The European Agency for the Evaluation of Medicinal Products
*Veterinary Medicines Evaluation Unit*

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# COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

## POTASSIUM AND SODIUM SALTS OF SELENIUM

## SUMMARY REPORT

1. Selenium is an essential micronutrient for both animals and man. Deficiency syndromes such as growth impairment, muscular degeneration, cardiomyopathy, hepatic degeneration and reproduction disturbances in ruminants and non-ruminants as well as exudative diathesis and encephalomalacia in poultry have been well documented. In veterinary medicine there is a widespread prophylactic and therapeutic use of sodium selenite salt, often in combination with vitamin E, against diseases and disorders related to selenium/vitamin E deficiencies. The recommended doses for sodium selenite vary between 0.01 and 0.08 mg $Se^{4+}$/kg bw (0.25-1.5 mg/kg feed) for the preparations used in medicated feed in the horse, cow and sheep as well as in swine and poultry. Selenium is also applied as sodium and potassium selenates (0.08-0.24 mg/kg bw). Dosing is given in a single application, divided into 3-5 daily doses or repeated every two or three weeks. In poultry supplemented feeding for 1-2 weeks with 2-3 week intervals is indicated. Selenium preparations (potassium selenate and sodium selenite) are also used for parenteral application (intramuscular) in cattle, swine and sheep as a single dose of 0.02-0.06 mg $Se^{4+}$/kg bw or repeated dosing with a one week interval. A slow-release bolus, indicated in ruminants, gives a daily dose 0.05 mg selenium/kg/day for 120 days. Sodium selenite and sodium selenate have also been approved as feed additives at a maximum concentration of 0.5 mg selenium/kg feed (complete feed). Selenium-based dietary supplements (up to 200 µg selenium/person/day) have a long history of use in human nutrition.

2. Selenium is ubiquitously present in soil in various chemical forms (selenites, selenates and elemental Se) but there is a great variation between different geographical areas. It is taken up by plants and via feed distributed in the tissues of food producing animals. In areas with low levels of selenium in the soil e.g. in the Nordic countries, feed is supplemented (0.1-0.3 mg/kg) in order to prevent development of deficiency syndrome in domestic animals.The foods of animal origin contain the highest selenium levels presumably in the form of selenomethionine and other organic selenocompounds. In grains and cereals selenium is generally low but much higher levels can be found in products from the seleniferous areas.

3. The principal mechanism of action for physiological and pharmacological effects of selenium is its antioxidative effect at the cell membrane against hydrogen peroxide and lipoperoxides. The effects are related to the enzymatic activity of glutathione peroxidases (GSHPx) which contain selenocysteine. Selenium's protective antioxidative action is partially linked to that of vitamin E. Selenocysteine is also an integral component of other functional proteins e.g. tetra- idothyronine-5´-I-deiodinase (involved in metabolism of thyroid hormones) but the full extent of the biochemical mode of action of selenium in the body still remains to be elucidated.

4. Most water-soluble selenium compounds (selenites, selenates, organocompounds) are readily absorbed (80-90%) in the gastrointestinal tract of mice, rats and dogs. Sheep and cows display a lower rate of absorption (30-35%) probably due to selenite reduction to elemental selenium by bacteria in the gastrointestinal tract of ruminants. A high degree of absorption after oral intake of selenite (40-85%), selenate (95%) and selenomethionine (75-97%) has also been shown in human studies. The retention

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of selenium in plasma after administration of selenite seems to be slightly higher in humans with poor selenium status.

5. In laboratory animals there is a rapid distribution of water-soluble selenium compounds to most organs. The specific organ accumulation in experimental animals was shown to be influenced by the selenium status as well as the chemical form of administered Se. The disposition of selenium in man appears to be similar to that of laboratory animals with the exception of distribution among blood components. Several studies have demonstrated that both inorganic and organic forms of selenium cross the placenta and enter milk in experimental animals and man.

6. Metabolic processes involving selenium are dependent on the chemical form and dose as well as on nutritional status. Major metabolites are methylated selenites. Following a reduction to selenide, inorganic selenium is also incorporated into aminoacids and functional proteins but the underlying biochemical processes are not yet fully understood.

7. Studies in laboratory animals indicate that, under normal conditions, urine is the major excretory pathway. However, faecal excretion may dominate in the cases of deficiency states. At high or toxic levels as much as 30-60% of selenium can be excreted via expired air predominantly as dimethylselenide. Available data suggest that man excretes selenium compounds in a way similar to the rat with 40-70% of all excreted selenium found in urine.

8. Various biological indicators of selenium exposure are used depending on the chemical form, level of exposure and nutritional status. Toxic levels of selenium in food-producing animals are reflected by increased blood levels of the element. In humans, at higher intake levels only, selenomethionine intake from food and supplements seems to be directly reflected in whole blood levels whereas high doses of selenite and selenate are related to the increase in urinary excretion.

   In experimental animals a biphasic pattern of selenium biological half-life has been identified with a rapid initial phase of about 3 days and 1.2 days in rat and dog respectively followed by a second phase of about 30-70 days in most species. In studies in humans three phases of elimination were found lasting 1 day, 8-20 days and 65-116 days respectively after selenite intake. There are indications that the half-life of the third phase may be longer when selenomethionine is used.

9. The water-soluble selenium compounds show a relatively high acute toxicity in laboratory animals. Oral $LD_{50}$ values for sodium selenite were 1.0 mg Se/kg in rabbit, 3.0 mg/kg in mouse and 4.8-7.0 mg/kg in rat. A selenium content of 25 mg/kg feed gives a rise to acute toxicity symptoms in most species tested. Gastrointestinal disturbances, cardiotoxic effects as well as signs of neurotoxicity, such as convulsions with an ultimate respiratory arrest, dominate the clinical picture. In farm animals "the blind stagger" syndrome has been described in livestock after an ingestion of plants known to accumulate selenium. The most pronounced clinical sign is restricted vision and neurotoxic effects.

10. According to earlier chronic toxicity studies cited in the literature, diets containing 5 mg Se/kg feed (corresponding to 0.25 mg/kg bw), usually given as selenite, resulted in growth reduction in the rat. At higher dietary levels 6.4-8 mg selenium/kg feed (corresponding to 0.3-0.4 mg selenium/kg bw) there were liver changes, anaemia, splenomegaly, pancreatic enlargement and increased mortality. Based on growth retardation and organ toxicity, a LOEL of 0.03 mg selenium/kg bw/day was suggested. In food-producing animals subclinical toxicity is believed to occur at 2-5 mg selenium/kg feed.

11. In areas with seleniferous soils an "alkali disease syndrome" can develop in horses, livestock and sheep after consumption of plants containing 5-25 mg Se/kg for periods of less than one month. The typical symptoms are emaciation, deformation and shedding of hooves, loss of long hair and erosions of joints of the long bones and eventually liver cirrhosis.

12. Teratogenic effects after exposure to inorganic forms of selenium were indicated in single studies in sheep and pigs but the results were inconclusive. The design of the studies did not conform with the

modern requirements for toxicological investigations. On the other hand according to literature the effects of selenium on reproduction and offspring observed in laboratory rodents were related to maternal toxicity and nutritional deprivation. Recent studies on macaques fed selenomethionine (3, 25, 150 and 300 µg selenium/kg bw/day during organogenesis) produced no signs of terata even although a dose dependent maternal toxicity was observed in this study.

Experimental studies in mice have also indicated a protective effect of selenium against e.g. radiation-induced teratogenicity. Taken altogether the data available do not indicate that there is a link between selenium exposure and toxic effects on the embryo or foetus. Contradictory results were also reported on reproductive toxicity of selenium compounds in laboratory animals. In an older study in mice a failure to breed in the third generation was seen after 0.57 mg selenium/kg/day (the only dose level tested) given in the drinking water as sodium selenate. In other published investigations no effects on sperm or oestrus cycle were observed in mice treated with sodium selenite (drinking water for 13 days in a dose of up to 7 mg selenium/kg bw). Based on the altered menstrual cycle after a daily administration of selenomethionine for 30 days to monkeys, a NOEL was calculated to 0.08 mg selenium/kg bw/day.

13. Both sodium selenite and selenate tested positive in some, but not all, *in vitro* studies in prokaryotic organisms such as *Salmonella typhimurium* (TA 100 without microsomal activation) and *Bacillus subtilis* recombination assay. Sodium selenite induced chromosomal aberrations as well as unscheduled DNA synthesis (UDS) and sister chromatid exchange (SCE) in eukaryotic test systems (Chinese hamster ovary (CHO) cells human fibroblasts). In *in vivo* tests an increased number of micronuclei was observed in the bone marrow of macaques treated by nasogastric intubation with selenomethionine at a dose 0.24 mg selenium/kg/day for 2 weeks. On the other hand chromosomal aberrations and sister chromatid exchange were not increased in healthy persons (n=5) given sodium selenite (0.025 mg selenium/kg/day) for 2 weeks or in patients (n=9) treated with sodium selenite injections (intramuscularly) or tablets (0.05-0.005 mg selenium/kg/day) for 1-13.5 months. These observations in humans were of limited value because this type of study is of low precision and the only parameters investigated were sister chromatid exchange and clastogenicity, with no consideration of possible gene mutations and possible changes in the number of chromosomes per cell. Consequently, there remains some concern that human exposure to selenium compounds may be associated with a mutagenic risk.

14. Several earlier studies indicated an increased incidence of tumours in laboratory animals after oral exposure to selenium. The significance of all these studies has been questioned because of serious shortcomings in design and conduct. On the other hand a number of investigations showed a protective effect against certain types of tumours. According to more recent international evaluations a collective view of the data seems to indicate that the compounds studied will not act as carcinogens at low or moderate doses (Nordic Council of Ministers, 1995).

15. Anecdotal cases of human acute poisoning after oral exposure to selenium compounds have been reported. However exposure levels associated with documented poisonings after ingestion of selenium are lacking. In one episode involving 12 persons, daily doses of 27-31 mg selenium (selenite) in "health" tablets with total doses of 27-2387 mg resulted in nausea, vomiting, hair loss, fatigue, irritability and garlicky breath. The highest serum levels reached 530 µg selenium/l four days after the last tablet. A high simultaneous intake of vitamin C might have alleviated the toxicity.

16. According to more recent studies involving 400 persons from seleniferous areas in China, typical signs of selenosis such as hair loss or nail loss, nail abnormalities, mottled teeth, skin lesions and changes in peripheral nerves were observed after a dietary intake of about 1200 µg selenium/day. The pathological changes were reversible and disappeared as soon as the diets were changed. Symptoms of selenosis were also seen in a man taking for two years 900 µg Se/day as selenite. Prolonged prothrombin times were observed in the chinese studies after a life-long daily intake exceeding 750-850 µg/day. Thus a dose of 750µg selenium/person/day may be regarded as LOEL. In a recent

American 2-year study on 142 persons no clinical signs of toxicity were observed after a dietary intake of 68-724µg selenium/day (mean intake 239 µg selenium/day). At the highest intake level no prothrombin time prolongation or other biochemical changes were seen except a slight increase of alanine aminotransferase enzyme in the serum. The latter values were however within the reference range and considered clinically insignificant. Thus a dose 724 µg selenium/person corresponding to 12 µg selenium/kg bw could be considered a NOEL but the data available do not permit the establishment of an ADI.

Based on the LOEL from human studies a Nordic expert group has recently proposed a safe and tolerable dietary intake of 4-5 µg selenium/kg/day corresponding to 240-300 µg selenium/person (Nordic Council of Ministers, Copenhagen, 1995). The United Kingdom Committee on Medical Aspects of Food Policy (COMA) recommended in 1991 a maximum safe intake of selenium from all sources of 450 µg selenium/person/day for adults. However in the controlled studies from seleniferous geographical areas in the United States of America (see above) on subjects with a nutrition status presumably similar to that of European consumers, there were no clinical side-effects observable after a long-term exposure to doses of up to 720 µg selenium/day. With regard to that an intake of 10 µg selenium/kg bw corresponding to approximately 600 µg/person/day may be considered as safe for human consumption.

The recommended dietary selenium intake established in various international expert bodies is at present between 20-70 µg/adult/day (e.g. Scientific Committee for Foods, European Union, 1993). In some geographical areas with a high selenium in soil in United States of America and China the estimated dietary intake has been reported to be 240 and 750 µg selenium/person/day respectively without any signs of selenosis. The most important sources of selenium are fish, edible organs, meat, dairy products and eggs. Animal products roughly contribute 50% to the total dietary selenium intake but the exact proportions of selenium depend on the composition of the diet and dietary habits. An average total dietary intake in European countries is estimated to range between 35-100 µg selenium/adult/day. A supplement to the human diet with Se-compounds, based on the postulated protective effect of the element against cardiovascular diseases, immunodeficiency and cancer, has been extensively debated but at the present time no internationally accepted recommendation has been adopted. In several countries selenium preparations have been marketed e.g. as "health foods" or nutritional supplements in recommended doses up to 200 µg selenium/person/day.

17. There are numerous reports on selenium tissue levels in various domestic animals after a continuous intake of feed supplemented either directly (additive) or through e.g. fertiliser with lower (prophylactic) doses of the element. However proper depletion studies after the application of selenium-based medicines to the indicated animal species seem to be lacking. The selenium contents of skeletal muscle and internal organs have been shown to be linearly increasing with intake and plateau with rising dose. Highest levels were found in the edible organs such as kidney and liver followed by lower concentrations in the muscle. However there seems to be a great variation both in the ratios between various tissues and with regard to the absolute concentrations depending on whether the selenium is supplied in the inorganic or organic (presumably present in plants) forms. The differences in bioavailability between various chemical forms present in different diets in various animal species have not yet been fully elucidated.

    In an investigation using radioactivity labelled $^{75}$Se-sodium selenite, groups of lambs (n=5) received varying doses of selenium (0.05 mg, 0.25 mg and 0.6 mg selenium/kg bw) given as a single intramuscular injection. 30 days and 56 days after injection the animal tissues were analysed for the content of selenium. There was a dose-dependent linear increase of selenium tissue concentration. The mean levels of selenium measured at 30 days after administration of the highest two doses were 0.005 mg and 0.011 mg/kg in the muscle, 0.112 mg and 0.470 mg/kg in the liver as well as 0.070 mg and 0.130 mg/kg in the kidney. The selenium concentration at the injection site did not differ significantly from that in the muscle. However it should be pointed out that selenium concentration at the injection

site after postinjection times shorter than 30 days has not been determined and there are at present no data available on depletion of selenium from the site of injection.

Studies in sheep (n=4) have shown that after the ingestion for 10 days of feed supplemented with 3 mg selenium/kg feed derived from sodium selenite (corresponding to approximately 0.12 mg selenium/kg bw) the concentrations were 1.4 mg selenium/kg in the kidney, 0.84 mg selenium/kg in the liver and 0.16 mg selenium/kg in muscle. In comparison, control animals (0.2 mg selenium/kg feed) had 0.34 mg selenium/kg in the kidney, 0.26 mg/kg in the liver and 0.08 mg selenium/kg in the muscle. In pigs (n=6) after 17 weeks of daily feeding, the diet supplemented with 2.6 mg selenium/kg feed from sodium selenite (corresponding to approximately 0.10 mg/kg bw) resulted in no increase in the muscle (0.4 mg selenium/kg), a 1.3 fold increase in the kidney to 2.15 mg selenium/kg and 4-fold increase in the liver to 2.43 mg selenium/kg as compared to control animals receiving 0.5 mg selenium/kg feed. The supplement in the two studies corresponded to the highest levels currently recommended for sodium selenite medical preparations used in feed. Considering the standard food package, the dietary intake via meat and edible organs from treated pigs would be approximately 490 µg selenium/person/day.

Using the lower level (corresponding to 4.5µg selenium/kg bw) of feed supplemented with sodium selenite or plant selenium for 90 days in milking cows (n=15), an American study showed a mean concentration in milk of 18µg selenium/l. Comparable results were obtained in a 2-year investigation in milking cattle (n=10) following feed supplemented with sodium selenite or yeast selenium in Sweden. Based on a daily consumption of 1.5 litre milk it would correspond to the intake of approximately 22 µg selenium/person/day. Levels of up to 60 µg selenium/litre milk were reported in earlier studies from Denmark.

It should be pointed out that in all the relevant studies a significantly higher increase in the tissue and milk selenium concentrations could be detected when supplement with the organic selenium (plant, yeast) was used as compared to feed fortification with sodium selenite.

18. In conclusion there is a great interspecies variation in the nature and severity of selenium induced toxic effects. The adverse effects reported in connection with human use of selenocompounds are therefore the most appropriate parameter to be used when assessing the NOEL. Based on the critical review of data available, the estimated safe level for a long-term ingestion of selenium in man appears to be 10 µg selenium/kg/day corresponding to 600 µg selenium/person/day.

Based on residue data available and a worst case scenario of animals slaughtered directly after a long-term continuous intake of feed medicated with recommended doses of sodium selenite, or sodium or potassium selenate, the consumer exposure to selenium from animal foods would be within the estimated safe daily intake. Considering a single dosing or short-term treatment most often recommended for the use of selenium-based medicines a significantly lower intake of selenium via animal foods can be expected in a normal consumer. There are however at present no data available on the depletion rate of selenium from the injection site after parenteral treatment of food-producing animals with selenium-based preparations. In order to avoid any possible risks to the consumer, withdrawal times for the injection preparation should be implemented by the national authorities.

**Conclusions and recommendation**

Having considered the criteria laid down by the Committee for the inclusion of substances in Annex II of Council Regulation (EEC) No 2377/90 and in particular that:

- selenium is an essential element and a normal constituent of the diet in man,
- animals are unlikely to be sent for slaughter immediately after treatment,
- the estimated safe intake of selenium is unlikely to be exceeded after exposure to residues of selenium in the foods of animal origin resulting from the treatment with the recommended doses of sodium selenite, sodium selenate or potassium selenate,
- the use of sodium selenite, sodium selenate or potassium selenate in prophylaxis and therapy of deficiency diseases in food-producing animals is not expected to increase substantially the long-term total dietary exposure of the consumer to selenium,
- it appears unlikely that the use of selenium derived from sodium selenite, sodium selenate or potassium selenate in veterinary medicine products represents any significant risk to the consumer;

The Committee concluded that there was no need to establish an MRL for selenium derived from the use of sodium selenite, sodium selenate or potassium selenate and recommend the inclusion of these three substances in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

| Pharmacologically active substance(s) | Target species | Other provisions |
|---|---|---|
| Potassium selenate | All food producing species | |
| Sodium selenate | All food producing species | |
| Sodium selenite | All food producing species | |

An accumulation of selenium residue at the injection site may exist shortly after treatment and therefore Member States should consider the establishment of withdrawal times for parenteral preparations.