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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

BARIUM SELENATE

SUMMARY REPORT

1. Barium selenate is used in a slow-release injectable preparation (oil suspension) for therapeutic and prophylactic use against diseases and disorders related to selenium deficiencies in sheep and cattle. The preparation is given as a single subcutaneous injection at a dose of 1 mg selenium/kg bw.

   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. Barium is present in the soil and plants and is a normal constituent of the human diet.

2. Selenium is ubiquitously present in soils in various chemical forms (selenites, selenates and elemental selenium) but there is a great variation between different geographical areas. It is taken up by the plants and via feed distributed in the tissues of food producing animals. In areas with low levels of selenium in the soil e.g. the Nordic countries, feed is supplemented (0.1 to 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 form of selenomethionine and other organic selenocompounds. In grains and cereals the level of 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, which contain selenocysteine. Selenium protective antioxidative action is partially linked to that of vitamin E. Selenocysteine is also an integral component of other functional proteins e g tetraiodothyronine-5'-iodo-deiodinase (involved in metabolism of thyroid hormones) but the full extent of the biochemical mode of action of selenium in the body remains to be elucidated.

4. Most water-soluble selenium compounds (selenites, selenates, organocompounds) are readily absorbed (80 to 90%) in the gastrointestinal tract of mice, rats and dogs. A high degree of absorption after oral intake of sodium selenite (40 to 85%), selenate (95%) and selenomethionine (75 to 97%) has also been shown in human studies.

5. In laboratory animals there is a rapid distribution of 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 selenium. The disposition of selenium in man appears to be similar to that of laboratory animals with 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.

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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 amino acids 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 deficiency states. At high or toxic levels as much as 30 to 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 to 70% of 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 an increase in urinary excretion.

   In experimental animals a biphasic biological half-life of selenium 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 to 70 days in most species. In studies on humans after selenite intake three phases of eliminantion were observed lasting 1 day, 8 to 20 days and 65 to 116 days, respectively. There are indications that the half-life of the third phase may be longer for selenomethionine.

9. Soluble barium compounds are absorbed to various degrees from the gastrointestinal tract in animals and man. The absorption depends on several factors such as chemical form, presence of barium sulfate in food and age. However, studies in rats have shown that after oral application of barium selenate there is negligible uptake in the blood and a very limited increase in urinary excretion with 60% of the administered dose (2.8 mg barium/animal) recovered in the faeces within 72 hours after treatment.

10. Water-soluble selenium compounds show a relatively high acute toxicity in laboratory animals. Oral LD_{50} values for sodium selenite were 1 mg selenium/kg in rabbit, 3 mg/kg in the mouse and 4.8 to 7 mg/kg in the rat. A selenium content of 25 mg/kg in feed gives 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 a restricted vision and neurotoxic effects.

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

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

13. Contradictory results were 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 and oestrus cycle were observed in mice treated with sodium selenite (drinking water for 13 days, doses up to 7 mg selenium/kg bw). Based on altered menstrual cycle after a daily administration of selenomethionine for 30 days to monkeys, a NOAEL of 0.08 mg selenium/kg bw/day was calculated.

14. Teratogenic effects after exposure to inorganic forms of selenium were suspected in single studies on sheep and pigs but the results were inconclusive. The study design did not conform with current requirements.

    On the other hand, according to literature, the effects of selenium on reproduction and offspring observed in laboratory rodents were related to the 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, although a dose dependent maternal toxicity was observed in this study. Studies in mice have also indicated a protective effect of selenium against e.g. radiation-induced teratogenicity. Overall the available data do not indicate that there a the link between selenium exposure and toxic effects on embryo or foetus.

15. Both sodium selenite and selenate tested positive in some, but not all, *in vitro* studies in prokaryotic organisms such as *Salmonella typhimurium* (strain TA 100 without metabolic activation) and *Bacillus subtilis* recombination assay. Sodium selenite induced chromosomal aberrations as well as unscheduled DNA synthesis and sister chromatide exchange in eukaryotic test systems (Chinese hamster ovary 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 bw/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 bw/day) for 2 weeks or in patients (n=9) treated with intramuscular sodium selenite injections or tablets (0.05 to 0.005 mg selenium/kg bw/day) for 1 to 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.

16. Several earlier studies indicated an increased incidence of tumors in laboratory animals after oral exposure to selenium. The significance of all these studies has been questioned because of serious shortcomings in design and conduction. On the other hand a number of investigations showed a protective effect against certain types of tumors. 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).

17. No information on the oral toxicity of barium was provided but a very low bioavailability observed in the experimental studies in rat makes a systemic toxicity of barium derived from barium selenate unlikely.

18. 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. According to a case report an intake of 250 mg selenium (chemical form unknown) as a single dose caused acute gastrointestinal disturbances and hair loss. In one episode involving 12 persons daily doses of 27 to 31 mg selenium (selenite) in "health" tablets with total dose of 27 to 2387 mg resulted in nausea, vomiting, hair loss, fatigue, irritability and garlicy breath. The highest serum levels reached 530 µg selenium/l 4 days after the last tablet. A high simultaneous intake of vitamin C might have alleviated the toxicity.

19. 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 selenium/day as selenite. Prolonged prothrombin times were observed in the chinese studies after a life-long daily intake exceeding 750 to 850 µg/day. Thus a dose 750 µg selenium/person/day may be regarded as LOAEL. In a recent American 2-year study on 142 persons no clinical signs of toxicity were observed after a dietary intake of 68 to 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 NOAEL but the data available do not permit the establishment of an ADI.

    Based on the LOAEL from human studies, a Nordic expert group has recently proposed a safe tolerable dietary intake of 4 to 5 µg selenium/kg bw/day, corresponding to 240 to 300 µg selenium/person (Nordic Council of Ministers, Copenhagen, 1995). The UK 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 United States of America (see above) on subjects with a nutrition status presumably similar to that of European consumers there were no clinical effects observable after long-term exposure to doses of up to 720 µg selenium/day. Thus, 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 to 70 µg/adult/day (e.g. Scientific Committee for Foods, EU, 1993). In some geographical areas in the United States of America and China with a high selenium concentration in the soil, 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 proportion of selenium depends on the composition of the diet and dietary habits. An average total dietary intake in European countries is estimated to range between 35 to 100 µg selenium/adult/day. A supplementation of human diet with selenium 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, for example as "health foods" or nutritional supplements, in recommended doses up to 120 µg selenium/person/day.

20. There are numerous reports on selenium tissue levels in various domestic animals after a continuous intake of feed supplemented either directly (additive) or through, for instance, fertilizer 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 shown a linear increase with the intake and plateau with rising dose. Highest levels were found in the edible organs such as kidney and liver followed by the 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 of various animal species have not yet been fully elucidated. Only limited studies are reported in the literature on the distribution of selenium after the application of barium selenate to the food producing animals.

21. In an investigation using radiolabelled (^{75}Se) barium selenate, groups of ewes (4 animals) received two dose levels of selenium (1.1 and 0.45 mg selenium/kg bw, respectively) given as a single, subcutaneous injection. Half of the animals were injected in the shoulder, the others in the base of the ear. Serum was collected in 7 day intervals and tissue samples were taken on day 148 post injection. There was a dose-dependent increase of selenium in serum and tissues. Significantly higher levels of the radiolabel were recorded throughout the experiment after injection in the shoulder as compared to application in the ear. The mean levels of selenium measured in the high dose group (shoulder application) were 35 µg/kg in muscle, 100 µg/kg in liver and 580 µg/kg in kidney, 148 days after the injection of barium selenate.

    In another study heifers (13 animals) received a single subcutaneous injection of barium selenate at a dose corresponding to 1 mg selenium/kg bw. Individual animals were slaughtered between 30 to 119 days after the treatment and tissues selenium content was measured using atomic absorption spectrometry. There was a statistically significant persistent increase of selenium in the liver of the treated animals with 400 mg selenium/kg (approximately 50 mg/kg in controls) detected 119 days post injection. In the muscle, the selenium (group mean) increased to 97 mg/kg as compared to 59 mg/kg in controls. No statistically significant differences were detected in the kidney. The amounts recovered from the injection site ranged from 459 to 556 mg selenium at 30 to 119 days after injection. This corresponds to a calculated 77 to 99% of the injected dose remaining at the injection site during the period of investigation. In sheep, approximately 35% of the injected selenium remained at the site of injection 90 days after the application of barium selenate (no details of the study given).

    No data were submitted on the transfer of selenium into milk after the treatment of lactating cows with barium selenate.

22. In an investigation on the distribution of barium in edible tissues of cattle and sheep, animals were injected subcutaneously with barium selenate at the recommended dose corresponding to 1 mg barium/kg bw. Groups of animals were slaughtered 6 (2 animals), 9 (2 animals) and 12 (4 animals) months after the injection**.** Two bovine and three ovine control animals receiving saline solution were also slaughtered at the 12 month time period. The slaughter times were based on the selenium levels measured in the serum 1, 3, 7, 10, 15, 21, 28, 56, 84, 112, 175, 238, 301 and 365 days post treatment. The mean concentrations in cattle showed a gradual increase from approximately 45 µg selenium/l (day 1) to a plateau of 60 µg selenium/l (day 300) and a subsequent decline to 50 µg selenium/l (day 365). A similar pattern of selenium distribution was seen in the sheep. Barium and selenium were measured in the collected tissue samples using inductively coupled plasma optical emmission spectroscopy and atomic absorption spectroscopy, respectively. Barium was present in all samples.

    In the cattle study there were great individual variations throughout the experiment with no indication of time related accumulation of barium in edible tissues. The highest barium levels detected in control cattle were 36, 63, 30 and 235 µg/kg in fat, muscle, liver and kidney, respectively. Comparable levels were also detected in the experimental animals with exception of liver and injection site. The liver of the treated animals showed elevated barium concentrations in the majority of the samples with the highest levels being approximately 2.5 to 16 times higher than in controls. Very high residues of barium in the animals given barium selenate were found at the injection site during the whole period of investigation with individual values ranging between 70 to 81 000 µg/kg. In control sheep, barium tissue levels were in the same range as those seen in cattle. The concentrations in edible tissues of barium selenate treated sheep showed a tendency to be somewhat higher than in controls with the highest increase 2 to 3 fold. The levels of barium detected at the injection site after treatment varied between 79 to 674 000 µg/kg as compared to 42 to 46 µg/kg barium found in the control animals.

    The data on the distribution of selenium in the tissues of cattle and sheep indicated a slight increase of tissue selenium in some of the treated animals with the highest increase (in the liver of cattle) 2 to 4 fold. The levels of selenium detected at the injection site after treatment varied between 86 to 243 000 µg/kg in sheep and 78 to 53 000 µg/kg in cattle.

Considering the standard food package, the dietary selenium intake via edible tissues (excluding the injection site) from treated cattle would be approximately 145 µg/person/day. Similarly, the daily exposure to barium from edible tissues of treated sheep and cattle can be calculated as 70 µg and 90 µg barium/person/day, respectively. However, a single exposure from the injection site immediately after administration may be expected to be in the range of 500 mg selenium and up to 900 mg barium per person, respectively.

23. In conclusion there is a great interspecies variation in 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 in the safety assessment. Based on the critical review of data available, the estimated safe level for long-term ingestion of selenium in man appears to be 10 µg selenium/kg bw/day, corresponding to 600 µg selenium/person/day. The available toxicological data are insufficient to establish an ADI for barium. However, with the exception of the injection site, the intake of barium from the edible tissues following the treatment with barium selenate is within the range of intake expected from the normal diet (500 to 1500 µg/person/day).

    Based on available residue data and a worst case scenario taking into account the highest levels of selenium detected after injection of barium selenate at recommended doses, the consumer exposure to selenium from edible tissues would be well within the estimated safe level. The expected dietary exposure from edible tissues of barium selenate treated animals should not exceed the normal intake of barium with food. Considering the very low oral bioavailability of barium selenate, even the ingestion of residues of selenium and barium from the injection site is unlikely to present a health risk for the consumer.

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,
- 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 barium selenate,
- barium is a normal constituent of the human diet and the estimated intake from edible tissues of barium selenate treated animals is not expected to increase substantially the long-term normal dietary exposure of the consumer to barium,
- oral bioavailability of barium from barium selenate is negligible,
- it appears unlikely that the use of selenium derived from barium selenate in veterinary medicine products represents any significant risk to the consumer;

the Committee for Veterinary Medicinal Products concluded that there was no need to establish an MRL for selenium or barium derived from the use of barium selenate and recommends the inclusion of this substance in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologially active substance(s)	Animal species	Other provisions
Barium selenate	Bovine, ovine	

An accumulation of selenium and barium at the injection site exists after treatment and therefore Member States may consider measures to make the injection site visible in order to avoid that the injection site be consumed.