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European public MRL assessment report (EPMAR)

Barium selenate (All food producing species)

Barium selenate is used for therapeutic and prophylactic treatment of diseases and disorders related to selenium deficiencies. The substance was previously evaluated by the Committee for Medicinal Products for Veterinary Use in 1999, leading to the establishment of a “No MRL required” classification in bovine and ovine species with no restrictions on the route of administration¹.

On 2 December 2013 the German Federal Ministry of Food, Agriculture and Consumer Protection submitted a request to the European Medicines Agency for a new opinion on barium selenate, in light of concerns relating to potential consumer exposure to residues at the injection site.

Based on the available data, the Committee for Medicinal Products for Veterinary Use recommended, on 10 April 2014, the modification of the maximum residue limit entry for barium selenate in Commission Regulation (EU) No. 37/2010, incorporating a restriction on the route of administration (“Not for administration by injection”) and extrapolating the entry to all food producing species.

Subsequently the Commission recommended, on 30 January 2015, the amendment of the entry for barium selenate in Commission Regulation (EU) No. 37/2010. This recommendation was confirmed on 20 February 2015 by the Standing Committee on Veterinary Medicinal Products and, on 17 March 2015, the European Commission adopted a Regulation² establishing maximum residue limits for barium selenate in all food producing species, valid throughout the European Union.

¹ Commission Regulation (EU) No 37/2010, O.J. L 15, of 20.01.2010

² Commission Implementing Regulation (EU) No 2015/446, O.J.L 74/18, of 18.03.2015



Summary of the scientific discussion for the establishment of MRLs

1. Introduction

Selenium is an essential micronutrient for both animals and humans. 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.

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 plants and so is present in feed, and is distributed to the tissues of food producing 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.

In areas with low levels of selenium in the soil e.g., in the Nordic countries, feed may be supplemented (0.1 to 0.3 mg selenium per kg feed) in order to prevent development of deficiency syndrome in domestic animals.

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

Barium selenate was previously assessed by the CVMP in 1999. At that time the Committee concluded that an intake of 600 µg selenium per person per day (10 µg/kg bw/day) could be considered as safe for human consumption. This figure was based on long-term exposure studies in humans. The data on barium did not allow the derivation of a safe level of intake but it was concluded that intake resulting from ingestion of food commodities derived from animals treated with barium selenate would be within the range of barium intake expected from the normal diet.

The 1999 CVMP summary report for barium selenate noted that ingestion of an injection site could lead to an intake of selenium greater than the safe level of 600 µg/person and an intake of barium in excess of that expected from the normal diet. The summary report therefore indicated that Member States may consider measures to make the injection site visible in order to avoid that the injection site is consumed. To date, adequate measures to ensure this have not been developed.

Currently, barium selenate is included in Commission Regulation (EU) No 37/2010 in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Barium selenate	NOT APPLICABLE	Bovine, ovine	No MRL required	NOT APPLICABLE	NO ENTRY	NO ENTRY

“No MRL required” classifications also exist for potassium selenate, sodium selenate and sodium selenite.

On 2 December 2013 the German Federal Ministry of Food, Agriculture and Consumer Protection submitted to the European Medicines Agency a request under Article 11 of Regulation (EC) No 470/2009 to issue a new opinion on the substance barium selenate. The request from Germany follows concerns raised with regard to the persistence of residues at the injection site and new data on the oral bioavailability of residues.

2. Scientific risk assessment

This evaluation focuses particularly on selenium rather than barium. This is appropriate as the risk to the consumer results from the selenium content of barium selenate, as the dose of barium selenate that would lead to the consumer being exposed to selenium at levels in excess of the safe level is far lower than the dose that would lead to the consumer being exposed to barium at levels in excess of the safe level.

2.1. Safety assessment

Since the original CVMP assessment of barium selenate in 1999 additional information relevant to the consumer safety evaluation of the substance has been generated. In particular, the European Commission's Scientific Committee on Food recommended a tolerable upper intake level for selenium and additional data relevant to the bioavailability of selenium from orally ingested barium selenate has become available.

2.1.1. Overview of pharmacological properties

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. Selenocysteine is also an integral component of other functional proteins such as 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.

Pharmacokinetic properties

Most water-soluble selenium compounds (selenites, selenates, organocompounds) are readily absorbed (80 to 90%) from 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.

In laboratory animals, following absorption, 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 in laboratory animals. Several studies have demonstrated that both inorganic and organic forms of selenium cross the placenta and enter milk in experimental animals and man.

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 (as selenocysteine) and cotranslationally into functional proteins. Although significant progress has been made in elucidating the biological role of selenium many aspects of the underlying biochemical mechanisms are not yet fully understood.

Studies in laboratory animals indicate that under normal conditions urine is the major excretory pathway for selenium. 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 humans excrete selenium compounds in a way similar to the rat with 40 to 70% of excreted selenium found in urine.

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 in humans after selenite intake three phases of elimination 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.

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 sulfate in food and age. However, studies in rats have shown that after oral application of barium selenate there is negligible uptake of barium into the blood and a very limited increase in urinary excretion with 60% of the administered barium (2.8 mg barium/animal) recovered in the faeces within 72 hours after treatment.

The potential bioavailability of barium selenate following oral administration can be estimated based on physicochemical considerations. Barium selenate is poorly soluble in comparison to other selenium salts. Its solubility product constant (K_{sp} [25 °C] 3.40×10^{-8}) suggests that approximately 52 mg barium selenate dissolve in one litre of water (25.279 mg/l Ba^{2+} and 26.315 mg/l SeO_4^{2-}), corresponding to approximately 15 (14.53) mg selenium/litre.

Furthermore, barium selenate can be dissolved in hydrochloric acid (HCl), from which chlorine may be released, leading to the formation of barium chloride and selenious acid.

The solubility of barium selenate will be temperature and pH dependent. At the physiological pH of gastric juice (ranging from about 1 to 4) the amount of soluble selenium would be expected to increase to more than the 15 mg/l estimated based on the solubility product.

It is also noteworthy that in the presence of sulphate ions barium sulphate ($K_{sp} 1.08 \times 10^{-10}$) will be formed and will precipitate. As sulphates are present in excess of selenates in normal food³ (for example, mineral water is a significant source of sulphate ions), the re-establishment of chemical equilibrium after precipitation of (acid) insoluble barium sulphate will enhance the release of (soluble) selenium ions from barium selenate.

Data on the absorption of selenium were also provided from a study using an *in vitro* gastrointestinal model simulating the successive processes in the stomach and the small intestine of humans after intake of food. Two different doses of barium selenate were used, equating to 35 mg and 140 mg selenium. Data from the model demonstrate that 5.0% and 10.4%, respectively, of the selenium (administered as barium selenate) was bioavailable. A number of weaknesses were identified in the model (for example, it does not take account of the fact that, *in vivo*, there would be a constant release of selenate ions from barium selenate as bioavailable selenium is absorbed) and consequently the data generated are considered to represent an estimate of the lower range of bioavailable selenium that would result from ingestion of barium selenate.

³ WHO (2004): Background document for development of WHO Guidelines for Drinking-water Quality WHO/SDE/WSH/03.04/114

Overall, theoretical considerations based on physico-chemical properties of barium selenate as well as recent *in vitro* and *in vivo* studies all consistently indicate that relevant amounts of selenate are bioavailable from orally ingested barium selenate. Data from the *in vitro* study simulating conditions in the human gastrointestinal tract indicate that a minimum of 5% to 10.4% of ingested selenium from barium selenate is bioavailable when present in a normal food matrix.

2.1.2. Calculation of pharmacological ADI, if relevant

Relevant pharmacological effects of barium selenate are considered to be adequately represented in the animal and human studies described below. No additional pharmacological effects that would need further characterisation have been identified. Consequently, and in line with the CVMP guideline on the approach to establish a pharmacological ADI, no pharmacological ADI is considered necessary.

2.1.3. Overview of toxicology

The toxicity of selenium (salts) is thought to occur following its absorption and reduction/metabolism by glutathione to hydrogen selenide (H_2Se) through selenodiglutathione and glutathionylselenol intermediates. Hydrogen selenide is the key metabolite derived from the inorganic forms of selenium, selenite and selenate. It is oxidised to selenium dioxide and probably causes toxicity due to the production of superoxide and other reactive oxygen species, which can induce cell damage. Selenium toxicity is also associated with non-specific incorporation of selenium in place of sulphur in functional proteins leading to protein malfunction and disruption of cellular processes. With acute poisoning, one explanation for selenium toxicity is the depletion of intermediate substrates, such as glutathione and S-adenosyl methionine.

Single-dose toxicity

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.

Repeated dose toxicity

No standard repeated dose toxicity studies performed with barium selenate were available.

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 established. In food-producing animals subclinical toxicity is believed to occur at 2 to 5 mg selenium/kg feed.

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.

Reproductive toxicity, including developmental toxicity

No standard reproductive toxicity or developmental toxicity studies performed with barium selenate were available.

Contradictory results have been reported on the 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 bw/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.

Teratogenic effects after exposure to inorganic forms of selenium were indicated in single studies on sheep and pigs but the results were inconclusive.

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. Studies on macaques fed selenomethionine (3, 25, 150 and 300 µg selenium/kg bw/day during organogenesis) produced no signs of teratogenesis, although a dose dependent maternal toxicity was observed. Studies in mice have also indicated a protective effect of selenium against for example, radiation-induced teratogenicity. Overall the available data do not indicate a link between selenium exposure and toxic effects on embryo or foetus.

Genotoxicity

No genotoxicity studies performed with barium selenate were available.

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 chromatid 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.

Carcinogenicity

No carcinogenicity studies performed with barium selenate were available.

Several earlier studies indicated an increased incidence of tumours in laboratory animals after oral exposure to selenium. The significance of 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. Overall, international evaluations conclude that the data seem to indicate that the compounds studied will not act as carcinogens at low or moderate doses⁴.

Studies of other effects including immunotoxicity and neurotoxicity

No studies of immunotoxicity or neurotoxicity were available.

2.1.4. Calculation of the toxicological ADI or alternative limit

The data available does not allow a toxicological ADI to be established for barium selenate. However, based predominantly on the available human data, a number of bodies have made recommendations on safe limits for selenium intake. The CVMP conclusion on the safe limit for selenium intake is presented in section 2.1.9 on the overall conclusions on the ADI.

2.1.5. Overview of microbiological properties of residues

No microbiological data were available which is acceptable as no microbiological effects are expected.

2.1.6. Calculation of microbiological ADI

As no microbiological effects are expected the establishment of a microbiological ADI is not relevant.

2.1.7. Observations in humans

In humans, cases of selenium poisoning have been described after oral ingestion of selenium although, in general, selenium exposure levels associated with documented poisonings are lacking. Gastrointestinal and neurological symptoms predominated. Intake of 250 mg selenium as a single dose or in multiple doses of 25–30 mg resulted in acute toxic effects, such as nausea, vomiting, nail changes, dryness of hair, hair loss, tenderness and swelling of fingertips, fatigue, irritability and garlicky breath. In Sweden, several cases of toxicity in children occur each year due to accidental overconsumption of selenium tablets. Acute symptoms such as vomiting have been observed. Clinically significant selenium toxicity was reported in 13 individuals after prolonged and regular ingestion of supplements containing 27.3 mg (27,300 µg) selenium per tablet due to a manufacturing error.

In an episode involving 12 people, ingestion of 'health' tablets led to daily doses of 27 to 31 mg selenium (selenite), with a total dose of 27 to 2387 mg, resulted in nausea, vomiting, hair loss, fatigue, irritability and garlicky 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.

In a review of selenium poisoning in humans (Nuttall, 2006⁵) case reports and corresponding estimated doses of selenium intake were given. The data indicate that oral doses of 5 mg to 22.3 mg selenium/kg bw (as sodium selenite or sodium selenate) were acutely toxic, sometimes resulting in a fatal outcome.

In 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

⁴ Alexander J, Melzer HM. Selenium. In: Oskarsson A (editor). Risk evaluation of essential trace elements – essential versus toxic levels of intake. Copenhagen: Nordic Council of Ministers Nord, 1995: 18: 9-54

⁵ Nuttall, KL (2006): Review: Evaluating Selenium Poisoning. Annals of Clinical and Laboratory Science, 36,4, 409-420.

and disappeared as soon as the diets were changed. The LOAEL for clinical selenosis was 900 to 1000 µg selenium per day. While one man taking 913 µg selenium per day (as selenite) exhibited clinical signs of selenosis, no clinical signs were seen in people with an intake estimated at approximately 850 µg/day. The NOAEL for clinical symptoms of selenosis was therefore considered to be 850 µg selenium/person per day. Marginally prolonged prothrombin times were observed in subjects estimated to have a selenium intake of around 850 µg/day.

In a 2-year American study on 142 persons no clinical signs of toxicity were observed after a dietary intake of 68 to 724 µg selenium/day (mean intake of 239 µg selenium/day). At the highest intake level no prothrombin time prolongation or other biochemical changes were seen except for 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.

2.1.8. Findings of EU or international scientific bodies

In 1991 The UK Committee on Medical Aspects of Food Policy (COMA) recommended a maximum safe intake of selenium from all sources of 450 µg selenium/person/day for adults.

A 1995 report (Nordic Council of Ministers, Copenhagen, 1995) proposed a safe tolerable dietary intake of 4 to 5 µg selenium/kg bw/day, corresponding to 240 to 300 µg selenium/person, based on effects seen in humans.

In 2000 the European Commission's Scientific Committee on Food recommended a tolerable upper intake level for selenium of 300 µg/person (5 µg/kg bw), based on effects seen in humans.

In 2006 the EFSA Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) accepted the tolerable upper intake level recommended by the Scientific Committee on Food.

In 2012 the European Commission's Scientific Committee on Health and Environmental Risks adopted a tolerable daily intake for barium of 0.2 mg barium/kg bw/day, which corresponds to 12 mg/person/day.

2.1.9. Overall conclusions on the ADI

Based on the data available an ADI cannot be established. However, both selenium and barium are naturally occurring constituents of the human diet and as such, safe limits for their intake have been established based on human exposure data. The safe level for selenium intake is much lower than the safe level for barium intake.

In its 1999 report the CVMP established a safe level for long term ingestion of selenium in man as 600 µg/person per day (10 µg/kg bw/day). This figure was based on consideration of the available human data. In particular, it was noted that in seleniferous geographical areas in United States of America (in which people are assumed to have a nutritional status similar to that of European consumers) no clinical effects were observed after long-term exposure to doses of up to 720 µg selenium/day.

In 2000 the European Commission's Scientific Committee on Food reviewed the available data on selenium and established a tolerable upper intake level of selenium at 300 µg/person per day. This figure was based on studies conducted in seleniferous areas in China (reported above) in which the LOAEL for clinical symptoms of selenosis was considered to be 900 to 1000 µg of selenium/person per day and the NOAEL was 850 µg/person per day. The Scientific Committee on Food considered that sensitive individuals were likely to have been included in the study and that consequently an uncertainty factor of 3 was appropriate for derivation of a tolerable upper intake level of selenium. The tolerable upper intake level of selenium was

therefore established as 300 µg/person per day, to cover intake from all sources (including food and supplements).

The CVMP supports the evaluation undertaken by the Scientific Committee on Food and confirms the tolerable upper intake level of 300 µg selenium/person per day as being appropriate for use in the consumer safety evaluation of selenium.

In addition the Scientific Committee on Health and Environmental Risks adopted, in 2012, a tolerable daily intake for barium of 0.2 mg barium/kg bw/day, which corresponds to 12 mg/person/day. This value is much higher than the tolerable upper intake level of 300 µg selenium/person per day established for selenium. Consequently the tolerable upper intake level for selenium is considered the appropriate consumer safety reference value for use in the MRL evaluation of barium selenate.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

In a study reported in 1961 in the publicly available literature using radiolabelled (⁷⁵Se) 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 injection in the base of 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. An additional group of ewes was administered sodium selenate subcutaneously. In these animals selenium was more rapidly absorbed and eliminated.

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

In a study in sheep barium selenate was administered orally at doses of zero (n=3), 100 (n=2) or 250 mg (n=2). Animals were slaughtered 27 days later and samples of blood, liver, kidney cortex and medulla, cardiac and skeletal muscle collected. Significant and dose-dependent increases in selenium concentrations in blood and tissues were seen following 100 mg or 250 mg of barium selenate compared to baseline concentrations in untreated sheep. In some tissues selenium concentrations were more than 100 times the levels seen in control animals.

The marked increase in selenium concentration of tissues indicates that selenium from the barium selenate was liberated and absorbed from the intestinal tract. Tissue levels were only measured on day 27. Given that the half-life for selenite in sheep liver is in the range of 2 to 3 days, it is clear that tissue concentrations at earlier time points must have been much higher than the levels seen at day 27 (though the chemical species of selenium present in liver was not known). The study authors conclude that residues of barium selenate in meat ingested by humans may represent a significant source of selenium.

2.2.2. Residue depletion studies

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 are lacking. The selenium contents of skeletal muscle and internal organs show 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) form. 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.

In a GLP compliant residue depletion study barium selenate, as a 50 mg/ml suspension for injection, was administered to cattle and sheep. Nine cattle and 10 sheep were treated with the recommended dose of 1 ml (one subcutaneous injection) per 50 kg bw (corresponding to 3.5 mg BaSeO₄/kg bw, or 1 mg selenium/kg bw). Five cattle and 6 sheep were treated with saline and served as controls. Mean serum 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. Two cattle and two sheep were slaughtered at 6 months and 9 months after treatment. At 12 months after treatment 4 treated cattle and 4 treated sheep were slaughtered, as well as 2 control cattle and 2 control sheep. Tissue samples were taken from liver, back fat, muscle, kidney and injection site. No surrounding injection site tissue was taken and sample size of core injection site was not specified.

Selenium concentrations in fat, muscle, liver and kidney were similar between treated and control animals in sheep and cattle. High residue concentrations were found at injection sites in both species. Selenium concentrations in injections sites were up to 6,900 times higher in treated sheep (up to 344.950 mg/kg) than in control animals and up to 601 times higher in treated cattle (up to 53.229 mg/kg) than in control cattle. Twelve months after treatment selenium concentrations at the injection site remained far above the concentrations measured in control animals. In addition, selenium concentrations in animals slaughtered after the same withdrawal period were extremely different, suggesting that injection site samples were not taken appropriately and representatively in all animals. Similar concentrations of barium were seen in fat, muscle and kidney samples from treated and control cattle. The liver of treated animals showed elevated barium concentrations in the majority of samples with the highest levels being approximately 2.5 to 16 times higher than in controls. Levels of barium at the injection site were very high in treated cattle throughout the investigation with individual values ranging between 70 and 81,000 µg/kg. In sheep barium levels were in the same range as those seen in cattle. The levels of barium detected at the injection site of treated sheep varied between 79 and 674,000 µg/kg versus 42 to 46 µg/kg in control animals.

In a second study three groups of sheep were administered barium selenate subcutaneously behind the left ear at doses of 0.8, 1.4 and 2.9 mg selenium/kg bw. On days 14, 28, 56, and 112 after treatment 2 ewes and 2 wethers (castrated rams) from each treatment group were slaughtered. Amongst other samples, skin and underlying tissue at the site of injection were removed for examination and determination of selenium concentrations. No surrounding injection site samples were taken and the core of the injection site was not specifically identified. Upon visual inspection of the tissue around the injection site, deposits of barium selenate were visible between the skin and the subcutaneous fat on slaughter days 14 and 28. After 56 days a mild tissue reaction was still evident in sheep given 1.4 and 2.9 mg selenium/kg bw as barium selenate, but by day 112 evidence of the injection had disappeared.

In this study more than 50% of the applied dose was present at the injection site of some sheep 112 days after treatment. At all slaughter days highly variable quantities of selenium were recovered. The study authors state that the variable recovery of selenium from the injection site may have occurred because part or all of the barium selenate administered was missed when the injection site was dissected from the neck.

In a further study thirteen pregnant heifers were administered 1 mg selenium/kg bw as barium selenate subcutaneously in the middle of the neck and another seven heifers remained untreated. The heifers were slaughtered at the end of pregnancy which varied between 30 and 119 days after treatment (the untreated up to 121 day). There was a statistically significant persistent increase in selenium in the liver of treated animals with 400 µg selenium/kg detected at 119 days post injection compared to approximately 50 µg/kg in controls. In muscle selenium increased to 97 µg/kg (group mean) as compared to 59 µg/kg in controls. No statistically significant differences were detected in the kidney.

Skin was removed from the neck and the site of injection was dissected and removed. This tissue was weighed and the whole mass wet ashed for the measurement of selenium. Palpation around the injection site showed that the preparation spread over an area of several square inches and that a palpable thickening of the subcutis appeared within a few days and remained palpable in all the injected animals throughout the study. After removal of the skin at slaughter a reaction to the injected material was visible over an area of approximately 12 square inches (approximately 77.4 cm²) and to a depth which varied from 0.5 to 1.0 inch (approximately 1.27–2.54 cm).

Doses of injected selenium were compared to the amount of selenium recovered from the injection site at slaughter. There was no observable decrease in the percent of selenium recovered with increasing time between treatment and slaughter. In cattle slaughtered at more than 100 days after administration more than 90% of the injected selenium remained present at the injection site. No correlation between the apparent rate of absorption of selenium from the injection site and the increase in plasma or liver concentration was seen. The study authors therefore conclude that the amount of residual barium selenate at the injection site is likely to have been underestimated in most cases, probably because of the wide area over which the material had diffused and the difficulty in recovering it all. The authors indicate that, in sheep, approximately 35% of the injected selenium remained at the injection site 90 days after the application of barium selenate, but no details of the sheep study were available.

Overall it is concluded that, following subcutaneous injection of barium selenate, selenium levels at the injection site remain elevated for a prolonged period of time (in one study 90% of injected selenium remained at the injection site 100 days after administration). Furthermore, the available data suggest that, even under controlled study conditions it was not possible to consistently identify and remove the injection site. Only very limited data on residue levels in tissues following oral administration of barium selenate are available. Limited data are available on residue levels seen following oral administration of sodium selenite and are reviewed in the CVMP summary report on potassium and sodium salts of selenium (EMA/MRL/249/97-FINAL). In that evaluation it is concluded that the available data indicate that, based on a worst case scenario of animals slaughtered directly after a long-term continuous intake of feed medicated with recommended doses of sodium selenate, potassium selenate or sodium selenite, the consumer exposure to selenium from animal produce would be below the estimated safe daily intake. As the oral bioavailability of selenium from barium selenate is far lower than the oral bioavailability of selenium from sodium selenate, potassium selenate and sodium selenite, it can be concluded that consumer exposure to selenium from food commodities derived from animals treated with oral barium selenate would also be below the estimated safe daily intake.

Selection of marker residue and ratio of marker to total residues

While it is not possible to distinguish between treatment and non-treatment related selenium in tissues, the only feasible marker residue would be selenium with a marker to total residues ratio definition of 1.

2.2.3. Monitoring or exposure data

No monitoring or exposure data other than that described elsewhere in this report are available.

2.2.4. Analytical method for monitoring of residues

No analytical method was provided in the original MRL application. This was considered acceptable since a 'No MRL status' was recommended. No analytical method is available for review.

2.2.5. Findings of EU or international scientific bodies

No relevant reports relating to residues of barium selenate were identified.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing

As the substance is not expected to possess antimicrobial activity no effect on microorganisms used for industrial food processing is expected.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

Selenium intake needs to be kept within a relatively narrow window as both deficiency and excess can have severe health consequences. Animal products provide approximately 50% of the total dietary selenium intake, with the most important sources being fish, edible organs, meat, dairy products and eggs. The average total dietary selenium intake in European countries is estimated to be between 35 and 100 µg per adult per day. Various international expert bodies have set recommended dietary selenium intake levels ranging from 20 to 70 µg/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 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.

In its 1999 evaluation the CVMP noted that selenium and barium may remain at the injection site after treatment and recommended Member States to consider measures to make the injection site visible in order to avoid its ingestion. However, techniques for allowing consistent identification of injection site residues have not been developed. Attempts have been made to stain the injection site but these are not considered adequate as there are no data to indicate that the stain diffuses and depletes in a similar way to barium selenate residues. Furthermore, the available residue studies reveal extremely variable residue concentrations at the injection site, indicating that even under controlled study conditions consistently identifying injection site residues is problematic. The difficulty would be far greater in slaughter houses, where information on how and where treatment has occurred may be lacking. Consequently the risk that consumers will be exposed to residues of barium selenate as a result of ingesting injection sites cannot be ruled out.

The option of controlling consumer exposure to barium selenate derived selenium by setting numerical MRLs is not appropriate in the case of barium selenate. This is because selenium is a naturally occurring element and consequently residue monitoring authorities would not be able to discriminate between residues of selenium resulting from selenium present naturally in feed and selenium derived from barium

selenate. In addition, selenium residues may also occur as a result of selenium administered in other veterinary medicinal products - potassium selenate, sodium selenate and sodium selenite all have a 'No MRL required' classification and all could, in principle, lead to selenium residues in animal produce. It is also noteworthy that a suitable analytical method validated for detection of selenium residues has not been provided.

It is acknowledged that selenium deficiency may lead to serious health consequences in livestock and that, because of its long acting properties, barium selenate provides a convenient tool for use in the treatment and prevention of selenium deficiency. However, other options do exist, including injections containing sodium or potassium selenite, long acting intraruminal devices containing sodium selenate, and dietary supplementation.

3.3. Elaboration of MRLs

In its 1999 evaluation the CVMP calculated a theoretical maximum daily intake (TMDI) of selenium from edible tissues (excluding the injection site) from cattle administered barium selenate at the recommended dose to be approximately 145 µg/person/day. The TMDI of barium resulting from ingestion of edible tissues from sheep and cattle was calculated as 70 µg and 90 µg barium/person/day, respectively. These values are well within the relevant limit values set for selenium and barium and consequently consumer exposure to residues of selenium and barium from non-injection site tissues is not considered to represent a health concern.

However, as discussed in the previous section, the possibility that consumers will be exposed to barium selenate derived selenium as a result of ingesting injection sites cannot be ruled out.

Most water-soluble selenium compounds (selenites, selenates, organocompounds) are readily absorbed (selenate ions are reported to be more than 90% bioavailable and selenite ions slightly less). However, barium selenate is relatively poorly soluble. Fifty two milligrams of barium selenate can be dissolved in each litre of water, which corresponds to approximately 15 mg of selenium (14.53 mg). In gastrointestinal fluid the solubility of barium selenate will be increased due to the effects of pH and temperature, and because liberated barium ions will react with sulphate ions and precipitate, leading to the liberation of additional barium and selenate ions. As barium selenate is injected at a dose of 1 mg selenium/kg bw and as adult cattle may weigh 500 kg and adult sheep may weigh 80 kg, it can be concluded that ingestion of an injection site could lead to exposure to barium selenate at levels considerably greater than 52 mg, resulting in a minimum of 15 mg of selenium becoming dissolved in each litre of gastrointestinal fluid. This dissolved selenium would be readily absorbed from the gastrointestinal tract. Exposure at this level would far exceed the established tolerable upper intake level of selenium of 300 µg/person per day.

This conclusion is supported by data from an *in vitro* gastrointestinal model, which indicate that a minimum of 5.0 to 10.4% of selenium ingested as barium selenate would be orally bioavailable. Even assuming absorption at the lower level of 5.0%, it can be concluded that ingestion of an injection site from treated sheep or cattle would lead to bioavailable selenium levels far in excess of the tolerable upper intake level.

Information on the absorption of selenium following administration of oral barium selenate is also provided from a study in which sheep were orally administered 250 mg barium selenate, equivalent to 77.4 mg selenium (the study is described in section 2.2.1 on pharmacokinetics in target species). At 27 days after administration (the day of slaughter) selenium concentrations in the liver of treated animals were up to 100 times the levels seen in control animals, indicating good oral absorption. Using the biological half-life for selenium of 3 days in sheep liver it can be estimated that the concentration of selenium in liver one day after administration would have been approximately 8,192 µg/kg. While there are substantial differences

between the gastrointestinal tracts of humans and sheep, the study nevertheless clearly indicates solubility and bioavailability of selenium from barium selenate.

It is noted that ingestion of injection site residues is not expected to occur on a daily basis and is likely to be a relatively rare event (as tissue into which an injection of barium selenate has been given will make up only a small fraction of the edible tissue harvested from a carcass). Consequently it can be argued that selenium exposure resulting from ingestion of injection site residues should be compared with an acute toxicity reference value (such as the acute reference dose). However, data to allow the derivation of such a reference value are not available. While the LD₅₀ does not represent an appropriate value for use in risk assessment it is noted that the margin of exposure (MoE) between the oral LD₅₀ in mice (3 mg/kg) and the bioavailable dose of selenium that would result from ingestion of an injection site is less than 10, which is clearly not adequate.

In relation to barium, the 1999 CVMP evaluation considered that, with the exception of the injection site, the intake of barium from the edible tissues following treatment with barium selenate is within the range of intake expected from the normal diet (500 to 1500 µg/person/day). In the current evaluation the oral bioavailability of barium selenate derived barium has not been further assessed as no specific concern was identified in relation to barium and as the tolerable intake level for barium is 40 times higher than the tolerable intake level for selenium.

Overall it is considered that ingestion of residues of barium selenate from edible tissues other than the injection site does not represent a consumer health hazard. However, there is a real risk that a consumer ingesting an injection site would be exposed to toxic levels of selenium. In view of this, measures are needed to ensure that consumers will not be exposed to injection site residues from barium selenate. The available data demonstrate that the residue depletion profile for barium selenate at the injection site is exceptional in that even six months after injection residues at the injection site remain largely unaltered. Consequently, the establishment of a muscle MRL or even an injection site residue reference value (ISRRV) would effectively prevent the use of injectable barium selenate as the withdrawal period required to ensure consumer safety possibly in excess of a year would not be practicable. In addition, as noted in the previous section, it is not possible to distinguish between naturally occurring selenium and barium selenate derived selenium and consequently the value of numerical MRLs would be of limited use for residue monitoring. In light of this, and in light of the fact that no other risk mitigation measures exist that would ensure that consumers are not exposed to injection site residues, it must be concluded that barium selenate is not an acceptable substance for administration by injection. In view of the risk to human health posed by injection site residues of barium selenate a restriction should be imposed to ensure that the substance is not given by injection.

The existing 'No MRL required' classification for barium selenate applies to all routes of administration. While concerns have been raised in relation to residues resulting from injections of the substance, similar concerns have not been raised in relation to administration by non-injection routes. However, residue data generated following administration of barium selenate by routes other than injection are not available, which is reflective of the fact that barium selenate is generally administered by injection. However, other selenium salts, in particular potassium selenate, sodium selenate and sodium selenite, are administered orally and also have 'No MRL required' classifications. The solubility of potassium selenate, sodium selenate and sodium selenite is far greater than that of barium selenate and, as a consequence, selenium from these salts will be readily absorbed from the gastrointestinal tract, with the result that consumer exposure to selenium from orally administered potassium selenate, sodium selenate and sodium selenite can be expected to be greater than that which would occur following oral administration of barium selenate. As the administration of these other selenium salts by non-injection routes is not considered to represent a consumer safety concern, it follows that administration of barium selenate by non-injection routes will not represent a consumer safety concern. The conclusion that MRLs are not needed for the protection of human

health in relation to non-injection administration of sodium selenate, potassium selenate and sodium selenite can therefore be applied also to barium selenate. There is therefore no basis for recommending a change to the MRL classification of barium selenate except with respect to administration by injection.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EC) No 470/2009, the CVMP considered the possibility of extrapolating its recommendation on maximum residue limits for barium selenate in bovine and ovine species to other food producing species and commodities. Taking into account the current scientific knowledge the recommendations on extrapolation are justified as described below.

Animal species/ food commodities	Extrapolation possible (Yes/ No)	Justification
All food producing species	Yes	The metabolism of barium selenate is not expected to differ substantially across species. Intake of barium selenate derived selenium resulting from administration of the substance by routes other than injection is expected to remain below the tolerable upper intake level. A 'No MRL required' status in all species is consistent with the accepted MRL status for sodium selenate, potassium selenate and sodium selenite.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Whereas:

- the most relevant hazards to human health associated with exposure to residues of barium selenate are due to the selenium content of the molecule,
- selenium is an essential element and a normal constituent of the diet in humans,
- a tolerable upper intake level of selenium has been established at 300 µg/person per day and is appropriate for use in the consumer safety evaluation of barium selenate derived selenium,
- the tolerable upper intake level of selenium will not be exceeded as a result of ingestion of non-injection site tissue from barium selenate treated cattle and sheep.

and having considered that:

- depletion of selenium from the injection site is extremely slow with the result that even one year after injection virtually no depletion of residues has occurred,
- based on the available data the oral bioavailability of selenium ingested as barium selenate is expected to be at least 5%,
- ingestion of an injection site or parts of an injection site will lead to selenium exposure far above the established tolerable upper intake level,
- there is a clear need to ensure that consumers do not ingest barium selenate injection site residues,

- no method for reliably identifying and removing barium selenate injection site residues has been identified,

the CVMP recommends, by consensus, the modification of the maximum residue limit classification for barium selenate in accordance with the following table:

Pharmaco- logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Barium selenate	NOT APPLICABLE	All food producing species	No MRL required	NOT APPLICABLE	Not for administration by injection	Alimentary tract and metabolism/mineral supplements

4. Background information on the procedure

Request for review

2 December 2013

Steps taken for assessment of the substance

Clock started:

2 December 2013

CVMP opinion adopted:

10 April 2014