selenium compounds (Butler *et al.,* 1990, reviewed ATSDR, 1996). This appears to be due to slightly increased absorption of selenomethionine as well as slower elimination as a consequence of incorporation into body proteins. Liver, muscle, kidney and blood concentrations of selenium were higher in rats given selenium orally as selenomethionine compared to selenite (Behne *et al.,* 1990). This was also apparent for plasma, erythrocytes, nails and hair (Salbe and Levander, 1990). In the rats fed selenomethionine, the levels of selenium in hair and nails increased as Se retention in muscle and liver increased with dose. However in rats fed selenite, the levels of Se in liver, hair and nails increased with dose but the levels in skeletal muscle did not. This is discussed in more detail below.

48. Selenium-deficient Wistar rats were dosed with ^{75} Se-labelled selenium in the form of selenite or selenomethionine at doses equivalent to 0.2 or 2 mg Se/kg diet (Behne *et al.,* 1990). Selenium levels in muscle and liver became elevated after consumption of the higher concentration of selenium. More selenium was retained following consumption of selenomethionine than selenite. Analysis of tissue proteins indicated that the higher tissue levels were as a result of non-specific incorporation into a large number of proteins. There were no differences between the two forms of selenium with regard to specific selenoproteins and the 10-fold increase in concentration resulted in only a small increase in the quantity of these proteins. The authors concluded that during consumption of normal amounts of selenium as selenite, the majority is incorporated into specific selenoproteins; however some non-specific incorporation also occurs. Part of the ingested quantity of selenomethionine follows the same metabolic route, but non-specific incorporation into protein in place of

methionine also occurs. In rats fed selenium as selenite or selenomethionine, retention of selenium in hair and nails was greater in rats also fed a restricted methionine diet (Salbe and Levander, 1990).

49. Finley (1998) investigated the ability of different forms of selenium (0.1 mg/Se kg diet) i.e. sodium selenite, sodium selenate, selenomethionine, and broccoli with high selenium levels; to replete selenium deficient rats (fed selenium deficient diets for 6 weeks). Se concentrations were determined in liver, liver cytosol, muscle, kidney, plasma, and RBCs and GSH-Px activity in liver cytosol and whole blood following Se repletion up to a maximum of 9 weeks. Selenium concentrations were also determined in faeces and urine, the authors reporting that the absorption of selenium from high Se-broccoli was less than from other forms and had a higher rate of urinary excretion. High Se-broccoli (even after adjustment for differences in absorption) was less effective than the other forms of selenium in restoring most measures of selenium status (there was little difference in kidney and plasma concentrations) and GSH-Px activity. The author concluded that these differences are because the metabolic pathway used by selenium from high Se-broccoli is different from the pathways used by other forms of Se.

50. In rats given drinking water supplemented with 0.5, 2, 6 or 15 ppm selenium (equivalent to 0.05, 0.2, 0.6 or 1.5 mg/kg bw/day- WHO, 1987) as selenite for up to 6 months (Crespo *et al*., 1990) plasma selenium levels were elevated at intakes above 0.5 ppm. There was a significant correlation between plasma selenium and selenium intake for the first month of treatment but this declined thereafter. The correlation between liver selenium and selenium intake was highly significant throughout the study.

51. Male weanling rats were injected with ^{75}Se-labelled selenium in the form of sodium selenite or selenomethionine (Beilstein and Whanger, 1986). Analysis of erythrocyte lysate by gel filtration chromatography revealed four labelled fractions: void volume proteins, GPX, haemoglobin and low molecular weight materials. In the selenite treated rats, the void volume proteins contained largely ^{75}Se-labelled selenocysteine. In the selenomethionine treated animals the label was initially present in both selenomethionine and selenocysteine, but by day 20, selenocysteine was the predominant form. GPX contained ^{75}Se in the form of selenocysteine in both treated groups. In haemoglobin, the label was present in selenomethionine and selenite treated animals as selenomethionine and two unidentified forms respectively. In liver hydrolysates, ^{75}Se was largely recovered as selenocysteine from the selenite treated animals. Although labelled selenomethionine was present in the selenomethionine treated animals, by day 5, selenocysteine was the predominant form. No differences were found in the deposition of the two forms of ^{75}Se in liver, kidneys, testes or plasma; approximately 3-fold greater retention of ^{75}Se was apparent in the muscles of selenomethionine treated animals.

52. Plasma and erythrocyte Se levels were higher in female Rhesus monkeys fed selenomethionine compared to those fed selenite for eleven months (Butler *et al* 1990). However, there were no differences in liver or muscle GPX activity. Approximately 34% and 64% of erythrocyte Se was associated with GPX activity in the selenite and

selenomethionine treated animals respectively. The correlation between blood selenium levels and erythrocyte activity was 0.94 in monkeys fed selenite and 0.37 in those fed selenomethionine. Gel filtration of plasma showed one major Se peak in the selenite treated animals but at least two in the selenomethionine treated animals. One of these peaks was thought to correspond to selenoprotein P.

53. In adult female macaques treated with oral selenomethionine (Willhite *et al.,* 1992) plasma, erythrocyte, hair and urinary Se levels were largely dose dependent. Plasma Se reflected more immediate Se exposure, while erythrocyte levels increased and decreased more slowly. Total tissue Se increased 13-28 fold in animals given the equivalent of 600 μg Se/kg (this was lethal to 2/4 animals in this treatment group) for 10-15 days with the liver and kidneys containing the highest Se concentration. It is noted that plasma and erythrocyte GPX activity did not vary with time or dose following a single dose of selenomethionine, however the data are not provided. In the continuous dose study (maximum dose equivalent to 300 μg Se/kg) erythrocyte but not plasma GPX activity showed a steady, dose related, increase. This was equivalent to twice the pre-dose activity and persisted for at least 13 days post-treatment.

54. Selenium has been found to cross the placenta and enter the foetus in a number of laboratory animal species (reviewed Bopp *et al.,* 1982).

55. Following oral exposure, selenium can be found the milk from mice, rats, dogs and monkeys (reviewed ATSDR, 1996).

Metabolism

56. Selenium metabolism is thought to be comparable to that of sulphur. The metabolic pathways of selenium are summarised in Figure 1 (adapted from Sunde, 1990).

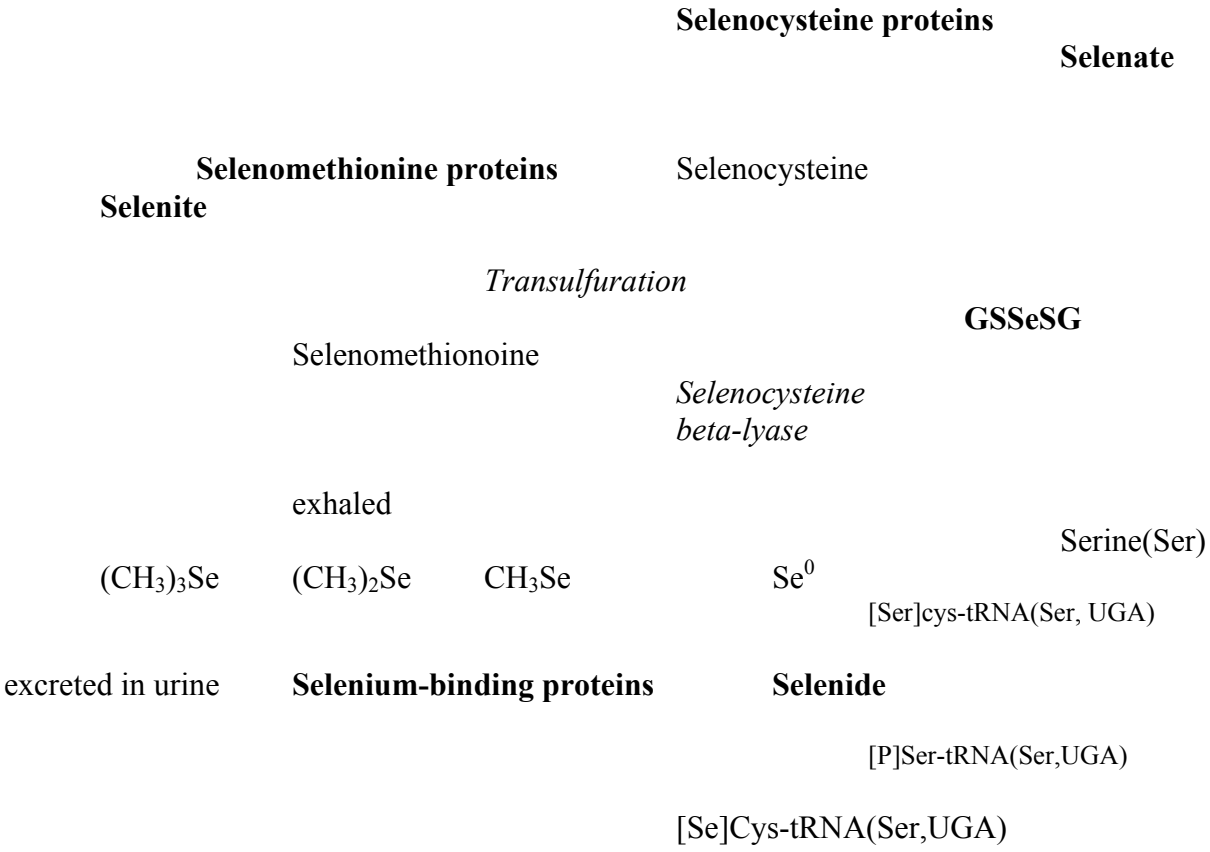
57. Selenomethionine is shown to follow the methionine transamination pathway though it is possible that an enzyme like L-methionine-γ-lyase might release methane selenol. Selenocysteine, however, does not follow cysteine metabolism with the oxidative release of selenite. Instead the enzyme selenocysteine lyase releases elemental selenium which is reduced to selenide.

58. Selenite is shown in a reduction pathway leading to selenide. Methylation of selenide leads to the formation of methylated selenium derivatives that are excreted in the breath and the urine. The methylation reaction proceeds as below (reviewed NTP, 1994). The formation of dimethyl selenide from selenite requires the presence of glutathione and is stimulated by NADPH. The following stages occur: 1) non-enzymatic reaction between selenite and glutathione to form the selenotrisulphide derivative; 2) reduction of the selenotrisulphide derivative to selenopersulphide non-enzymatically in the presence of excess glutathione or by means of NADPH and glutathione reductase; decomposition of selenopersulphide to hydrogen selenide by the glutathione reductase system, and; 4)

methylation of hydrogen selenide by methyltransferase to form dimethyl selenide. Trimethylselenonium is the urinary metabolite of selenite and is thought to be formed by the addition of a third methyl group to dimethyl selenide. Erythrocytes take up and metabolise selenite, and then transport the selenium back into the plasma as selenide (reviewed, Sunde, 1990). Selenide may be the common intermediate in the metabolism of the amino acid and inorganic forms of selenium.

59. Methylation and excretion of selenium in rats is limited by vitamin B12 deficiency (Chen *et al* 1993). Rats were fed a basal diet supplemented with 5, 7 or 9 mg/kg selenium as selenite and with or without vitamin B12. In the animals fed the vitamin B12 deficient diet, significant histological changes were apparent in the liver. In the rats in the top dose group with vitamin B12, lower selenium levels were measured in the liver and kidney, but blood levels were higher, suggesting that deficiency of vitamin B12, a co-factor of methionine synthetase, reduced excretion and increased tissue levels of the element. Primary hepatocytes from vitamin B12 deficient rats volatilised 15% of the selenite in the incubation medium in 5 hours compared to volatilisation of 49% in the control rats (Chen and Whanger, 1993). *In vitro* methylation of selenium with liver extract occurred at one third to one half of the rate in control rats. The reduction in methylation is thought to occur because the methyl donor *S*-adenosylmethionine is necessary for selenium methylation; vitamin B12 is important in its synthetic pathway.

Figure 1. The Metabolic Pathways of Selenium (adapted from Sunde, 1990)



Adapted from Sunde, 1990

60. The model described by Sunde (1990) leads to classification of selenoproteins into four groups. These are selenomethionine or selenocysteine specific proteins, where selenomethionine or selenocysteine is deposited into the peptide chain during translation. There are selenium specific proteins such as glutathione peroxidase, which contain selenocysteine but obtain the selenium moiety from selenide and serine precursors, and selenium binding proteins. The selenocysteine moiety of the selenium specific proteins is specifically coded for by the codon UGA. In addition to the GPX family, the UGA codon for selenocysteine has also been found in the gene for type 1 iodothyronine deiodinase (reviewed Cohen and Avissar 1993).

61. Animals are unable to synthesise selenomethionine directly from inorganic selenium (reviewed Sunde, 1990). Selenocysteine in the tissues of animals fed only inorganic forms of selenium may arise solely from the synthesis of GPX and other selenoproteins. In the rat liver, selenocysteine can be synthesised from selenomethionine by the enzyme cystathionase, but no evidence has been found for the formation of selenocysteine from selenide. It has been reported that up to 80% of body's Se may be in the form of selenocysteine and that this may be synthesised in conjunction with the synthesis of other selenoproteins. It has been reported (Hasegawa, 1995) that following an oral dose of seleneocysteine, a selenocysteine-containing metabolite is produced in the small intestine as a result of a reaction with glutathione. The metabolite is incorporated into blood plasma and transported to the liver.

62. Selenomethionine is readily incorporated into rat liver protein since the formation of selenomethionine transfer RNA is only slightly less favourable than the formation of methionine transfer RNA. This is thought to explain why supplementation with selenomethionine results in a more marked increase in erythrocyte selenium levels than in GPX, whereas supplementation with selenite results in a parallel increase in both parameters.

Excretion

63. The main route of selenium excretion in the rat is urine, but volatile metabolites, which are excreted into expired air, are also produced. Dimethyl selenide present in expired air is responsible for the garlic odour in the breath of animals poisoned with selenium. (Burk *et al.,* 1971). The production of the urinary metabolite trimethylselenonium is directly related to dietary selenium intake and the conversion of dimethylselenide to trimethylselenonium is the rate limiting step under conditions of selenium excess. Where large doses of selenium are given, dimethylselenide accumulates and is exhaled. Studies using radiolabelled selenium in human volunteers indicate that there is early urinary excretion of ingested selenium compared to native plasma pools (Janghorbani *et al.,* 1981).

64. Small quantities (up to about 10%) of selenium are excreted into the bile of rats and dogs (reviewed Bopp *et al.,* 1982). Although the primary route of excretion is via the urine,

appreciable quantities of selenium are also excreted in the faeces (Bopp *et al.,* 1982). This becomes more prominent when the selenium is administered chronically. When given as a tracer dose, the major route of selenium excretion is the urine. However, approximately equal proportions of naturally occurring dietary selenium are excreted via the urine and the faeces (Nordic Project Group, 1995).

Humans

65. A 10 μg dose of ^{75} Se labelled selenite was rapidly excreted in the urine of three female volunteers (Thomson and Stewart, 1974). Peak excretion occurred within 2 hours. 7 to 14% of the dose (14-20% of the absorbed tracer) was excreted in the urine in the first 14 days following dosing. 3-4% of the dose (4-6% of the absorbed tracer) was excreted in the faeces in the 14 days following dosing. There was no evidence for excretion of selenium in sweat.

66. Approximately 15% of a 200 μg dose of selenomethionine was excreted by human volunteers in the twelve days following dosing (Swanson *et al*., 1991).

Animals

67. In rats administered 4 μg/ml Se as selenite in drinking water for 30 days, a rapid rise in urinary selenium occurred (Janghorbani *et al*., 1990). This reached a plateau within a few days of dosing and accounted for approximately 54% of the intake. Excretion of trimethylselenonium increased from 2% to 35-40% of urinary Se in the control and treated animals respectively. Calculation of selenium balance indicated that 35% of the ingested Se could not be accounted for by urinary or faecal losses or retention in the carcass. This suggested that other excretory pathways might occur. These pathways could include the loss of volatile selenium compounds in the breath.

68. In female macaques treated with oral selenomethionine (Willhite *et al.,* 1992), the maximum rate of Se excretion occurred within the first 3-12 hours of dosing regardless of the duration of exposure. The rate of Se elimination was constant in the subsequent 12-24 hours for the first 15 days, but increased relative to the controls in days 16-30. Faecal Se elimination increased with dose and exposure, the highest concentration generally being found 12-24 hours after dosing

Toxicity

69. The toxicity of different selenium compounds varies. Selenite, selenate and selenomethionine are among the most toxic selenium compounds whereas selenium sulphide is much less toxic due to its insolubility. Selenium toxicity is cumulative.

Human Toxicity

Acute Toxicity

70. The acute effects of selenious acid toxicity have been summarised by Mack (1990). Patients suffer from hypersalivation, repetitive copius emesis (which may contain blood), diarrhea and garlic odour to the breath. Burns and erosions may occur in the mouth and upper gastro-intestinal tract. Extreme restlessness, muscle spasms, tachycardia, pulmonary oedema and toxic cardiomyopathy may also be experienced. In some patients, rather than hypertension, a state of severe shock develops, possibly as a result of decreased contractility secondary to the cardiomyopathy as well as lowered peripheral vascular resistance. Stupor, respiratory depression and death can occur several hours post ingestion.

71. A 3 year old child was fatally poisoned following ingestion of Gun Blue, a lubricant containing selenious acid (Carter, 1966). The child was admitted in a deep coma with peripheral circulatory failure and died 45 minutes later. Salivation and a strong smell of garlic on the breath were also noted. At autopsy, grey mucoid fluid was found in the stomach and small intestine. The lungs were diffusely and intensely congested and oedematous and contained widespread focal haemorrhages. The heart was normal with the exception of tight contraction of the ventricles and the brain was slightly oedematous. The liver parenchymal cells were packed with glycogen and slight lipoid accumulation was observed in the Kupffer cells. Similar findings were reported following a fatal suicidal ingestion of Gun Blue (Matoba *et al.,* 1986). It was thought that approximately 90 ml of the solution (which contained 4% selenious acid and 2% copper sulphate in a hydrochloric acid carrier) had been ingested. Tissue selenium levels were estimated to be 9-90 times those in normal subjects, the highest levels being found in the stomach contents, lung, liver and kidneys (18.0, 12.7, 5.4 and 14.2 mg/kg wet weight respectively).

72. Diarrhoea was observed in a 15-year old girl who had swallowed sheep drench containing a dose of approximately 22 mg Se/kg. This occurred despite the induction of vomiting shortly after exposure (Civil and McDonald, 1978). Serum bilirubin and alkaline phosphatase levels were elevated indicating possible liver damage; the elevated bilriubin persisted for six days. An ECG showed diffuse T-wave flattening consistent with a generalised myocardial abnormality. Serial ECGs demonstrated definite T-wave inversions in the interior and lateral leads along with a prolonged Q-T interval, consistent with some form of anterolateral myocardial damage. These findings were at a maximum, three days after exposure and gradually resolved over two weeks.

73. A patient thought to have been criminally poisoned with an unspecified quantity of Gun Blue presented with a sudden onset of profuse watery diarrhoea (Ruta and Haider, 1989). This was followed by weight loss, cramping, alopecia and a purple-red colouration at the base of the fingernails. The patient was also dehydrated and mildly jaundiced; after some weeks, the patient was too weak to leave bed. A full recovery was made subsequently.

74. Five men consumed an unknown quantity of selenium in dietary supplements and developed nausea, diarrhoea, abdominal pains, chills and tremor. Serum levels were 400 - 800 μg/l and 1175-4365 μg selenium was excreted in the following 24 hours (quoted Nordic Project Group, 1995). Ingestion of breakfast cereal containing 120 mg selenite and 230 mg unknown selenium compounds caused vomiting, diarrhoea, cramps and a numb sensation in the arms of a female patient. On recovery, the patient had irregular menstrual cramps and marked hair loss (quoted Nordic Project Group, 1995).

75. Consumption of super-potent selenium supplements containing 27.3 mg Se/tablet (the declared quantity was 150 μg) led to marked hair loss by day 11 of treatment leading to almost total alopecia by two months (Jensen *et al.,* 1984). Following approximately one month of treatment horizontal streaking, swelling and purulent discharge from the nail bed was observed in some fingertips. Over a three-week period the effects occurred in all fingernails, with the complete loss of the fingernail on the left fifth digit. Episodes of nausea, vomiting, breath odour and fatigue were also reported. Following cessation of consumption approximately 10 weeks later, serum selenium was reported to be 528 ng/ml, approximately 4 times higher than normal. No long term follow up is reported.

Chronic Toxicity

76. A number of adverse effects have been reported in subjects living in selenium-rich areas. These include gastrointestinal disturbances, icteroid discolouration of the skin, decayed teeth and pathological changes in the nail, loss of hair and dermatitis. Endemic selenosis in China has been studied by Yang and colleagues (1983). The incidence of morbidity was approximately 50% in villages with a daily intake of approximately 5000 μg Se/day. The main symptoms observed included brittle hair with intact follicles, new hair with no pigment, thickened nails and brittle nails with spots and streaks. In more severe cases fluid effused from the nail bed. Skin lesions were common on the backs of hand and feet, the outer sides of the legs, the forearms and the neck. Skin was red and swollen followed by blistering and eruptions. Neurological symptoms (peripheral anaesthesia, acroparasthesia, pain and hyperreflexia; numbness, convulsions and paralysis developed at a later stage) were also observed in 18 of the 22 inhabitants of one particularly affected village. One fatality was reported, with symptoms such as loss of appetite, headaches, dizziness, numbness and hemiplegia (paralysis of one half of the body) being reported. Subjects recovered on evacuation from the area and the consequent change in diet. Intake of selenium was estimated to be 3200 to 6690 μg/day. The mean hair selenium level in the selenosis area was 32.2 μg/g (0.41 μmol/g), the mean blood level 3200 μg/l (40.5 μmol/l) and the mean urine level 2680 μg/L (33.9 μmol/l).

77. Yang *et al.,* (1989a) also investigated a variety of parameters in subjects in areas where there were low, medium or high selenium intakes (the selenium content of the soil was 0.37 to-0.48, 0.73 to 5.66 and 7.06 to 12.08 mg/kg respectively). It was estimated that average daily selenium exposures (based on lifetime exposure) were 70, 195 and 1438 μg for adult males

and 62, 198 and 1238 μg for adult females respectively. When the increasing range of intake was compared to corresponding tissue (whole blood, hair, fingernail, toenail, breast milk and urine) levels it was found that whole blood selenium reflected most closely the physiological range of selenium intake. However, at higher levels of selenium intake (1238.5 and 1438.2 μg/day in females and males respectively) hair, fingernail and toenail levels reflected the range of intake more closely. It was suggested that these may act as excretory organs when excess amounts of selenium are ingested.

78. In a follow up study in 400 individuals, the relationship between blood Se concentrations, dietary Se intake and the clinical signs of selenosis was explored (Yang *et al.,* 1989b). Morphological changes in the fingernails were used as the main diagnostic criterion and this largely occurred in adults. There was no correlation found between blood selenium and clinical signs because of the day to day variation in selenium intake. However, persistent symptoms of selenosis were observed in five subjects with blood levels of 1.054 to 1.854 mg/l. A regression equation (details not provided, but presumably the one described in Yang *et al.,* 1989a) was then used to calculate the corresponding level of selenium intake, which was found to be greater than 910 μg Se/day. It was considered by the authors that the minimal blood Se concentration of 1.054 mg/l would have to be taken as approximately the marginal level of selenium toxicity since such a level could not be tolerated by these susceptible subjects. No comment is made about the lack of correlation in the study, presumably because previous work (Yang *et al.,* 1989a) demonstrated a significant correlation between annual average whole blood selenium and annual average dietary selenium. It was observed that the incidence of selenosis was lower in the younger age groups, even though selenium intake per kg bodyweight and blood selenium levels were higher in these groups compared to affected adults. Yang suggested that since the children had not experienced the heavy prevalence of selenosis that had occurred 20 years previously due to an outbreak of Se intoxication, they may be less susceptible. Thus, the level of selenium intake not producing clinical signs among subjects in a high Se area does not necessarily mean such intakes are safe, while the blood level that cannot be tolerated by the susceptible cases would be closer to the marginal level of Se toxicity. Whether this would apply to areas where selenium intakes are traditionally much lower e.g. UK, is not discussed. However, as noted in Yang *et al.,* (1989a) at high selenium intakes, concentrations in integumentary tissues reflect high intakes more closely than whole blood concentrations. Thus it is conceivable that those older subjects exhibiting selenosis have a higher selenium status than the younger subjects and are exhibiting the signs resulting from long term selenium intoxication. A range of biochemical parameters were also measured by Yang, who reported that prolonged plasma prothrombin time and decreased glutathione levels were observed at dietary intakes in excess of 750 to 850 μg Se/day corresponding to blood levels of 1 mg/l Se. Overall, Yang *et al.,* concluded that 400 μg/day was the maximum safe daily intake of selenium (applying a safety factor of 2). In a subsequent follow up study (Yang and Zhou, 1994) a decrease in average blood selenium levels from 1346 to 968 μg/l had occurred. This was associated with an absence of clinical signs of selenosis. The corresponding safe selenium intake was equivalent to 816 ± 126 (approximately 800 μg/day), which was suggested to be the mean NOAEL, the lower limit of the confidence interval

being equivalent to 600 μg/day, the approximate maximum individual safe selenium intake. Based on these data, Yang and Zhou confirmed the previous recommendation that 400 μg/day was the maximum safe daily dietary intake.

79. In a study by Swanson *et al*., (1990) of 44 adult subjects in a seleniferous area of the US, no adverse effects were reported at average intakes of up to 174 μg Se/day (range 42 to 444 μg Se/day). Selenium intake during the year of the study was assessed by duplicate meal collection on 2 non-consecutive days each quarter.

80. In the study conducted by Longnecker *et al*., (1991), 142 subjects from areas of high selenium intake in the US were followed for a two year period (in the first year, 78 subjects were enrolled and in the second year an additional 64 were enrolled). Some of the participants were randomly selected but others were ranchers from ranches where selenium toxicity had occurred. The mean age of the subjects was 50 (range 22-82 years) and 47% were male. Subjects completed health questionnaires, underwent physical examinations (observations included a general health assessment with special attention given to abnormalities reported with by other workers including loss of nails, neurologic abnormalities and abnormal liver function tests) and provided blood samples for clinical analysis. Samples of blood, urine, toenails and duplicate-plate food collections were taken for analysis of the selenium content. The average selenium intake was 239 μg/day (the range 68 to 724 μg/day). About half of 142 subjects had selenium intakes greater than 200 μg/day. Physical findings of selenium toxicity or clinically significant changes in laboratory tests were not present even in subjects with a selenium intake as high as 724 μg/day. In this study, no association was found between pro-thrombin time and selenium intake but a positive association between selenium intake and serum alanine aminotransferase (ALT) levels was apparent. However, the values were within the reference range and the finding was not considered to be clinically significant. The author quotes data from a Danish study which showed that in a small group of arthritic patients receiving 250 μg selenium/day as a supplement, serum somatomedin C was reduced compared to the placebo group; however this was not observed in the Longnecker study.

Human Supplementation studies

81. In a study by Longnecker *et al.,* (1993) groups of 4 healthy males were fed bread (2 slices/day) containing control, medium or high dose levels of selenium for 1 year to examine the utility of using toenail samples as a marker of selenium intake. The bread contained 32.4, 206 or 388 μg selenium per 2 slices respectively. Prior to the study, the subjects were consuming approximately 80 μg Se/day. The authors concluded that toenail samples could be a useful alternative to blood when a measure of long-term average selenium intake was desired. No adverse effects were reported in the study but the subjects do not appear to have undergone any formal examination.

82. In an investigation 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 or

selenium-poor wheat. In the treatment group the bread provided 200 μg Se/day per subject. No adverse effects were reported, though this information was not specifically sought. Although platelet GPX activity was increased in the treated group compared to the controls, platelet aggregation was not increased. In a study by Meltzer *et al.,* consumption of 100, 200 or 300 μg Se/day in bread for six weeks by female volunteers was not associated with any adverse effects.

83. In a randomised, double-blind, placebo-controlled study by Clark *et al.,* (1996), the effect of selenium supplementation on the prevention of skin cancer was investigated. 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/day selenium or a placebo for up to 10 years (mean duration 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 ng/ml, which was at the lower end of the range of normal plasma levels reported in the US. Plasma selenium levels in the placebo group remained constant throughout the study. In the treatment group, plasma selenium levels rose to 190 ng/ml within 6-9 months of beginning treatment. The safety end points investigated included known signs of frank selenosis including garlic breath, pathologic nail changes and brittle hair. Patients were assessed every 6 months and the authors observed no dermatological or other signs of selenium toxicity.

84. Several studies report on the selenium supplementtion of elderly subjects, who tend to have a lower selenium status than healthy young subjects; Simonoff *et al*., (1992) attribute this to reduced dietary intakes in the elderly. For example, Simonoff *et al*., (1992) reported plasma selenium concentrations in healthy elderly subjects of 66 ng/ml compared to 83 ng/ml for healthy young adults. Following 1 month 120 μg/day selenium supplementation of young (30 years old), middle aged (45 years old) and elderly (82 years old) subjects, the increase in plasma selenium concentration in the first two groups was less than 20% but >80% in the elderly group. Bortoli *et al.,* (1991) supplemented 67 elderly female subjects (>80 years of age) for 30 days with 66 μg/day. Plasma selenium concentrations significantly increased following supplementation; there was also a slight increase in RBC GSH-Px activity. No adverse side effects were reported.

85. Peretz *et al*., (1991) supplemented 22 elderly subjects (>66 years of age) with either placebo or 100 μg/day Se (as enriched yeast) for 6 months (background intakes of a similar population were reported as 40 μg Se/day). During and at the end of the supplementation period the lymphocyte proliferative response to mitogens (phytohaemogglutunin (PHA), the antibody OKT3 and Pokeweed Mitogen (PWM)) was assessed. The proliferative response to PWM increased during the period in the selenium supplemented group, reported to reach the upper limit of usual range of healthy adults (Se supplementation had no effect on responses to OKT3 and PHA). Data on the overall health of the subjects is not reported.

Carcinogenicity

86. An adequately performed epidemiology study to assess the carcinogenic risk of selenium compounds in humans is not available.

Adverse Drug Reactions

87. Suspected adverse reactions to medicinal products are reported to the Committee on Safety of Medicines/Medicines Control Agency. Many factors influence the number of reports received, and in most situations there is considerable "under-reporting" of reactions. All of the adverse reactions reported for oral products containing selenium relate to multiconstituent products, and may not, therefore, be directly attributable to the mineral.

Vulnerable Groups and children

88. Selenium supplementation may exacerbate the effects of severe iodine deficiency. There is no evidence that children are particularly sensitive to selenium toxicity.

Genetic Variation

89. No genetic variations affecting response to selenium have been identified

Toxicity in Laboratory Animals

Acute Toxicity

90. Single doses of 1.5 to 6 mg/kg bw of selenite, selenate and selenomethionine are lethal to laboratory animals (Nordic Project Group, 1995). The critical system is the central nervous system, while necrosis and degenerative changes in the liver are also apparent. The most characteristic sign of acute selenium toxicity is the development of a garlic breath odour due to the pulmonary excretion of volatile selenium compounds. Acute exposure results in adverse effects on the lungs; pulmonary congestion, haemorrhage and oedema have been reported. This may occur as a result of the exhalation of active metabolites.

91. Elemental selenium is less toxic than sodium selenite or selenate as a result of its lower solubility; an oral LD50 of 6700 mg/kg bw has been reported for elemental selenium in Swiss Webster mice (Cummins and Kimura, 1971). Similarly, selenium sulphide and disulphide are less acutely toxic as a result of reduced solubility. An oral LD50 for selenium disulphide (dissolved in methyl cellulose) of 138 mg/kg has been reported in Sprague Dawley rats and an LD50 of 3,7000 mg/kg sodium sulphide (dissolved in carboxymethylcellulose) in Swiss Webster mice (Cummins and Kimura, 1971).
92. An oral LD50 of 76 mg/kg (equivalent to 35.9 mg/kg selenium) has been reported for selenocysteine in mice (Sayato *et al.*,, 1993). Liver and kidney damage was reported but

this was considered to be transient since the levels of a range of renal and hepatic biochemical markers declined in the surviving animals in the days post-treatment.

93. Groups of 5 male and 5 female Swiss mice were given a daily gavage dose of 0.5 ml containing up to 64 ppm Se as sodium selenite for three days, and observed for a further seven days (Jacobs and Forst, 1981). All male mice survived to the end of the study. One female in the 32 ppm group died after the second dose and all females in the 64 ppm group died following the third dose. Pale and slightly necrotic livers were observed at autopsy. Small organs with apparent atrophy accompanied the stunted growth observed in the high dose groups.

Sub-chronic toxicity

94. Administration of 6 ppm Se as sodium selenite in drinking water for 4-6 weeks resulted in the deaths of 4/6 male rats (Palmer and Olson, 1974). Using data from the paper, the dose can be estimated to be 0.48 mg Se/kg bw/day at the end of the study. The same concentration of selenium as selenate resulted in the deaths of 1/6 animals (this can be estimated to be 0.42 mg Se/kg bw/day at the end of study). At levels of 2 and 3 ppm there was a small decrease in body weight gain, compared to the controls but no mortality occurred; there were no differences between the toxicities of the two forms of selenium at these doses. In animals fed a rye rather than a corn-based diet, there was no difference in the toxicity of the two forms of selenium administered at 6 ppm. The authors concluded that the toxicity of the two forms of selenium and the likely metabolic pathway were similar. Sodium selenite in drinking water at a dose of 0.28 mg/kg bw/day resulted in the deaths of 25/50 male rats (Schroeder and Mitchener, 1971). However, male rats appear to be more sensitive to sodium selenite toxicity than females. The same treatment level of 0.28 mg/kg bw/day did not result in any deaths in the females, even following a year's treatment.

95. Groups of 5 Sprague Dawley rats of each sex were treated with 0, 1, 4, 8, 16 or 64 ppm selenium as sodium selenite in drinking water for 5 weeks (Jacobs and Forst, 1981b). In rats that were 5 weeks old at the start of treatment, survival was unaffected in the 1 and 4 ppm groups. One female in the 8 ppm group, 3 males and 4 females in the 16 ppm group and all the animals in the 64 ppm group died during the study. A second set of rats that were 12 weeks old at the start of treatment, were more resistant to the effects of selenium, one female in the 16 ppm group and all the animals in the 64 ppm group died during the study. Body weight gain was reduced in all treated groups in a dose related manner. It is uncertain whether the decreases seen in the 1 ppm groups are significant. Serum alkaline phosphatase and glutamic oxaloacetic transaminase (SGOT) levels were increased in animals treated with 16 ppm selenite. At necropsy, the stunted growth seen in the rats treated with high doses of selenium was accompanied by small organs with apparent atrophy. Microscopical examination did not reveal any striking abnormal pathology. Data on Se intake are not provided but the dose can be estimated to be 0.18, 0.7, 1.4, 2.8

or 11.3 mg/kg bw/day in the 5 week old rats and 0.07, 0.29, 0.57, 1.1, 4.6 mg/kg bw/day for the 12 week old rats[1].

96. Drinking water supplemented with 1, 4, 8, 16, 32 or 64 ppm selenium in the form of selenite was administered to groups of 5 male and 5 female Swiss mice for 46 days (Jacobs and Forst, 1981). In the animals who were 7 weeks old at the start of the study, one animal of each sex in the 64 ppm group died during week 4 of the study. No further deaths occurred. In a second set of animals who were 18 weeks old at the start of treatment, 2 males and one female in the 32 ppm group and 4 males and 2 females in the 64 ppm group died during the study. This was taken to indicate that males were more susceptible to selenite toxicity. Body weights were significantly reduced in the 64 ppm group only. Serum alkaline phosphatase was elevated in the top two dose groups. The baseline level and subsequent dose related increase was lower in the younger group of animals. Serum glutamic-oxaloacetic transferase was elevated in the low dose animals compared to the high dose group. General and widespread necrosis in the liver was observed on histopathological examination. Data on Se intake are not provided but can be estimated to be 0.2, 0.7, 1.4, 2.9, 5.7 or 11.4 mg/kg bw/day in the 7 week old mice and 0.1, 0.4, 0.9, 1.8, 3.6, and 7.1 mg/kg bw/day for the 18 week old mice[2].

97. In male Wistar rats given drinking water supplemented with 0.5, 2, 6 or 15 ppm selenium as selenite (equivalent to a total intake of 24, 65, 125 or 211 μg Se/rat/day) for 1, 3 or 6 months, no treatment-related differences in body weights or survival were observed (Crespo *et al.,* 1992) in most of the dose groups. In the group treated with 15 ppm for 6 months, survival was reduced. It is reported that toxic manifestations were observed in biochemical markers of lipid status and hepatocyte function but the data are not provided.

98. Thirty Wistar rats aged 2-3 weeks were randomised into three groups (Grønbæk *et al.,* 1995). Group A were treated with 3.3 mg/l selenium (as sodium selenite) in drinking water. Group B were fed and were given unsupplemented water *ad libitum*. Group C were pair-fed relative to Group A. The treatment period was 5 weeks. Body weight gain was reduced in selenium treated animals compared to the controls. Tibia lengths were significantly reduced compared to both control groups. Selenium treatment induced a significant decrease compared to both control groups in insulin-like growth factor I and insulin-like growth factor-binding protein 3 (IGFBP-3) by the end of the study. IGFBP-1 and/or IGFBP-2 were also significantly reduced in the selenium treated animals compared to the *ad libitum* fed controls. Levels were decreased compared to the pair-fed controls but this was not significant. It was noted that the reduction in insulin-like growth factor and IGFBP-1 could not be due to reduced caloric intake and appeared to be a specific effect of selenium.

[1] Assuming body weights of 85 and 350 g and water consumption of 15 and 25 ml for 5 and 12 week old rats respectively.

[2] Assuming water consumption of 5ml and body weights of 28 and 45 g for 7 and 18 week old mice respectively.

99. Gavage treatment of male mice with greater than 14.2 mg/kg bw/day Se as selenocysteine for 30 days resulted in the deaths of all 15 treated animals (Sayato *et al.,* 1993). No deaths occurred in the 9.4 mg/kg group. Histopathological changed observed in the livers of the animals that died included necrosis or vacuolisation of hepatocytes. Plasma levels of the enzymes aspartate aminotransferase and alanine aminotransferase were elevated in the group treated with 9.4 mg/kg Se. Renal damage was not apparent.

100. Halverson *et al.,* (1966) fed groups of 8 male post-weanling Sprague-Dawley rats with diets containing added sodium selenite or seleniferous wheat for 6 weeks. The controls received a basal diet. The doses used were 1.6, 3.2, 4.8, 6.4, 8.0, 9.6 or 11.2 ppm selenium (the 11.2 ppm dose was in the form of seleniferous wheat only). The doses of selenium used are approximately equivalent to 0.16, 0.32, 0.48, 0.8, 0.96 and 1.12 mg/kg bw/day (WHO 1987). At the end of the dosing period, blood haemoglobin was measured in all dose groups and serum bilirubin and glutamic-oxalacetic transaminase levels measured in selected treatment groups. The growth rate in the first 4 weeks of the experiment was reduced in rats fed with 6.4 ppm or greater selenium from either source. Growth rate was also reduced in the animals receiving 4.8 ppm selenium as sodium selenite. Mortality occurred after 4 weeks of treatment; 1/8 and 1/10 rats receiving 8.0 or 9.6 ppm Se as sodium selenite and 1/8, 5/8 8/8 rats receiving 8.0, 9.6 or 11.2 ppm Se as seleniferous wheat died during the experiment. There were no deaths among the controls. Food consumption was reduced in the 9.9 and 11.2 ppm groups.

101. Relative spleen weights were significantly increased at doses of >6.4 ppm Se compared to the controls. At doses of >8.0 ppm Se, the haemoglobin content of blood was significantly reduced and the relative pancreas weights significantly increased. The reduction in haemoglobin levels was stated to be most marked in animals that died later in the study. Liver weights were reduced in animals treated with 9.6 or 11.2 ppm Se. The livers of the animals receiving 6.4-11.2 ppm Se showed roughness, mottling and discolouration. In the animals treated with 8 ppm Se, serum glutamic-oxalacetic transaminase was slightly increased and serum bilirubin markedly increased compared to the controls. It was also noted that selenium from seleniferous wheat was more readily deposited in the tissues than selenium in the form of sodium selenite.

102. Long-tailed macaques were administered selenomethionine by nasogastric intubation as part of a 30-day study (Cukierski *et al.,* 1989). In some cases, the doses given to animals in the 300 and 600 μg selenomethionine/kg bw/day groups had to be reduced. Thus, the time weighted average doses were 0, 25, 62-117, 150, 188-203 and 300 μg selenomethionine kg bw/day. Two out of four animals in the 600 μg/kg group died of anorexia or aspirated vomitus secondary to emesis and gastritis after 10 or 15 days of treatment. In these high dose animals, histopathological changes were observed in the liver and kidney that were compatible with the macacque fatal fasting syndrome. One animal in the 300 μg/kg group was removed from the study due to selenium induced hypothermia. Six animals receiving >188 μg selenomethionine/kg bw/day had to be given non-scheduled supplementary fruit and dietary supplementation to prevent death.

As the dose and duration of exposure increased, the incidence of anorexia, gastrointestinal distress, mucocutaneous toxicity of varying severity and frequency of hypothermia increased. At the larger dose, disturbances of the menstrual function was observed and were accompanied by decreases in serum progesterone levels, reduced luteal phase lengths, increased inter-menstrual intervals and decreased oestrogen excretion. No changes in dentition, hair or nails were observed in treated animals, nor were there any differences in breath odour between the control and treated groups. A maximum tolerated dose of 150 μg selenomethionine /kg bw/day was identified.

103. In a 13 week National Toxicology Program (NTP, 1980) study, groups of 10 F344 rats/sex received 0, 3.2, 5.6, 10.0, 17.8 or 31.6 mg/kg bw/day selenium sulphide by gavage. No effects were found on survival or growth rate and no gross pathological lesions were observed. Urine stains were observed in some of the males and most of the females in the top dose group but there were no differences in other clinical signs. Focal coagulation necrosis with infiltration by inflammatory cells was apparent in the livers of the top dose animals.

104. Groups of 10 B6C3F1 mice/sex received 0, 21.6, 46.4, 100.0, 216 or 464 mg/kg bw/day selenium sulphide by gavage for 13 weeks (NTP, 1980). There was a moderate decrease in body weights in the top dose females compared to the controls. One death occurred in the top dose males and 4 in the top dose females. With the exception of one death in a 216 mg/kg female, no effects were observed in the lower doses. The top dose animals were thin and/or appeared hunched. No compound related gross pathological effects were observed at necropsy but an increase in the incidence and severity of microscopic interstitial nephritis was observed in the top dose groups.

105. In a 13 week National Toxicology Program study (NTP, 1994), groups of 10 male and female F344 rats and B6C3F1 mice received sodium selenate in drinking water at levels of 3.75, 7.5, 10, 15, 30 or 60 ppm, equivalent to selenium doses of 0.1 to 1.1 mg/kg bw/days in rats and 0.3 to 2.6 mg/kg bw/day in mice. All rats in the high dose group died and the body weights of the rats in the 30 ppm group and the mice in the 30 and 60 ppm groups were reduced compared to controls. Water consumption was decreased in both species at 15 ppm, leading to secondary effects on the blood and urine.

106. In the rats, relative brain, lung (females only) and right testis weights were increased and absolute heart, right kidney (males only), liver, thymus (females only) and lungs (males only) were decreased. These were considered to be secondary to the decrease in body weights. Increases in relative kidney weights in the 15 and 30 ppm groups were considered to be a physiological response to dehydration. A number of changes occurred in haematology, clinical chemistry and urinalysis parameters. These were largely concentration dependent and many changes occurred early in the study but were resolved towards the end. In the top dose group, gross lesions observed at necropsy included decreased thymus, seminal vesicle and uterus size. Histopathological changes in these groups included cellular depletion of the bone marrow, lymph nodes, thymus and spleen,

atrophy and degeneration of the reproductive organs, metaphyseal atrophy of the bone, minimal single-cell necrosis of hepatocytes and atrophy of the acinar structure of the pancreas and salivary glands. These changes were also considered to be secondary to the decreased body weight in the top dose group. The changes were also apparent in rats in the 30 ppm dose group that had marked decreases in body weight gains. Renal papillary degeneration, to which dehydration may have contributed, was observed in rats receiving 7.5 ppm or more selenium.

107. All male and female mice treated with selenate survived to the end of the study. Final mean body weights of both sexes and mean body weight gains of males exposed to 15 ppm selenate or greater were lower than those of controls. The body weight gains of females in the 30 and 60 ppm groups were also lower than controls. No lesions related to sodium selenate exposure occurred in mice. Water consumption by males and females in the three top dose groups was lower than that of controls. The estimated NOAEL for sodium selenate was 0.4 and 0.8 mg Se/kg bw/day for rats and mice respectively.

108. As part of the same NTP study, groups of 10 male and female F344 rats and B6C3F1 mice received sodium selenite in drinking water at concentrations of 2, 4, 8, 16 or 32 ppm, equivalent to selenium doses of 0.08 to 0.09 mg/kg in rats and 0.14 to 1.6 mg/kg in mice. Two female rats in the top dose group died and the body weights of both species were reduced.

109. In the rats, absolute and relative thymus weights were significantly decreased and relative right kidney weights were increased in the 32 ppm dose group. Other changes in organ weights were considered to be related to the decreased body weights. In rats, changes in haematology, clinical chemistry and urinalysis parameters were similar to those found in rats treated with sodium selenate but were minimal and sporadic. Treatment-related gross lesions observed at necropsy in the 32 ppm group included decreased thymus, seminal vesicle and uterus size. Histopathological changes in these groups included atrophy and cellular depletion of the thymus, lymph nodes, bone marrow, spleen, salivary gland, pancreas, liver, mammary gland, uterus, clitoral gland and metaphyseal plate of the femur. These were considered to be secondary to the decreased body weight in the top dose group. There was a dose- related decrease in water consumption and renal papillary degeneration, to which dehydration may have contributed, was apparent at levels of 4 ppm or greater in rats.

110. All male and female mice treated with selenite survived to the end of the study. Final mean body weights of both sexes and mean body weight gains of males and females in the 16 and 32 ppm groups were lower than those of the controls. Water consumption by males and females in the three top dose groups was lower than that of controls. The estimated NOAEL for sodium selenite was 0.4 and 0.9 mg Se/kg bw/day for rats and mice respectively.

111. Liver nodules, suggestive of nodular regenerative hyperplasia were found in Sprague Dawley rats fed 4 ppm selenium in the diet for two months (Bioulac-Sage *et al*., 1992).

112. Groups of 10 ICR male mice were treated with 0, 5, 10 or 15 mg/kg bw selenocysteine for 30, 60 or 90 days (Hasegawa *et al.,* 1994). No deaths occurred during the study but body weight gain decreased with dosage. Levels of serum aspartate aminotransferase and alanine transferase were significantly elevated at the 15 mg/kg level at 60 days and at the 10 and 15 mg/kg bw level at 90 days. It is reported that histopathological examination did not reveal any hepatic changes related to selenocysteine exposure, though the data are not provided. Examination of the sub-cellular distribution of selenium, showed that the major part was found in the cytosolic fraction. The distribution was not affected by increasing time or dose. The level of acid-volatile selenium (such as hydrogen selenide) increased with dose and time, possibly occurring as a result of selenocysteine being broken down by selenocysteine lyase to produce alanine and hydrogen selenide. It was also suggested that this could be related to hepatic damage.

Chronic toxicity

113. Domestic animals develop a condition known as blind staggers when fed plants that have accumulated selenium. The animals have impaired vision, depressed appetite and a tendency to wander in circles, eventually this leads to paralysis and death from respiratory failure (Nordic Project Group, 1995). Selenium is also associated with alkali disease in which animals become emaciated, stiff and lame and lose hair from the mane and tail (ATSDR, 1996). Alkali disease is associated with atrophy in the heart and liver while congestion and focal necrosis of the liver is prominent in blind staggers. The most sensitive indicator of chronic selenium poisoning in animals appears to be growth inhibition. Doses of 4-5 mg/kg feed, equivalent to 0.2-0.25 mg/kg bw/day is necessary to achieve this effect in rats fed a normal diet.

114. Syrian Hamsters were fed diets containing low, adequate (0.1 ppm Se, equivalent to 0.009 and 0.01 mg/kg bw/day in males and females respectively) or excessive (5 ppm Se, equivalent to 0.46 and 0.52 mg/kg bw/day in males and females respectively) selenium (as sodium selenite) supplementation throughout their lifetime (up to 144 weeks) (Birt *et al.,* 1986). Mortality was unaffected by the level of selenium supplementation but the body weights of the male hamsters in the 5 ppm group were reduced compared to the controls. Erythrocyte and plasma GPX activity and blood selenium levels increased with the increments in dietary selenium at both the 54 and 79 week time points.

115. Weanling Long Evans rats were given 2 ppm selenium as selenate or selenite in drinking water over their complete lifespan (Schroeder and Mitchener, 1971). Assuming body weights of 500 and 350 g for males and females and water consumption of 25 and 20 ml/day this is approximately equivalent to 0.1 and 0.11 mg/kg bw/day in males and females respectively). After 1 year the dose was increased to 3 ppm (equivalent to 0.15

and 0.17 mg/kg bw/day in males and females respectively). An epidemic of pneumonia occurred when the rats were 21 months old, causing considerable mortality. Selenite was considered to be more toxic than selenate with regard to suppressed growth and mortality (50% mortality in the males by day 58). The toxic effect of selenite was more apparent in males than females. Selenate was considered to be tumorigenic in older rats with an incidence of 30/48 (41.7%) tumours in the treated animals and 20/65 (16.9%) in the controls. The incidence is not broken down by sex. This study would not now be considered adequate to assess the carcinogenicity of selenium because of the low numbers of animals used, the relatively low dose used and because not all the animals were examined histopathologically.

116. Groups of 50 Swiss mice of each sex were given drinking water supplemented with 1, 4 or 8 ppm selenium as sodium selenite (equivalent to an intake of 0.0103, 0.0323 or 0.0498 mg Se/day) for 50 weeks (Jacobs and Forst, 1981a). Survival was improved in the treatment groups compared to the controls. Body weight gain in the top dose group was markedly reduced. Interim sacrifices showed that liver selenium levels gradually increased over the duration of the study. Glutathione peroxidase activity was increased in the treated groups compared to the controls but this was not dose-related. Both the basal and increased levels were reduced at 50 weeks compared to 25 weeks treatment. The white blood cell count was reduced and the levels of the enzymes serum glutamic-oxaloacetic transferase and alkaline phosphatase were increased in the 8 ppm group suggesting a mild toxic effect at this level. No signs of neoplasia were observed on histopathological examination. The non-toxic dose level was stated to be 4 ppm.

117. Groups of 17 male Sprague Dawley rats were treated with 0 or 4 ppm selenium as sodium selenite in drinking water for two years starting at 5 weeks of age (Jacobs and Forst, 1981b). (Assuming body weights of 500 and 350 g for males and females and water consumption of 25 and 20 ml/day, 4 ppm Se in drinking water is approximately equivalent to 0.2 mg/kg bw/day in males and 0.22 mg/kg bw/day in females.) A second set of animals was treated for one year beginning at 8 weeks of age. Following one year of treatment, survival was reduced in the younger group compared to the untreated controls (63% vs 94%) but increased in the older group (95% vs 90%). Weight gain was slightly reduced in the treated animals. In a parallel experiment, female rats were treated with 4ppm selenium for 1 year starting at 5 weeks of age. Weight gain at 1 year was reduced compared to the treated males, but survival (90%) was increased. White blood cell count was increased in the treated rats in the younger group but this was thought to reflect the poor condition of these animals. Similarly, the age related decline in white blood cell count seen in the older animals was reduced in the treated group. No other significant changes in haematological parameters were apparent. No significant changes in serum enzymes were observed as a result of treatment. After one year of treatment, liver selenium concentration was two-fold higher in the treated animals. A decrease in liver GPX activity was measured in the animals treated with selenium; this was considered to be partly due to the changes in the relative contributions of selenium and non-selenium dependent GPX to the assay. No significant adverse effects were observed

following gross and microscopical examination at necropsy. Overall the authors concluded that 4 ppm selenium was not toxic.

118. Groups of 50 F344 rats of each sex were administered doses of 0, 3 or 15 mg/kg bw/day selenium sulphide, dissolved in carboxymethylcellulose, by gavage (NTP, 1980). Surviving animals were killed at week 104 or 105. Body weights were reduced in the top dose group compared to the controls from approximately week 16 onwards. In males survival to the end of the study was 40/50, 38/50 and 40/50 in the control, low and high dose groups respectively. In the females survival was 38/50, 39/50 and 38/50. A significant increase in the incidence of hepatocellular carcinomas and neoplastic nodules was apparent in the high dose group in both sexes. In the males 14/49 animals in the high dose group had carcinomas and 15/49 had neoplastic nodules compared to 1/48 and 3/48 in the controls. In the females 21/50 animals in the high dose group had carcinomas and 25/50 had neoplastic nodules. These lesions were not observed in the controls. A compound related increase in pigment was found in the lungs of 47/49 high dose and 1/50 low dose males and in 45/50 high dose and 36/50 low dose females. The lesion was not observed in the controls of either sex.

119. Groups of 50 B6C3F1 mice of each sex were administered doses of 0, 20 or 100 mg/kg bw/day selenium sulphide, dissolved in carboxymethylcellulose, by gavage (NTP, 1980). Surviving animals were killed at week 104 or 105. In males survival to the end of the study was 30/50, 33/50 and 35/50 in the control, low and high dose groups respectively. In the females, survival was 43/50 in the controls and 39/50 in both treated groups. No differences in body weights were found between the treated and control groups. The incidence of hepatocellular carcinomas and adenomas was significantly increased in the high dose female mice (25/49) compared to the controls (0/50) and slightly increased (23/50) in the high dose males compared to 15/50) in the control males. The number of primary lung tumours (alveolar/bronchiolar carcinomas) was significantly increased in the high dose females (22/49 compared to 2/50 in the controls) and slightly increased in the high dose males (23/50 compared to 17/50 in the controls).

120. The NTP concluded that selenium sulphide was carcinogenic in F344 rats and in B6C3F1 female mice.

Immune toxicity

121. Johnson *et al*., (2000) compared the effects of inorganic selenium (sodium selenite) and organic selenium (selenomethionine) on the immune system of mice. Male BALB/c mice were exposed to either forms of Se in their drinking water at concentrations of 0, 1, 3, or 9 ppm for 14 days (equivalent to 0, 0.2, 0.5, and 1.6 mg/kg bw/day – assumes mice weigh 28g and consume 5ml water/day). Splenic cellularity, basal and mitogen induced (Con-A, PHA-P and LPS) lymphocyte proliferation and cytokine (TNF-α and IL-1) production/secretion by LPS stimulated macrophages was assessed. Splenic cellularity was significantly reduced in mice treated with 9 ppm sodium selenite (spleen and thymus weight was also significantly

reduced in this group). Basal and mitogen induced lymphocyte proliferation was also non-significantly increased in this treatment group; the authors speculated that the basal increase was a compensatory mechanism for the reduced spleen weight. The production (measured by mRNA) and secretion of proinflammatory cytokines by LPS stimulated macrophages was increased in the 9 ppm sodium selenite group; the authors speculate that the increased production of IL-1 may be responsible for the increase in PHA-P stimulated lymphocyte proliferation. Immunostimulatory effects were also observed in elderly patients supplemented with selenium (paragraph 85). Selenomethionine had no effect on any of the immune parameters assessed. The immune effects of selenium are briefly discussed by Johnson who notes that selenium enhances the primary humoral response to sheep erythrocytes in mice, increases the delayed-typre hypersensitivity response in rats and reduced Coxsackie virus B3-induced mortality in mice, possibly via natural killer (NK) cells. The macrophage is thought to be a potential target for selenium toxicity. The effects of organic selenium compounds on immune function are unknown.

Reproductive toxicity

122. Selenite, selenate, selenomethionine and selenocysteine are teratogenic in birds and fish. Teratogenic effects have also been reported in sheep and pigs (reviewed ATSDR, 1996). Adverse effects have been reported in some studies in rats and mice. Abnormalities have been observed in hamsters but only at doses where there is overt maternal toxicity. Selenomethionine is not teratogenic in macaques. This is discussed further below.

123. Groups of 2-4 male and 6-8 female Sprague Dawley rats aged 70 days, were fed one of two Torula yeast diets or a casein diet (2 similarly dosed experiments) containing different quantities of selenium (as selenite) supplementation (Halverson, 1974). Yeast diet A contained 0, 0.1, 1, 2.5 or 5 ppm selenium (approximately equivalent to 0, 0.01, 0.1, 0.25 and 0.5 mg/kg bw/day, WHO 1987), yeast diet B 0, 0.1, 1, or 2.5 ppm selenium (approximately equivalent to 0, 0.01, 0.1 or 0.25 mg/kg bw/day, WHO 1987), and the casein diet contained 0, 1.25, 2.5 or 3.75 ppm (approximately equivalent to 0, 0.0125, 0.25 or 0.375 mg/kg bw/day, WHO 1987),. The animals (F0) were kept separate until they were 90 days old when the breeding programme began. The offspring (F1) were followed up to postnatal day 77. No consistent effects were found on the number of pregnancies per group, the number of live young per litter, pup weight or mortality up to day 21 (when the animals were weaned and then fed the same diet as the F0 generation) at doses of up to 5 ppm selenium (the authors note that Se had a slight beneficial effect on survival up to day 21). At 21-77 days of age, there was an increase in mortality in the offspring of animals given yeast diet A plus 2.5 or 5 ppm selenium. The animals fed the casein diets were retained as breeding stock for a second generation (F2) and three successive matings (F2a, F2b, F2c) were then observed. No significant effects were reported in the first two matings. In the third reproduction (F2c), fewer litters were born and survival was reduced in the control group. In the first experiment where the doses of selenium were 1.25, 2.5 and 3.75 ppm, selenium had a protective effect on the number of litters at all dose levels. In a second experiment using the same doses of selenium, this protective effect was less apparent and did not occur at the lowest dose level.

Growth rates were reduced in the offspring (F1) of the animals given yeast diet A plus 5ppm selenium, and in the offspring of those given the casein diet with 3.75 ppm selenium for two generations (growth rates were also reduced in the F2 generation at 2.5 ppm). Conversely, at levels of 0.1 and 1 ppm selenium in the yeast diets, the growth rate of the males of the F1 generation was increased compared to untreated controls. In animals fed yeast A plus 5 ppm selenium, kidney and spleen weights were increased at necropsy on postnatal day 77, and surface roughness, fissuring and discoloration was apparent in the liver and mild liver atrophy was observed in males. At lower doses of 2.5 and 3.75 ppm in al dietary groups, similar effects were observed but at a lower frequency. The authors concluded that Se concentrations of 0.1 ppm (on the basis of the yeast diet) were adequate for growth (it appears that this study was designed primarily to investigate the effects of selenium deficiency on reproduction - no NOEL/LOEL has been identified).

124. Kunming mice were treated with drinking water containing 0.25 ppm selenium (approximately 0.06 mg/kg bw/day) in the form of κ-selenocarrageenan from 22 days of age (Chiachun *et al.,* 1991). The selenium content of the normal diet is not given. When the animals reached 87 days of age they were placed into groups of 2 males and 5 females for breeding. The interval between first insemination and calving was significantly reduced by 3.2 days and the number of pups per litter (8 versus 5.2) was significantly increased in the treated animals compared to the controls. The offspring were followed for up to 56 days after birth. In the treated mice, whole blood and liver selenium levels were increased by 17 and 5% respectively. GPX activity in these tissues was increased by 198 and 8% respectively. Few other details are provided.

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

length. However, foetal body weights and lengths were reduced in a dose-dependent manner following treatment with single oral doses of selenomethionine. At the higher doses of selenomethionine, maternal toxicity was pronounced; lethargy, inanition, weight loss and severely reduced food consumption were observed but no mortality occurred in the maternal animals.. The authors concluded that it was not possible to assign a specific teratogenic effect to selenium since it was confounded by maternal toxicity.

126. Ten-day old male rats were given sub-cutaneous injections of a range of selenium compounds (Oštádalová, 1980). These were sodium selenate (10-30 μmol/kg bw (1.89 –5.67 mg/kg)), D,L-selenomethionine (20-100 μmol/kg bw, (3.92-19.6 mg/kg)), D,L-selenocystine (5-80 μmol/kg bw (1.67-26.72 mg/kg), dimethyl selenide (20,000-55,656 μmol/kg bw (2140-5955 mg/kg)), and trimethylselenonium ion (250-750 μmol/kg bw (30.26-90.76 mg/kg). Control animals were not injected and all compounds were tested into the lethal range.. Cataracts of the lens were induced by treatment with sodium selenate, D,L-selenomethionine or D,L-selenocystine. The cataractogenic effect was dose-related and became evident at 20, 40 and 10 μmol/kg respectively. It was suggested that this effect might be due to the selenium compounds interfering with glutathione metabolism in the lens tissue.

127. Groups of six male rats captured from the wild were fed 0, 2 or 4 ppm dietary selenium as sodium selenite for 5 weeks (equivalent to 0.1 and 0.2 mg/kg bw, WHO 1987). A dose-dependent reduction in body weight, testicular and cauda epididymidus weights was measured (Kaur and Parshad, 1994) in the treated animals. A dose-dependent reduction was also found in the concentration, motility and percentage of live spermatozoa, along with an increase in the number of abnormal forms. The percentage of abnormal spermatozoa was 1.39%, 3.89% and 24.64% in the control, 2 and 4 ppm dose groups respectively. Ingestion of 4 ppm selenium had no significant effect on the head and neck regions but abnormalities of the midpiece region and multiple abnormalities increased significantly. Analysis of the stages of differentiation suggested that the abnormalities were induced mainly in the midpiece region of the flagellum, which is a site of energy production. Other than the decrease in body weights, no information is provided on the clinical condition of the rats so it is uncertain whether the effects are secondary to generalised toxicity. The authors discuss studies that indicate that there is no correlation between selenium levels and sperm count or motility in humans but note that selenium levels can be high in the semen of infertile men.

128. In the NTP study described above (paragraph 101), adverse effects on reproductive parameters have been observed in F344 rats and B6C3F1 mice given sodium selenate for 13 weeks. It was observed that at all selenium doses, treated female rats spent more time in dioestrous and less time in other oestrous stages than the controls. Male rats in the 30 ppm group showed a decrease in sperm motility. Doses of 3 ppm (390 μg/day) selenium as selenate fed to CD mice through four generations did not cause adverse effects in the maternal animals (Schroeder and Mitchener, 1971). However there was an increase in deaths of the young in the F1 generation and an increase in the number of runts in generations F1 to F3; by the F3 generation there was also a decrease in the number of breeding events. Doses

of 75, 125 or 375 μg/day selenium (as potassium selenate) were administered to male and female rats through five breeding cycles. In the mid-dose group there was decreased fertility in the females, decreased numbers of surviving offspring and reduced growth rate in the young rats.

129. In the NTP study described in paragraphs 101 to 108, adverse effects were observed in rats and mice receiving selenium as sodium selenite. Male rats receiving 4 ppm or greater sodium selenite had a decreased epididymal sperm concentration. Female rats in the 16 ppm group spent more time in dioestrous and less time in other oestrous stages than the controls and the oestrous cycle was extended in the 32 ppm female mice. However doses of selenite up to 780 μg/kg for 30 days did not have adverse effects on IVCS mice, other than a 6% reduction in the number of surviving fetuses (cited ASTDR, 1994). Selenite did not cause adverse effects in hamsters (Ferm *et al.,* 1990). Selenomethionine was associated with embryonic and fetal toxicity in hamsters and rabbits, but only at doses where overt maternal toxicity occurred (reviewed Nordic Project Group, 1995).

130. Seleno-methionine did not have adverse reproductive effects in macaques at doses of up to 300 μg/kg/day where dose-related maternal toxicity was observed (Tarantal *et al.,* 1991). In this study, groups of 10 pregnant long-tailed macaques were dosed, via naso-gastric intubation, with 0, 25, 150 or 300 μg L-selenomethionine kg/bw on days 10-50 of gestation (organogenesis). The pregnancies of 2-3 dams/group were followed to term, but the remainder were scheduled for hysterotomy on day 100 of gestation. A standard fetal examination, both visceral and skeletal, was then conducted. Neonates delivered at term remained with the dams but were removed periodically for morphometric, ophthalmologic and behavioural evaluation. Dose-related, maternal toxicity indicated by vomiting, anorexia and significant weight loss increased with the duration of dosing. Non-scheduled supplementation of the diet was necessary for some of the animals in the higher dose groups. One early-embryonic death occurred in a compromised dam and two intra-uterine fetal deaths occurred in the 150 μg/kg dose group (one was followed by the death of the dam). In the top dose group, one fetal death and one spontaneous abortion occurred. Total pregnancy loss in the treated groups was 0/10, 0/10, 3/10 and 2/20 for the control, 25, 150 and 300 μg/kg dose groups respectively. However, this was not considered to be different to the incidence in the concurrent or historical controls. No statistically significant treatment-related findings were apparent at necropsy. The data suggest a small, non-significant decrease in a range of measurement parameters in the fetuses exposed to 300 μg/kg, examined at day 100 of gestation. However this is not apparent in the offspring examined at term. The authors do not comment on this finding. One infant exposed to 150 μg/kg was found to have a unilateral cortical cataract, which was considered to be a spontaneous finding.

Carcinogenicity

131. Although tumours have been observed in some animal studies, the results of these have been questionable as a result of a variety of problems including infection and contamination

of the diet with a known carcinogen (discussed Nordic Project Group, 1995). In studies in mice in which selenite or selenate (3 mg/l) or selenium oxide (2 mg/l) were administered in the drinking water, the incidence of tumours in the treated animals was the same or lower than that in the controls. Other workers have reported that certain selenium compounds have an anti-carcinogenic effect. Selenium sulphide given by gavage induced hepatocellular tumours in rat and mice. However, when applied dermally to mice, selenium sulphide did not cause an increase in tumour incidence (external or internal) compared to controls. Although selenium sulphide is taken up to an insignificant extent in man, selenosis following the handling of a shampoo containing selenium sulphide has been described, thus the Nordic Project Group could not rule out the relevance of this to man. The mechanism underlying the tumours associated with selenium sulphide is uncertain.

Genotoxicity

132. The results of genotoxicity studies on selenium compounds are conflicting and are reviewed more fully by ATSDR (1996) and Shamberger (1985). Selenite and selenate are weakly mutagenic in Salmonella strain TA 100. Unscheduled DNA synthesis, sister chromatid exchange and chromosomal aberrations have been observed in *in vitro* systems in the presence of glutathione. In an *in vivo* study in hamster bone marrow, an increase in sister chromatid exchange was observed but at doses of selenite which were toxic. It has been suggested (Nordic Project Group, 1995) that glutathione is required for the production of reactive oxygen metabolites, and that this may be a concentration dependent effect. Conflicting results on the co-mutagenic effects of selenium have also been reported.

In Vitro

133. In the *Salmonella typhimurium* reversion assay (Ames test) sodium selenite and sodium selenate were weakly mutagenic in strain TA100 in the absence of S9 metabolic activation (Noda *et al.,* 1979). No mutagenic effect was found in strains TA 98 or TA 1537 indicating that the mutation was a base-pair substitution. Selenite was found to be slightly more mutagenic than selenate producing 0.2 and 0.05 revertants/nmole respectively. It is noted that this is in contrast to the results of other workers (Lofroth and Ames, 1978) who found selenate to cause base-pair substitutions at approximately 0.3 revertants/nmole but did not find any indication that selenate was mutagenic. It is suggested that the differences could be due to the instability and relative insolubility of selenate in water. It has been proposed that the mutagenic activity of selenite in *Salmonella typhimurium* is due to the ability of the compound to act as an oxidizing agent (Kramer and Ames, 1988). Selenite is thought to react with sulphydryl-containing macromolecules to produce hydrogen peroxide and superoxide anion. Pre-treatment of strain LT2 with hydrogen peroxide induces the production of stress proteins and resistance to subsequent damage by selenite and vice versa. Selenite was found to be mutagenic in *Salmonella* strain TA 104 in which a number of other oxidising agents have also been mutagenic. Strain *oxy*R1 which over-produces

proteins that protect from oxidative stress such as catalase was resistant to high doses of selenite as would be expected if hydrogen peroxide and superoxide anion were involved.

134. Sodium selenite and sodium selenate were weakly mutagenic in the *Bacillus subtilis Rec* bacterial mutation assay (Noda *et al.,* 1979). However, selenite but not selenate was reported to be positive in the same assay by Nakamuro *et al* (1976).

135. Sodium selenite was tested for mutagenic and recombinogenic effects in two diploid yeast strains, *Saccharomyces cerevisiae* D_7 and BZ 34 (Anjaria and Madhvanath, 1988). Selenite induced gene conversion in strain BZ 34 and a variety of genetic events, back mutation, gene conversion, mitotic crossing over, aberrant colony formation and toxicity in strain D_7. In the BZ 34 strain, the presence of glutathione during treatment enhanced the convertogenic and toxic effects of selenite.

136. The effect of sodium selenite, sodium selenide and sodium selenate on the induction of sister chromatid exchange (SCE) in the presence and absence of S9 metabolic activation was investigated in Chinese Hamster V79 cells *in vitro* (Sirianni and Huang, 1983). The most effective inducer was sodium selenide, followed by sodium selenite. Sodium selenate did not increase the induction of SCE compared to the controls. A similar pattern was seen with regard to growth inhibition.

137. Five selenium compounds were tested for their ability to induce chromosome aberrations in human leukocytes *in vitro* (Nakamuro *et al.,* 1976). Chromosmome-breaking activity was greater in 4-valent compounds compared to 6-valent compounds. In decreasing order, efficacy was selenous acid > sodium selenite > selenium oxide > selenic acid. The increase in the number of aberrations was largely dose dependent and was statistically significant for selenous acid, sodium selenite, selenium oxide and the highest dose of selenic acid. Sodium selenate did not cause an increase in chromosome aberrations. High concentrations of sodium selenite (Ray *et al.,* 1978) caused a three-fold increase in the frequency of sister chromatid exchange (SCE) in human whole blood cultures. Red blood cell lysate was necessary for the increase in SCE to occur (Ray and Altenburg, 1978). The SCE frequency in human lymphoblastoid cell lines was not affected by the same concentrations of sodium selenite. However if these cells were incubated with red blood cell lysate, an increase in SCE occurred. High concentrations of sodium selenite produced DNA fragmentation, DNA-repair synthesis, chromosome aberrations and mitotic inhibition in cultured human fibroblasts (Lo *et al.,* 1978). Induction of chromosome aberrations was enhanced by the addition of mouse S9 fractions. Sodium selenate did not cause an increase in chromosome aberrations in the presence or absence of mouse S9 activation.

138. Biswas *et al.,* (2000) investigated the ability of sodium selenite and sodium selenate to cause chromosomal aberrations in human lymphocytes (in the absence of metabolic activation). Both sodium selenite and sodium selenate caused dosed related aberrations though sodium selenite was reported to be more clastogenic than sodium selenate.

139. The induction of Unscheduled DNA synthesis (UDS) in cultured human skin fibroblasts by inorganic selenium compounds was strongly enhanced by glutathione (Whiting *et al.,* 1980). No UDS was detected in cells treated with selenomethionine or selenocystamine. However, a low level of UDS was induced by selenocysteine and this was enhanced by the presence of glutathione. Similarly, glutathione enhanced the number of chromosome aberrations and the cytotoxicity of inorganic selenium compounds *in vitro.* The authors conclude that reduction may be important in the conversion of selenium species into mutagenic forms.

140. Rat lymphocytes exposed to sodium selenite *in vitro* showed a significant dose-related increase in the number of abnormal metaphases (Newton and Lilly, 1986). Human lymphocyte cultures were treated with increasing concentrations of sodium selenite or selenomethionine (Khalil, 1989). The yield of abnormal metaphases was dose dependent. The number of aberrant cells reached 53.5% for selenite and 43% for selenomethionine. The chromosomal aberrations observed were largely of the chromatid type and included breaks and fragments; chromosomal exchanges were less frequent and included triradials and quadriradials.

In Vivo

141. Selenium sulphide at doses of up to 140 mg/kg produced equivocal levels of unscheduled DNA synthesis (UDS) in rat hepatocytes following *in vivo* treatment (Mirsalis *et al.,* 1989). There was a significant increase in the number of cells in repair but the net grain counts never exceeded zero.

142. Sodium selenite did not cause a significant increase in the number of abnormal metaphases in the lymphocytes of Wistar rats treated with two doses of up to 6 mg/kg bw (Newton and Lilly, 1986). However two doses of 5 or 6 mg/kg bw caused an increase in the number of abnormal metaphases in the bone marrow cells of the rats. The authors suggest that the chemical is acting as an "S" dependent chemical, giving a delayed response (initial lesions formed at the G_0 stage of the cell cycle are repaired at stages G_0 and G_1 leaving few lesions left at S phase to form aberrations). The small amount of damage represents unrepaired lesions. It is also noted that because of the high toxicity of selenium there is only a brief concentration range where chromosome damage is observed.

143. Sodium selenite caused an increase in the number of chromosome aberrations and sister chromatid exchange in the bone marrow of Chinese hamsters (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 such that the treated animals died some hours after dosing. The numbers of aberrations in these dose groups were 13-35% compared to 0.9-1% in the controls and 6.7-11.4 SCE per cell compared to 3.4-4.4 in the controls. Doses of 0.3, 0.6, 1 or 2 mg Se/kg did not cause any clastogenic effects.

144. As part of developmental toxicity study in long-tailed macaques described above, a transplacental bone marrow micronucleus assay was conducted in the fetuses of the treated animals (Choy *et al.,* 1993). Selenium in the form of selenomethionine was administered to pregnant macaques at doses of 0, 150 or 300 μg/kg bw/day. Although showing clear signs of selenosis, selenium levels in maternal plasma did not exceed 4 ppm (3.7 pppm in erythrocytes) and cord blood levels did not exceed 0.1 ppm (1.1 ppm in erythrocytes). Fetal bone marrow smears were prepared and did not show any increase in the number of micronuclei. A micronucleus assay was not performed on the maternal animals since it was though that the traumatic nature of bone marrow aspiration might adversely affect the pregnancy outcomes. The finding is contrasted with previous work by the same authors (Choy *et al.,* 1989) which demonstrated a seven-fold increase in the incidence of micronuclei in the bone marrow of a non-pregnant macaque dosed with 600 μg/kg bw/day for 15 days (a lethal dose) compared to the control animal. In this case, plasma selenium levels were 7.3 ppm (5.7 ppm in erythrocytes). In animal treated with 300 μg/kg bw/day no increase in the number of micronuclei was observed. The animals used were part of the study described by Cukierski *et al*., (1989)

Co-mutagenic effects

145. A concentration of 2.9 x 10^{-5} but not 1.16 x 10^{-5} g selenite increased the *N*-methylnitrosourea (MNU) and *N*-methyl-*N*-nitro–*N*-nitrosoguanidine (MNNG) induced mutation rate in strain TA1535 in the *Salmonella typhimurium* assay in the absence of metabolic activation (Balansky, 1991). Pre-treatment of the bacteria with selenite alone did not affect the spontaneous mutation rate. The induction of chromosome aberrations in CHO cells *in vitro* by MNNG or tobacco smoke also in the absence of metabolic activation was increased by pre-treatment with selenite. A similar effect was observed *in vivo*; pre-treatment of male BDF1 Or CC57W mice with 10 ppm selenite added to drinking water for 7-14 days resulted in a 2-3 fold increase in urethane induced damage in the bone marrow. In addition, pre-treatment with selenite increased by 43.8% the number of mitomycin C induced micronuclei in the bone marrow of BDF1 mice.

Mechanisms of Toxicity

146. A variety of mechanisms are thought to be involved, including redox cycling of auto-oxidisable metabolites, glutathione depletion, inhibition of protein synthesis, depletion of S-adenosyl-methionine and the general replacement of sulphur and reactions with critical sulphhydryl groups of proteins and co-factors. The toxicity of selenite *in vitro* and *in vivo* is enhanced by vitamin B12 deficiency (Chen and Whanger, 1993, Chen *et al.,* 1993). It has been suggested (Hasegawa *et al.,* 1993) that the production of hydrogen selenide following the reaction of a selenocysteine containing metabolite and selenocysteine β-lyase in the liver may contribute to the oral toxicity seen in mice.

Regulatory Considerations

147. In the UK a maximum level for selenium is 10µg for cereal based foods in The processed cereal-based foods and baby foods for infants and young children regulations, 1997[3].

Recommendations on maximum intake levels

148. Reviewing the situation in the US, Levander (1988) proposed that infant formula should provide an intake of 10-45 µg/day selenium. This was based on extrapolation from studies in adults which indicated that 10 µg/day was sufficient to meet the nutritional requirement of the infant while providing a reasonable margin of safety. Extrapolation of the work by Yang was taken to suggest that intakes of 75-160 µg/day Se could be harmful to infants, but it was also noted that in the seleniferous areas of the US, human milk provided selenium intakes of up to 47 µg/day without adverse effect.

149. In the 1991 Dietary Reference Value report, the Committee on the Medical Aspects of Food Policy (COMA) gave brief guidance on high intakes of selenium. The COMA panel agreed that 450 µg Se/day was the maximum safe intake of selenium from all sources. This corresponds to 6 µg/kg bw/day for a 75 kg male.

150. The Nordic Project Group (1995) considered an intake of 4-5 µg Se/kg bw/day to be safe and tolerable. For a 70 kg adult the acceptable dose of 4-5 µg Se/kg bw/day was equivalent to an intake of 280-350 µg Se/day.

151. The United States Environmental Protection Agency (US EPA, 1994) used the Reference Dose (RfD) method to establish a maximum safe level for selenium of 5 µg Se/kg bw/day.

152. The World Health Organisation (1996) recommended that the upper limit of the safe range of population intake was 400 µg Se/day.

Recommendations on maximum supplementation levels

153. The 1991 report "Dietary Supplements and Health Foods - Report of the Working Group ("The Denner Report") was produced by the Department of Health and the Ministry of Agriculture, Fisheries and Food. In this, it was proposed that for isolated or highly purified products such as vitamin and mineral supplements, only one tenth of an identified "undesirable" dose should be present in dietary supplements as a daily dose. For selenium it was noted that adverse effects had been observed at intake levels of 1000 µg/day and this was considered to be the undesirable dose. The maximum level for selenium supplements would therefore be 100 µg/day.

[3] The Processed Cereal-based Foods and Baby Foods for Infants and Young Children Regulations 1997.

154. The European Federation of Health Food Manufacturers recommended a maximum upper safe level for supplementation of 200 μg Se/day (Shrimpton, 1994 and 1997). Consumers for Health Choice (1998) also recommended 200 μg Se/day as the maximum upper safe level for supplementation. The Council for Responsible Nutrition, a UK Trade Association, recommend a maximum limit of 200 μg Se/day for long term supplementation and 700 μg Se/day for short term supplementation.

Summary

155. Selenium is an essential trace element, which is necessary for the functioning of the enzyme glutathione peroxidase, which protects against intracellular oxidative damage. Other selenoproteins exist and the element may also be involved in thyroxine metabolism. Human exposure to selenium is largely through food. In the UK the population average intake of selenium from food is 29-39 μg/day (this excludes intake from dietary supplements).

156. Selenium deficiency in animals results in effects such as growth retardation, reproductive failure and degenerative organ changes. In humans selenium deficiency is thought to be involved in Keshan disease, a cardiac myopathy and, possibly, Kashin-Beck disease a condition of the joints and muscles. In the UK, the Reference Nutrient Intake (RNI) for selenium recommended by the Committee on the Medical Aspects of Food Policy (COMA) is 60 and 75 μg/day for females and males respectively. This is considered to be sufficient to permit functional saturation of glutathione peroxidase.

157. In humans, acute selenium toxicity is characterised by gastrointestinal disturbance, hair loss, numbness in the arms, fatigue and garlic-smelling breath. In China where endemic selenosis occurs, symptoms such as brittle and pigmentless hair, skin lesions, pathological changes to the nails and neurological disturbances are observed. Investigations by Yang *et al.,* (1989 a and b) have investigated the dietary intake levels at which symptoms of selenosis are not apparent. Similar work has been conducted in the US by Longnecker *et al*., (1991).

158. The toxicity of different selenium compounds varies. Selenite, selenate and selenomethionine are more toxic than selenium sulphide. In animals, acute toxicity is characterised by central nervous system toxicity and degenerative changes in the liver. As a result of the excretion of volatile selenium compounds, garlic-smelling breath also occurs. Adverse effects on growth rates, the kidneys and reproductive parameters have been reported in rats and mice dosed with selenium compounds chronically and sub-chronically. Domestic animals develop a condition known as blind staggers, involving impaired vision and eventual respiratory failure. Selenite, selenate and selenomethionine are teratogenic in birds and fish. Some adverse reproductive effects have also been reported in rats and mice fed selenium compounds. Selenomethionine does not have adverse reproductive effects in macaques. Selenium compounds are not genotoxic in the majority of studies reported.

References

Agency for Toxic Substance and Disease Registry (ATSDR) (1996) Toxicological Profile for Selenium (Update). US Department of Health and Human Services.

Anjaria, K. B. and Madhvanath, U. (1988). Genotoxicity of Selenite in Diploid Yeast. Mutation Research 204, 605-614.

Anonymous, (1982). Essential Trace Elements and Thyroid Hormones, The Lancet, 339, 1575-1576.

Balansky, R. M. (1991). Comutagenic and coclastogenic effects of selenium in vivo and in vitro. Mutation Research. 263, 231-136.

Behne, D *et al* (1990). Effects of Chemical Form and Dosage on the Incorporation of Selenium into Tissue Proteins in rats. Journal of Nutrition, 121, 806-814.

Beilstein, M.A. and Whanger, P. D (1986). Chemical Forms of Selenium in Rat Tissues after Administration of Selenite or Selenomethionine. Journal of Nutrition, 116, 1711-1719.

Benton D. and Cook R. (1991). The impact of selenium supplementation on mood. Biol Psychiatry, 29(11), 1092-8.

Bioulac-Sage, P. *et al*, (1992). Nodular Regenerative Hyperplasia in the Rat Induced by a Selenium enriched diet: Study of a model. Hepatology, 16, 418-425.

Birt, D. F. *et al*, (1986). Tolerance of Low and High Dietary Selenium Throughout the Life Span of Syrian Hamsters. Ann. Nutr. Metab 30, 233-240.

Biswas, S. Talukder, G. Sharma, A. (2000). Chromosome damage induced by selenium salts in human peripheral lymphocytes. Toxicology vitro, 14, 405-408.

Bopp, B. A. *et al*, (1982). Metabolic Fate of Selected Selenium Compounds in Laboratory Animals and Man. Drug Metabolism Reviews. 13, 271-318.

Bortoli, A. Fazzin, G. Marchiori, M. Mello, F. brugiolo, R. Martelli, F. (1991). Selenium status and effect of selenium supplemenation in a group of elderly women. J Trace Elements Electrolytes health Dis, 5, 19-21.

Bruce A. (1986). Swedish views on selenium. Ann Clin Res, 18(1), 8-12.

Burk, R. F. *et al*, (1971) Influence of Dietary and Injected Selenium on Whole-body Retention, Route of Excretion, and Tissue retention of $^{75}O_3^{2-}$ in the Rat. Journal of Nutrition, 102, 1049-1056.

Butler, J. A *et al* (1990), Metabolism of Selenite and Selenomethionine in the Rhesus Monkey. Journal of Nutrition, 120, 751-759.

Carter, R. F. (1966). Acute Selenium Poisoning. Medical Journal of Australia, 1, 525-528.

Chen, C. L. and Whanger, P. D. (1993). Effect of Vitamin B12 Status on Selenium Methylation and Toxicity in Rats: *In Vivo* and *In Vitro* Studies. Toxicology and Applied Pharmacology, 118, 65-72.

Chen, C. *et al* (1993) Effect of vitamin B12 on performance and tissue selenium content in rats fed sub-toxic levels of selenite. Toxicology, 85, 101-115.

Chiachun, T. *et al*, (1991). The Effects of Selenium on Gestation, Fertility, and Offspring in Mice. Biological Trace Element Research. 30, 227-231.

Choy, W. N. *et al,* (1989) Primate Micronucleus Study of L-methionine. Environmental and Molecular Mutagenesis. 14, 123-25.

Choy, W. N. *et al*, (1993) Incorporation of a Micronucleus Study Into a Developmental Toxicology and Pharmacokinetic Study in Nonhuman Primates. Environmental and Molecular Mutagenesis. 21, 73-80.

Civil, I. D. S. and McDonald, J. A. (1978). Acute Selenium Poisoning: Case Report. New Zealand Medical Journal, 87, 354-356.

Clark, L.C., et al (1996), Effects of Selenium Supplementation for Cancer Prevention in Patients with Carcinoma of the Skin. JAMA, 276, 1957-1963.

Cohen, H. J. and Avissar, N. (1993) Molecular and Biochemical Aspects of Selenium Metabolism. Essential and Toxic Trace Elements in Human Health and Disease: An Update. Pp 191-202. Wiley Liss.

COMA (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy (COMA). Department of Health RHSS 41.

COMA (1998a). Statement from the Committee on Medical Aspects of Food and Nutrition Policy on selenium. Food Safety Information Bulletin No. 93, MAFF:DH, 1998.

COMA (1998b). Nutritional Aspects of the Development of Cancer. Report of the Working Group on Diet and Cancer of the Committee on Medical Aspects of Food Policy (COMA). Department of Health RHSS 48.

Combs, G. F. and Combs, S. B. (1987). Selenium Effects on Drug and Foreign Compound Toxicity. Phrmac. Ther. 33, 303-315.

Consumers for Health Choice (1998). A Summary of the Science of Vitamin and Mineral Supplement Safety.

Contempre, B. *et al*, (1991). Effect of Selenium Supplementation in Hypothyroid Subjects of an Iodine and Selenium Deficient Area: The Possible Danger of Indiscriminate Supplementation of Iodine-Deficient Subjects with Selenium. Journal of Clinical Endocrinology and Metabolism. 133, 99-109.
Contempre, B. *et al* (1995) Effects of selenium Deficiency on Thyroid Necrosis, Fibrosis and Proliferation: a Possible Role in Myxoedematous Cretinism. European Journal of Endocrinology. 133, 99-109.

Crespo, A. *et al*, (1992) Plasma and Liver Selenium Levels in the Rat During Supplementation with 0.5, 2, 6 and 15 ppm Selenium in Drinking Water. Biological Trace Element Research. 38, 139-147.

Cukierski, M. J., *et al* (1989). 30-Day Oral Toxicity Study of L-selenomethionine in Female Long-Tailed Macaques (*Macaca fascicularis*). Fundamental and Applied Toxicology. 13, 26-39.

Cummins, L. M. and Kimura, E. T. (1971) Safety Evaluation of Selenium Sulfide Anti-Dandruff Shampoos. Toxicology and Applied Pharmacology, 20, 89-96.

Ferm, V. H. *et al*, (1990). Embryotoxicity and Dose-Response Relationships of Selenium in Hamsters. Reproductive Toxicology. 4, 183-190.

Finley, JW. (1998). The absorption and tissue distribution of selenium from high selenium broccoli are different from selenium from sodium selenite, sodium selenate, and selenomethionine as determined in selenium deficient rats. J Agric Food Chem, 46, 3702-3707.

Finley, JW. (1999). The retention and distribution by healthy young men of stable isotopes of selenium consumed as selenite, selenate or hydroponically grown broccoli are dependent on the isotopic form. J Nutr, 129, 865-871.

Finley, JW. Duffield, A. Ha, P. Vanderpool, RA. Thomson, CD. (1999). Selenium supplementation affects the retention of stable isotopes of selenium in human subjects consuming diets low in selenium. Brit J Nutrition, 82, 357-360.

Finley, JW. Davis, CD. Feng, Y. (2000). Selenium from high selenium broccoli protects rats from colon cancer. J Nutr, 130, 2384-2389.

Ganther, HE. (1999). Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. Carcinogenesis, 20, 1657-1666.

Ge, K. and Yang, G. (1993). The epidemiology of selenium deficiency in the etiological study of endemic diseases in China. American Journal of Clinical Nutrition (Supplement) 57, 259S-63S.

Grønbæk, H. *et al*, (1995). Effect of sodium selenite on growth, insulin-like growth factor-binding proteins and insulin-like growth factor-I in rats. Journal of Endocrinology. 145, 105-112.

Halverson, A. W., (1974). Growth and Reproduction With Rats Fed Selenite-Se. Proceedings of the South Dakota Academy of Science. 53, 167-177

Halverson, A.W. *et al,* (1966). Toxicity of selenium to Post-weanling rats. Toxicology and Applied Pharmacology. 9, 477-484.

Hasegawa, T *et al*, (1994). Toxicity and Chemical Form of Selenium in the Liver of Mice Orally Adminstered Selenocysteine for 90 Days. Archives of Toxicology. 68, 91-95.

Hasegawa, T. *et al*, (1993) Chemical Form of Selenium-containing Metabolite in Small Intestine and Liver of Mice Following orally administered selenocysteine. Archives of Toxicology, 69, 312-317.

Hasselmark L. *et al*. (1993). Selenium supplementation in intrinsic asthma. Allergy, 48(1), 30-36.

Hathcock, J.N., (1993) Safety Limits for Nutrient Intakes: Concepts and Data Requirements. Nutrition Reviews, 51, 278-285.
Hathcock, J.N (1996). Safety limits for Nutrients. J Nutr. 126: 2386S -2398S

Ip, C. *et al,* (1990) Chemical Form of Selenium, Critical Metabolites, and Cancer Prevention. Cancer Research, 51, 595-600.

Jacobs, M. and Forst, C. (1981a). Toxicological Effects of Sodium Selenite in Swiss Mice. Journal of Toxicology and Environmental Health, 8, 587-598.

Jacobs, M. and Forst, C. (1981b). Toxicological effects of Sodium Selenite in Sprague Dawley Rats. Journal of Toxicology and Environmental Health. 8, 575-585.

Janghorbani, M. *et al*, (1981). Selenium Metabolism in Healthy Adults: Quantitative Aspects Using the Stable Isotope $^{74}SeO_3^{2-}$. American Journal of Clinical Nutrition. 35, 647-654.

Janghorbani, M. *et al*, (1990) Effect of chronic selenite supplementation on Selenium Excretion and Organ Accumulation in Rats. Journal of Nutrition, 120, 274-279.

Jensen, R. *et al* (1984) Selenium Intoxication – New York. JAMA, 251 (15), p1938.

Johnson, VJ. Tsunoda, M. Sharma, RP. (2000). Increased production of proinflammatory cytokines by murine macrophages following oral exposure to sodium selenite but not seleno-L-methionine. Arch Environ Contam Toxicol, 39, 243-250.

Juhlin L. *et al*. (1982). Blood glutathione-peroxidase levels in skin diseases: effect of selenium and vitamin E treatment. Acta Derm Venereol, 62(3), 211-4.

Kaur, R. and Parshad, V. R. Effects of Dietary Selenium on Differentiation, Morphology and Functions of Spermatozoa of the House Rat, *Rattus rattus* L. Mutation Research. 309, 29-35.

Khalil, A. M. (1989) The Induction of Chromosome Aberrations in Human Purified Blood Lymphocytes Following In Vitro Exposure to Selenium. Mutation Research. 224, 503-506.

Kramer, G. F. and Ames, B. N. (1988) Mechanisms of mutagenicity and toxicity of sodium selenite (Na_2Se0_3) in *Salmonella typhimurium*. Mutation Research. 201, 169-180.

Lane, H. W. *et al*, (1990). Effect of Chemical Form of Selenium on Tissue Glutathione Peroxidase Activity in Developing Rats. Journal of Nutrition, 121, 80-86.

Levander, O. A. (1988) Upper limit of Selenium in Infant Formulas. Journal of Nutrition, 119, 1869-1873.

Lo, I. W. *et al* (1978). The Mutagenicity and Cytotoxicity of Selenite "Activated" Selenite and Selenate for Normal and DNA Repair-Deficient Human Fibroblasts. Mutation Research. 49, 305-312.

Lofroth, G. and Ames, B. N. (1978). Mutagenicity of inorganiccompounds in *Salmonella typhimurium*: aresnic, chromium and selenium. Mutation Research. 53, 65-66.

Longnecker, M.P., et al (1991), Selenium in diet, blood and toenails in relation to human health in as seleniferous area. Am. J. Clin. Nutr., 53, 1288-94.

Longnecker, M.P., (1993), A 1-y trial of high-selenium bread on selenium concentrations in blood and toenails. Am. J. Clin. Nutr. 57, 408-413.

Mack, R. B. (1990). The Fat lady Enters Stage Left. Acute Selenium Poisoning. NCMJ, 51, 636-638.

Matoba, *et al* (1986). An Autopsy Case of Acute Selenium (Selenious Acid) Poisoning and Selenium Levels in Human Tissues. Forensic Science International, 31, 87-92.

McGuire, M. K. *et al*, (1993a). Selenium Status of Lactating Women is Affected by the Form of Selenium Consumed. American Journal of Clinical Nutrition. 58, 649-652.

McGuire, M. K., *et al,* (1993b). Selenium Status of Infants is Influenced by Supplementation of Formula or Maternal Diets.

Meltzer, H. M., *et al* (1990). The Form of Selenium Determines the Response to Supplementation in a Selenium Replete Population. European Journal of Clinical Nutrition. 44, 435-446.

Meltzer, H.M., *et al,* (1992). Supplementation with wheat selenium induces a dose-dependent response in serum and urine of a Se-replete population. British Journal of Nutrition 67, 287-294.

Mertz, Abernathy and Olin (Eds). Risk Assessment of Essential Elements (1994). ILSI Press, Washington D. C.

Ministry of Agriculture Fisheries and Food (1994). Food Surveillance Information Sheet no 141.

Ministry of Agriculture Fisheries and Food (1997a). 1994 Total Diet Study: metals and other elements. Food Surveillance Information Sheet no 131.

Ministry of Agriculture Fisheries and Food (1997b). Dietary intake of selenium. Food Surveillance Information Sheet no 126.

Ministry of Agriculture, Fisheries and Food, (1998). Cadmium, Mercury and other Metals in Food. The 53rd report of the Steering Group on Chemical aspects of Food Surveillance.

Ministry of Agriculture Fisheries and Food & Department of Health (1991). Dietary supplements and Health Foods - Report of the Working Group.

Mirsalis, J. C. *et al*, (1989). Measurement of Unscheduled DNA synthesis and S-phase Synthesis in Rodent Hepatocytes Following *In Vivo* Treatment: Testing of 24 Compounds. environmental and Molecular Mutagenesis. 14, 155-164.

Nakamuro, K. *et al* (1976). Studies on Selenium-Related Compound. V. Cytogenetic Effect and Reactivity with DNA. Mutation Research. 40, 177-184.

Nevé, J. (1995). Human Selenium Supplementation as Assessed by Changes in Blood Selenium Concentration and Glutathione Peroxidase Activity. Journal of Trace Elements in Medicine and Biology. 9, 65-73.

Newton, M. F. and Lilly, L. J. (1986). Tissue-specific clastogenics effects of chromium and selenium salts in vivo. Mutation Research, 169, 61-69.

Noda, M. *et al*, (1979). Mutagenic Activity of Selenium Compounds. Mutation Research, 66, 175-179

Nordic Project Group (1995). Risk Evaluation of Essential Trace Elements - Essential versus toxic levels of intake. Report of a Nordic project group. Nord 1995:18

Norppa, H. *et al* (1980). Chromosomal Effects of Sodium Selenite In Vivo. III Aberrations and Sister Chromatid Exchange in Chinese Hamster Bone Marrow. Hereditas. 93, 101-105.

NTP (1980). Bioassay of selenium sulfide (gavage) for possible carcinogenicity.

NTP (1994). Sodium Selenate and Sodium Selenite administered in drinking water to F344/N rats and B6C3F1 mice. Toxicity Report Series, No 38.

Oštádalová, I.(1980). Toxic Effect of Various Selenium Compounds on the Rat in the Early Postnatal Period. Archives of Toxicology. 45, 207-211.

Palmer, I. S. and Olson, O. E. (1974). Relative toxicities of Selenite and selanate in the Drinking Water of Rats. Journal of Nutrition, 104, 306-314.

Peretz, A. neve, J. Desmedt, J. Duchateau, J. Dramaix, M. Famaey, JP. (1991). Lymphocyte response is enhanced by supplementation of elderly subjects with selenium enriched yeast. Am J Clin Nutr, 53, 1323-8.

Ray, J. H. and Altenburg (1978). Sister-Chromatid Exchange Induction by Sodium Selenite: Dependence on the Presence of Red Blood Cell or Red Blood Cell Lysate. Mutation Research. 54, 343-354.

Ray, J. H., *et al* (1978). Effect of Sodium Selenite and Methyl Methanesulfonate or *N*-Hydroxy-2-Acetylaminofluorene C--Exposure on Sister-Chromatid Exchange Production in Human Whole Blood Cultures. Mutation Research. 57, 359-368.

Roti, E. *et al*, (1993). Selenium Administration Does not Cause Thyroid insufficiency in Subjects with Mild Iodine Deficiency and Sufficient Selenium Intake. Journal of Endocrinological Investigation. 16, 481-484.

Ruta, D.A. and Haider, S., (1989). Attempted murder by selenium poisoning. British Medical Journal, 299, 316-7

Salbe, A. D. and Levander, O. A, (1990). Effect of various dietary factors on the Deposition of Selenium in the Hair and nails of Rats. Journal of Nutrition, 120, 200-206.

Sayato, Y. *et al* (1993). Acute and Subacute Oral Toxicity of Selenocysteinein Mice. Japanese Journal of Toxicology an Environmental Health. 39, 289-296.

Schrauzer G.N. and Sacher J. (1994). Selenium in the maintenance and therapy of HIV-infected patients. Chem Biol Interact, 91(2-3), 199-205.

Schroeder, H. A. and Mitchener, M. (1971) Selenium and Tellurium in Rats: Effect on Growth, Survival and Tumours. Journal of Nutrition. 101, 1531-1540.

Shamberger, R. J. (1985). The Genotoxicity of Selenium. Mutation Research. 154, 19-48.

Shrimpton, D.H (1995). Essential Nutrients in Supplements - European Federation of Associations of Health Product Manufacturers.

Shrimpton, D.H., (1997). Vitamins and Minerals. A Scientific Evaluation of the Range of Safe Intakes. European Federation of Health Product Manufacturers.

Simonoff, M. Sergeant, C. Garnier, N. Moretto, P. Llabador, Y. Simonoff, G. Conri, C. (1992). Antioxidant status (selenium, vitamins A and E) and aging. Free Radicals Aging, 368-397.

Sirianni, S. R. and Huang, C. C. (1983). Induction of Sister Chromatid Exchange by Various Selenium Compounds in Chinese Hamster Cells in the Presence and Absense of S9 mixture. Cancer Letters. 18, 109-116.

Spencer, R. P. and Blau, M. (1962). Intestinal transport of Selenium-75 Selenomethionine. Science, 136, 155-156.

Sunde, R. A. (1990). Molecular Biology of Selenoproteins. Annual Review of Nutrition. 10, 451-474.

Swanson, C. A. *et al*, (1990). Selenium Intake, Age, Gender, and Smoking in Relation to Indices of Selenium Status of Adults Residing in a Seliniferous Area. American Journal of Clinical Nutrition. 52, 858-862.

Swanson, C. A. *et al*, (1991). Human [^{74} Se] Selenomethionine Metabolism: a Kinetic Model. American Journal of Clinical Nutrition. 54, 917-926.

Tarantal, A. F. *et al*, (1991) Developmental Toxicity of L- selenomethionine in *Macaca fascicularis.* Fundamental and Applied Toxicology, 16, 147-160.

Thomson, C. D. and Stewart, R. D. H. (1976). Metabolism of [^{75} Se] selenite in young women. British Journal of Nutrition. 32, 47-57.

Vanderpas, J. B. *et al*, (1990). Iodine and Selenium Deficiency Associated with Cretinism in Northern Zaire. American Journal of Clinical Nutrition. 52, 1087-93.

Van Dokkum, W., *et al* (1992). Supplementation with selenium-rich bread does not influence platelet aggregation in healthy volunteers. European journal of Clinical Nutrition. 46, 445-450.

Whiting, R. F. *et al* (1980). Unscheduled DNA Synthesis and Chromosme Aberrations Induced by Organic and Inorganic Selenium Compounds in the Presence of Glutathione. Mutation Research. 78, 159-169.

Willhite, C. C. *et al*, (1992) Absorption, distribution and elimination of selenium as L-selenomethionine in non-human primates. Food and Chemical Toxicology, 30, 903-913.

World Health Organization (1987). IPCS Environmental Health Criteria no 70. Principles for the safety assessment of food additives and contaminants in food

World Health Organization. Trace Elements in human nutrition and health (1996). WHO, Geneva.

Yang, G. and Zhou, R. (1994). Further Observations on the human Maximum Safe Dietary Selenium Intake in a Seleniferous area on China. J. Trace Elemnt. Electrolotes. Health Dis. 8, 159-165.

Yang, G., et al, (1989a). Studies of Safe Maximal Daily Selenium Intake in a Seleniferous area in China. I. Selenium Intake and Tissue Selenium Levels of the inhabitants. J. Trace Elemnt. Electrolotes. Health Dis. 3, 77-87.

Yang, G., et al, (1989b). Studies of Safe Maximal Daily Selenium Intake in a Seleniferous area in China. II. Relation between Se Intake and the manifestation of Clinical Signs and Certain Biochemical Alterations in Blood and Urine. J. Trace Elem. Electrolytes. Health Dis. 3, 123-130.

Yu S.Y. *et al*. (1997). Protective role of selenium against hepatitis B virus and primary liver cancer in Qidong. Biol Trace Elem Res, 56(1), 117-24.

Yu S.Y. *et al*. (1989). Chemoprevention trial of human hepatitis with selenium supplementation in China. Biol Trace Elem Res, 20 (1-2), 15-22.

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TOXICITY DATA ON SELENIUM

Human Toxicity

Acute and Short Term Toxicity

Subject	Endpoint	Dose	NOAEL/LOAEL	Duration of Exposure	Comment	Reference
3 year old child Poisoned with Gun Blue (contains selenious acid)	Fatal. Salivation, lung congestion, circulatory failure Contracted ventricles and oedema in brain	Unknown	LOAEL	Single dose	Mixed exposure	Carter, 1966
Adult male, poisoned with Gun Blue	As above	90 ml of a 4% selenious acid in HCl solution	LOAEL	Single dose	Mixed exposure	Matoba *et al*, 1986
15 year old female poisoned with sheep drench	Diarrhoea, ECG irregularities	Approx 22 mg/kg.	LOAEL	Single dose	Vomiting rapidly induced	Civil and MacDonald, 1978
Adult male criminally poisoned with Gun Blue	Diarrhoea, cramping, hair loss, alterations to fingernails	Unknown	LOAEL	Unknown-weeks to months		Ruta and Haider, 1989

Five males	Nausea, diarrhoea, cramping, chills, tremor	Unknown, though 1.1-4.4 mg Se excreted in the 24 hours post admission	LOAEL	Unknown		Quoted, Nordic Project Group, 1994
Adult	Vomiting, diarrhoea, cramping, hair loss, numbness	350 mg (equivalent to 5.8 mg/kg in 60 kg adult)	LOAEL	Unclear		Quoted, Nordic Project Group, 1994
Adult	Hair loss, swelling and discharge from nail bed, nail loss	27.3 mg/day (equivalent to 0.5 mg/kg in 60 kg adult)	LOAEL	10 weeks		Jensen *et al* 1984

Chronic Toxicity

Exposure and Subjects	**Endpoint**	**Dose**	**NOAEL/LOAEL**	**Duration of Exposure**	**Comment**	**Reference**
Adult villagers in seleniferous areas of China	Brittle and pigmentless hair, changes to nails, fluid in nail bed, skin lesions and neurological disturbances	3.2 –6.7 mg/day in area with endemic selenosis. 0.24-1.51 mg/day in non –selenosis area.	**NOAEL = 0.75 mg/day (range 0.24-1.51)**	**Lifetime**		**Yang *et al* (1983)**
400 Adult villagers in	Morphological changes in	0.07, 0.195 and 1.38 mg/day	>0.91 mg/day = NOAEL(by	**Lifetime**	Biochemical changes	Yang et al (1989b)

seleniferous and non-seleniferous areas of China	fingernails. Increased prothrombin time, decreased glutathione levels.	(males) and 0.06, 0.198 and 1.24 mg/day (females)	regression analysis). Can be regarded as minimal effect level as could not be tolerated by sensitive subjects		(?NOAEL) at >0.75-0.85. Significance uncertain	
44 Adult subjects in seleniferous area of US		0.042-0.444 mg Se/day (mean 0.174)	NOAEL	Up to lifetime exposure but study duration 1 year		Swanson *et al* (1994)
142 Adult subjects in seleniferous area of US	Examined for general health, changes in hair and nails, neurological and hepatic abnormalities	0.068-0.724 mg Se/day (mean 0.239)	No effects observed	Up to lifetime exposure but study duration 1-2 years	Se intake associated with serum ALT, but values in reference range and not significant.	Longnecker *et al* (1991)
22 elderly subjects (institutionalised)	Examined for effects on lymphocyte response	0.1 mg/day in yeast or placebo	No adverse effects reported.	6 months	No specific examination made	Peretz *et al* (1991)
67 elderly female subjects (institutionalised)	Plasma selenium levels	0.066 mg/day supplement or placebo	No adverse effects reported.	30 days	Unclear how lack of adverse effect ascertained.	Bortoli *et al* (1991)
20 young adult, 6, middle aged and	Whole blood, plasma and erythrocytes	0.12 mg/day as yeast or sodium	No adverse effects reported.	1 month	No specific examination made	Simonoff *et al* (1992)

10 elderly subjects.	selenium concentrations	selenite				
4 male volunteers/group fed bread with control, medium or high Se.	Toenail selenium concentrations	0.032, 0.206 or 0.388 mg Se/day from bread plus other Se intake (0.08 prior to study)	No effects observed	1 year	No specific examination made	Longnecker *et al* (1993)
6 male volunteers/group fed bread with Se poor or rich flour.	Platelet GPX activity	0.2 mg/day from bread in treatment group plus other Se intake	No effects observed	6 Weeks	No specific examination made	Van Dokkum *et al* (1992)
Female volunteers fed Se in bread		0.1, 0.2 or 0.3 mg/day from bread plus other Se intake	No effects observed	6 Weeks	No specific examination made	Meltzer *et al*
1312 Adults in cancer prevention trial	Examined for selenosis (garlic breath, nail and hair changes)	0.2 mg Se/day supplement plus other Se intake	No effects observed	Up to 10 years	Randomised double-blind trial	Clark *et al* (1996)

Animal Toxicity

Sub-chronic toxicity

Species	Endpoint	Dose	NOAEL/LOAEL	Duration of Exposure	Comment	Reference
Groups of 6 male rats	Decreased weight gain, death at top doses.	2, 3 or 6 ppm Se in drinking water (6 ppm equivalent to 0.48 mg/kg)	LOAEL= 2 ppm	4-6 weeks		Palmer and Olson, 1974
Groups of 50 rats	Mortality in males only	Se in drinking water at 0.28 mg/Kg	LOAEL	Up to 1 year	Males more sensitive?	Schroeder and Mitchener, 1971
Groups of 5 Sprague Dawley rats aged 5 or 12 weeks at start of study	Decreased weight gain and organ weights death at top doses. Increases in liver enzymes.	Up to 64 ppm Se in drinking water. (64 ppm can be estimated to be 11.3 or 3.7 mg/kg bw/day in the 5 and 12 week animals).	NOAEL = 1 ppm	5 weeks	Younger animals more sensitive but could be a body weight effect.	Jacobs and First 1981b
Groups of 5 Swiss mice aged 7 or 18 weeks at start of study	Decreased weight gain, 1 death in top dose. Increases in liver enzymes.	Up to 64 ppm Se in drinking water (64 ppm can be estimated to be 12.8 or 9.2 mg/kg bw/day in the 7 and 18 week animals).	NOAEL = 16 ppm (?SGOT increased in low doses)	5 weeks	Males more sensitive	Jacobs and First 1981a

Groups of male Wistar rats	Survival, body weights, biochemical markers.	Up to 15 ppm Se in drinking water (15 ppm can be estimated to be 0.21 mg/kg bw/day)	NOAEL ?= 6ppm (approximately 0.8 mg/kg bw) Toxic effects on biochemical markers noted but data not provided	6 months		Crespo *et al* (1992)
Wistar rats	Reduced tibia lengths and IGFBP-1/2 levels	3.3 mg Se/l in drinking water	LOAEL	5 weeks		Gronbaek *et al* 1995
Groups of 15 male mice	Lethal at top dose, liver damage, increased plasma AST and ALT	9.4 and 14.2 mg/kg Se as selenocysteine by gavage	LOAEL	30 days		Sayato *et al* 1993
Groups of 8 male Sprague Dawley rats	Increased mortality, decreased growth rate, decreased spleen and liver weights, increased pancreas weights, decreased haemoglobin levels, gross changes to liver.	Up to 11.2 ppm Se in diet as selenite or wheat (11.2 ppm can be estimated to be 1.12 mg/kg bw/day)	NOAEL= 3.2 ppm	6 weeks		Halverson *et al* 1966
Groups of 4 macaques	Deaths in top dose group (anorexia and following emesis and gastritis) pathological changes to liver,	Up to 0.6 mg/kg Se as selenomethionine	NOAEL= uncertain. Maximum tolerated dose = 0.15 mg/kg	30 days		Cukierski *et al* 1989.

	anorexia, hypothermia, disruption of menstrual function					
Groups of 10 F344 rats	Pathological changes in liver	Up to 31.6 mg/kg selenium sulphide by gavage	NOAEL= 17.8 mg/kg	13 weeks	Compound poorly soluble	NTP, 1980
Groups of 10 B6C3F1 mice	Decreased body weights and survival, increased interstitial nephritis	Up to 464 mg/kg selenium sulphide by gavage	NOAEL= 100 mg/kg	13 weeks	Compound poorly soluble	NTP, 1980
Groups of 10 F344 rats	Reduced survival and body weights, changes in organ weights secondary to decreased body weights and dehydration. Pathological changes in immune and reproductive organs, bone liver and pancreas. Renal papilliary degeneration.	Up to 60 ppm (1.1mg/kg Se as selenate)	NOAEL=0.4mg/g bw (15ppm)	13 weeks		NTP, 1994
Groups of 10 B6C3F1 mice	Decreased body weights	Up to 60 ppm (2.6 mg/kg Se as	NOAEL =0.8 mg/kg bw	13 weeks		NTP, 1994

		selenate)				
Groups of 10 F344 rats	Reduced survival and body weights, decreased thymus and right kidney weights other changes in organ weights secondary to decreased body weights Pathological changes in a variety of tissues secondary to decreased body weights. Renal papilliary degeneration.	Up to 32 ppm (0.9 mg/kg Se as selenite)	NOAEL = 0.4 mg/kg bw/day	13 weeks		NTP, 1994
Groups of 10 B6C3F1 mice	Reduced body weights	Up to 32 ppm (1.6 mg/kg Se as selenite)	NOAEL = 0.9 mg/kg bw/day	13 weeks		NTP, 1994
Groups of 10 ICR male mice	Increased AST and ALT at high doses.	Up to 15 mg/kg selenocysteine	NOAEL + 5mg/kg	Up to 90 days		Hasegawa *et al* (1994)

Chronic Toxicity

Species	Endpoint	Dose	NOAEL/LOAEL	Duration of Exposure	Comment	Reference
Groups of Syrian Hamsters	Decreased body weights	"low", 0.1 or 5 ppm Se (0.4-0.5mg/kg as selenite)	NOAEL=0.1 ppm	Up to 144 weeks		Birt *et al* (1996)
Long Evans Rats	Increased mortality, decreased body weights. Selenite more generally toxic, but selenate more tumorigenic	2 ppm Se (as selenite or selenate) in drinking water (approximately 0.1 mg/kg) raised to 3ppm (0.15-0.17 mg/kg) after 1 year	LOAEL.	Lifetime	Pneumonia outbreak, low number of animals. Inadequate study.	Schroeder and Mitchener, 1971
Groups of 50 Swiss mice	Reduced body weight, WBC count. Increased enzymes levels. No signs of neoplasia.	Up to 8 ppm Se as selenite in drinking water (equivalent to 0.05 mg/kg bw/day)	4ppm ="non toxic" dose	2 years		Jacobs and Forst, 1981a
Groups of 17 Sprague Dawley rats	Changes in survival, weight gain, WBC count	0 or 4 ppm Se as selenite in drinking water (equivalent to 0.22 mg/kg bw/day)	4ppm ="non toxic" dose	1-2 years	Small numbers of animals	Jacobs and Forst, 1981b
Groups of 50	Increased incidence	Up to 15 mg/kg	LOAEL= 3	104-5 weeks		NTP, 1980

F344 rats	of hepatocellular carcinomas and neoplastic nodules.	selenium sulphide by gavage	mg/kg bw			
Groups of 50 B6C3F1 mice	Increased incidence of hepatocellular carcinomas and adenomas and primary lung tumours.	Up to 100 mg/kg selenium sulphide by gavage		104-5 weeks		NTP, 1980

Reproductive Toxicity in Rodents

Species	**Type of study and endpoint**	**Dose**	**NOAEL/LOAEL**	**Duration of Exposure**	**Comment**	**Reference**
Sprague Dawley rats	3 generation study. Reduced growth rates. Gross effects on liver.	Up to 5 ppm (0.5 mg/kg bw) Se in the diet.		90 days before breeding to day 77 of offspring		Halverson *et al* 1974
Kunming mice	1 generation study. Increase in litter size, reduced interval between insemination and birth	0.25 ppm Se as selenocarrageenan		65 days before breeding to day 47 after birth	Few details provided.	Chiachun *et al* (1991)
Syrian golden hamsters	Teratogenesis study. Increased malformation mainly encephalopathies at	A range of doses, compounds and routes.		"Critical stages of embryo-genesis"		Ferm *et al* (1990)

	maternally toxic doses. Increased resorptions					
10 day old male rats	Cataracts	A range of doses and compounds by sc injection.	NOAEL = 1.67 mg Se/kg			Ostadalova, 1980
Wild male rats	Reduced body and testes weights. Decreased sperm concentration and motility	Up to 4 ppm Se in diet (equivalent to 0.5 mg/kg bw/day)	2ppm = LOAEL	5 weeks	Few details provided	Kaur and Parshad, 1994
F 344 rats and B6C3F1 mice	Disruption to oestrous cycles and decreased sperm motility and concentration.	Rats: Up to 60 ppm (1.1 mg/kg bw Se) as selenate or up to 32 ppm (0.9 mg/kg bw Se) as selenite. Mice: Up to 60 ppm (2.6 mg/kg bw Se) as selenate or up to 32 ppm (1.6 mg/kg bw Se) as selenite.		13 weeks		NTP, 1994
CD mice	3 generation study. Increased deaths in F1 and runts in F3	3 ppm Se (0.39 mg/kg bw/day)	LOAEL			Schroeder and Mitchener 1971
IVCS mice	6% reduction in fetal survival	Up to 0.78 mg/kg as selenite				Cited ASTDR, 1994

Macaques	No effects except maternally toxic doses	0.3 mg/kg bw/day as selenomethionine	NOAEL = 0.025mg/kg	Day 10-50 gestation		Tarantal *et al* (1991)

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Intakes of Selenium from food

Concerns have been raised that selenium intakes in the UK are falling, with the trend towards European rather than North American wheat, which has a higher selenium content, being suggested as a contributory factor, together with a decline in consumption of cereals, particularly bread.

Information on selenium intakes has not been obtained from dietary surveys due to the lack of reliable information on the selenium content of foods. Instead, selenium intakes are monitored by analysing samples from the Total Diet Study, a model of the national average domestic diet in the UK[4]. The Total Diet Study provides estimates of population average intake of selenium. No information is available on selenium intakes of specific population age groups or on intakes from dietary supplements.

Selenium levels were determined in 1995 Total Diet Study samples by two laboratories using different methods. Average intakes of selenium were calculated from the concentrations of selenium found in each food group and the population average consumption of each food group as estimated from the National Food Survey. Results from the two laboratories agreed well indicating that population average intakes were in the range 29-39µg/day[5]. This intake barely meets the lower end of the reference range for adults (LRNI 40µg/day). Table 1 shows the concentration of selenium in each food group and its relative contribution to intake. The food groups that contributed most to the intake of selenium were meat and meat products, bread, dairy products and fish.

Table 1: Concentrations of selenium in Total Diet samples and estimated average intake 1995

[4] A total of 119 categories of food and drink are specified for inclusion in the Total Diet. These are assigned to one of twenty broad food groups. The quantities and relative proportions of each food that make up the Total Diet are largely based on data from the National Food Survey and are updated annually. Food samples are purchased fortnightly from different locations representative of the UK as a whole and prepared and cooked according to normal consumer practice. The constituents of each group are then homogenised and frozen. Samples can be analysed for a range of food constituents. Average intake of a particular food constituent can be estimated from its concentration in each food group and consumption of each group as determined by the NFS.

[5] Range based on lower & upper level intakes. Lower level intake - concentrations < limit of detection taken as zero; upper level intake - concentrations < limit of detection taken as limit of detection.

	1995 CSL[6]		1995 LGC[7]	
Food group	Se content mg/kg	Se intake[8] mg/person/day	Se content mg/kg	Se intake[5] mg/person/day
Bread	0.053	0.006	0.05	0.005
Miscellaneous cereals	0.022	0.002	0.02	0.002
Carcase meat	0.08	0.002	0.08	0.002
Offals	0.42	0.000	0.45	0.000
Meat products	0.099	0.004	0.07	0.003
Poultry	0.16	0.003	0.11	0.002
Fish	0.32	0.004	0.3	0.004
Oils and fats	<0.004	0.000	<0.01	0.000
Eggs	0.16	0.002	0.21	0.003
Sugars and preserves	0.009	0.001	<0.004	0.001
Green vegetables	0.007	0.000	0.01	0.000
Potatoes	0.007	0.001	0.02	0.003
Canned vegetables	0.008	0.000	0.01	0.000
Other vegetables	0.015	0.001	0.02	0.002
Fruit	<0.002	0.000	<0.01	0.000
Fruit products	<0.002	0.000	<0.01	0.000
Beverages	<0.001	0.000	<0.004	0.002
Milk	0.010	0.003	<0.01	0.001
Dairy products	0.031	0.002	0.03	0.002
Nuts	0.52	0.001	0.54	0.001
Total	-	0.034	-	0.033
Total intake (µg/day)		**34**		**33**

[6] CSL Food Science Laboratory
[7] Laboratory of the Government Chemist
[8] middle level intake, i.e. concentrations below limit of detection taken as 0.5 x limit of detection

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Selenium: Summary table of selected nutrition related information and existing guidance on intakes

Unit of usage	**µg /d**		**µg /100 kcal**	**µg /100g**
	male	female		
UK DRV[9] for adults (19-50+) LRNI RNI	40 75	40 60		
EU labelling RDA[10]	None			
Supplemental doses	50-300µg			
Regulations Weight reduction[11] whole daily diet replacement meal replacement	55µg 18µg			
Maximum total safe daily intake COMA 1991[1] EHPM 1997[12]	450µg/d for adult males, corresponding to 6µg/kg/day. Upper safe level = 200µg Long term consumption Upper limit = 750µg Short term consumption			

[9] Committee on Medical Aspects of Food and Nutrition Policy (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects 41. London: HMSO.
[10] The Food Labelling Regulations 1996
[11] The Foods Intended for Use in Energy Restricted Diets for Weight Reduction Regulations 1997.
[12] Vitamins and Minerals A Scientific Evaluation of the Range of Safe Intakes. European Federation of Health Product Manufacturers 1997.