



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### PHYTOMENADIONE (VITAMIN K<sub>1</sub>) AND MENADIONE (VITAMIN K<sub>3</sub>)

#### SUMMARY REPORT

1. The term vitamin K is used for a range of naphthoquinone compounds which are necessary for the biosynthesis of blood clotting factors. Vitamin K activity is associated with at least two distinct natural substances designated as phytomenadione (vitamin K<sub>1</sub>) and vitamin K<sub>2</sub>. Phytomenadione (CAS No. 84-80-0), is 2-methyl-1, 4-naphthoquinone; vitamin K<sub>2</sub> represents a series of compounds, menaquinones, in which the phytyl side chain of phytomenadione has been replaced by a side chain built up of 2 to 13 phenyl units. The menaquinones are synthesised by Gram-positive bacteria in the gastrointestinal tract. The phytomenadione (the major dietary source), occurs in plants and is the only natural vitamin K available for therapeutic use. Green leafy plants and cured roughage are excellent sources of phytomenadione. Fish meal and liver contain significant amounts. Menadione (CAS No. 58-27-5) (2-methyl-1,4-naphthoquinone) is the synthetic form of vitamin K. In the body menaquinones are partly formed from menadione. Menadione is also a metabolisation product of phytomenadione formed by bacteria in the gastrointestinal tract. Menadione, on a molar basis, at least as active as phytomenadione.

In veterinary medicine, vitamin K preparations are obtained from synthetic and natural sources. Vitamin K compounds are used in the treatment and prevention of haemorrhage associated with vitamin K deficiency and also as antagonists for anticoagulant poisoning (e.g. rodenticide toxicity and sweet clover poisoning).

Phytomenadione as a prophylactic agent, is given intravenously, intramuscularly and orally at doses of 1 mg/kg bw/day in cattle and sheep and at doses of 2 to 4 g/l water or 0.5 to 1 mg/kg in the diet of chickens. In the treatment of haemorrhagic disorders phytomenadione may be given intravenously and orally at doses of 0.5 to 2.5 mg/kg bw per day in cattle, sheep, horses and swine. Menadione may be used intravenously, intramuscularly and orally at doses of 1 to 2 mg/kg bw per day in cattle, sheep, horses, and swine. Likewise menadione is used for treatment of chickens at doses of 8 mg/l water.

In humans, phytomenadione may be given orally and parenterally at doses up to 25 mg per day and a water soluble derivative of menadione at oral doses in a range of 10 to 40 mg per day.

2. Vitamin K is a cofactor in the post-ribosomal synthesis of clotting factors including II (prothrombin), VII (proconvertin), IX (plasma thromboplastin component), and X (Stuart factor) factors as well as of proteins C and S that are involved in the production and inhibition of thrombin. Vitamin K acts on all clotting factor precursors to convert glutamyl residues into  $\gamma$ -carboxyglutamyl residues. During this process of  $\gamma$ -carboxylation, phytomenadione is converted to phytomenadione-2,3 epoxide, an inactive metabolite. There exists evidence that a number of other proteins containing  $\gamma$ -carboxyglutamic acid require vitamin K for their biosynthesis, notably osteocalcin in bone.
3. Vitamin K is synthesised and absorbed in sufficient amounts in the digestive tract of all animal species. It is subsequently absorbed in the small intestine of ruminants and may be acquired by coprophagy or absorption from the lower intestinal tract in other mammals.

Phytomenadione is reasonably well absorbed in the proximal small intestine, but only poorly from the colon. Like all fat-soluble vitamins, its absorption in substantial amounts depends upon normal production of bile acids and pancreatic enzymes. Phytomenadione is absorbed only if bile salts are present and is transported via the thoracic lymph duct. Phytomenadione and menaquinones are absorbed almost entirely by way of the lymph; menadione and its water-soluble derivatives enter the blood stream directly. Phytomenadione is absorbed by an energy-dependent, saturable process in proximal portions of the small intestine. Menaquinones and menadione are absorbed by diffusion in the distal portions of the small intestine and colon.

In humans, the half-life of phytomenadione after intravenous administration is 1.4 to 2.2 hours. The bioavailability of phytomenadione given orally at a dose of 10 mg is 10 to 63% and leads to a maximum plasma concentration of 115 to 407 ng/ml. The phytomenadione half-life in plasma after oral use is 1 to 4 hours and its volume of distribution is 0.07 l/kg.

Phytomenadione is rapidly metabolised to more polar metabolites that are excreted in the bile and urine. The major urinary metabolites result from shortening of the side chain to five or seven carbon atoms, yielding carboxylic acids that are conjugated with glucuronate prior excretion. Menadione is apparently reduced to the diol (hydroquinone) form and excreted as glucuronide and sulphate conjugates.

There is little storage of vitamin K in the body and there appears to be a fairly rapid turnover. The limited stores of the vitamin K present in tissue are slowly broken down.

4. Acute dose toxicity studies were performed with mice and rats. In mice, the subcutaneous and oral LD<sub>50</sub> values were higher than 1 g/kg bw and 25 g/kg bw for phytomenadione and 138 mg/kg bw and 500 mg/kg bw for menadione, respectively. Thus menadione can be considered as moderately toxic and phytomenadione as practically nontoxic. In the rat, the intravenous LD<sub>50</sub> value for menadione was 800 mg/kg bw.

The administration of large doses of menadione and its derivatives to animals resulted in the production of anaemia, polycythemia, splenomegaly, renal and hepatic damage, and death.

5. While no studies on repeated dose toxicity, reproductive toxicity including embryotoxicity/foetotoxicity, mutagenicity and carcinogenicity were available, submission of such information was not considered necessary since vitamin K has a long history of safe use in human and veterinary medicine.
6. No tolerance studies in target species were available. Phytomenadione and menaquinones are nontoxic to animals.

In horses, severe adverse effects were occasionally observed when menadione was administered intravenously (rate of intravenous administration and the composition of preparation not stated). Depression, anorexia, muscle tremors, colics, renal impairment, increased blood urea nitrogen and serum creatinine concentrations with increased kidney volume were presented. Menadione is considered nephrotoxic at dosage in a range of 1 to 2.5 g.

7. No specific studies on immunotoxicity and other effects were provided.
8. In man, rapid intravenous administration of phytomenadione produced flushing, dyspnoea, chest pains, cardiovascular collapse, and rarely death. Whether these reactions were due to the vitamin itself or to the agents used to disperse and emulsify the preparation is not clear.

Menadione and its derivatives have been implicated in producing haemolytic anaemia, hyperbilirubinemia, and kernicterus in the newborn, especially premature infants. Phytomenadione has only rarely caused this. Menadione also can induce haemolysis in individuals who are deficient in glucose-6-phosphate dehydrogenase.

In patients who have severe hepatic disease, the administration of large doses of phytomenadione or menadione may further depress function of the liver.

9. Reports of the Scientific Committee for Food (EU) in 1992 indicate that it is not easy to estimate human requirements because of the difficulty of inducing vitamin K deficiency in normal subjects. In one experiment, young healthy subjects consumed a diet, from which foods rich in vitamin K had been removed, giving approximately 50 µg/day of phytomenadione. Blood clotting appeared normal, but there were signs that prothrombin biosynthesis was not optimal, and there was a decrease in γ-carboxyglutamic acid excretion. The Committee concluded that the requirement for dietary vitamin K is about 1 µg/kg bw/day. On the basis of the daily recommended intake, the safety margin is large. Side effects of vitamin K are rare. Moreover, the minimum dose responsible for an oral adverse effect is unknown.
10. No residue depletion studies in target species were provided. The possibility of residues in meat and milk is considered to be of no concern due to the fact that both natural and synthetic forms of vitamin K are rapidly metabolised and largely excreted as glucuronide or sulphate conjugated. Vitamin K has a short elimination half-life and a low volume of distribution. Furthermore, it should be noted that vitamin K (phytomenadione and menaquinones) is synthesised in the digestive tract. In treated animals tissue concentrations can be expected in the range of concentrations naturally occurring in food derived from plant or fish.
11. No routine analytical method for the determination of vitamin K in tissues of target animals was provided. This information was not considered necessary.

### Conclusions and recommendation

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

- phytomenadione and menadione are endogenously available substances,
- phytomenadione is a normal component in the diet of humans and animals,
- phytomenadione and menadione are used in veterinary medicine only for short-term therapy in individual animals,
- the treated animals are unlikely to be sent for slaughter during or immediately after treatment,
- the pharmacokinetic data indicate that the substances are largely metabolised and rapidly excreted;

the Committee considers that there is no need to establish an MRL for phytomenadione and menadione and recommends their inclusion in Annex II of Council Regulation (EEC) No. 2377/90 in accordance with the following table :

Pharmacologically active substance(s)	Animal species	Other provisions
Phytomenadione	All food producing species	
Menadione	All food producing species	