

EXPERT GROUP ON VITAMINS AND MINERALS

REVISED REVIEW OF VITAMIN K

The attached review is an updated version of paper presented to the Group at the meeting in October 2001. It has been amended to reflect Members' comments.

The following annexes are also attached:

- Annex 1 Figures and tables referred to in the review
- Annex 2 Intakes of Vitamin K from foods in the UK. This annex contains unpublished data and cannot be released at this time.
- Annex 3 Summary table of selected nutrition related information and existing guidance on intakes

Expert Group on Vitamins and Minerals Secretariat
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Vitamin K

Chemistry

1. Vitamin K¹ is not a single substance but a homologous group of fat-soluble vitamins consisting of 2-methyl-1,4-naphthoquinone derivatives (Basu and Dickerson, 1996).

Natural Occurrence

	Name	Synonyms/chemical name	Source
Vitamin K ₁	Phylloquinone	2-methyl-3-phytyl-1,4-naphthoquinone phytomenadione phytoandione phytylmenadione	Plants
Vitamin K ₂	Menaquinone	Menatetranone Menaquinone K4 Vitamin MK-4	Gram +ve bacteria
Vitamin K ₃	Menadione	2-methyl-1,4-naphthoquinone	Synthetic
Vitamin K ₄	Menadiol	Menaquinol 2-methyl-1,4-naphthoquinol 2-methyl-1,4-naphthohydroquinone reduced menadione	Synthetic

2. Phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) is designated as vitamin K₁. It is the only naturally occurring homologue of vitamin K synthesised by plants.
3. A second series of vitamin K homologues synthesised by various Gram-positive bacteria are called menaquinones and are collectively designated as vitamin K₂. The menaquinone family of homologues is a large series of vitamins containing unsaturated side-chains in the 3-position of the 2-methyl-1,4-naphthoquinone nucleus; the side chains differ in the number of isoprenyl units. Most of the menaquinones contain 6-10 isoprenyl units (Basu and Dickerson 1996). Individual compounds are designated menaquinone-*n* (MK-*n*) according to the number of

¹ In this paper, "vitamin K" refers to all naturally occurring substances with "vitamin K-like activity" where the form is unspecified by the original author or where the text applies to phylloquinone and menaquinones. Where possible the form has been specified. Menadione and menadiol are referred to by name throughout.

prenyl units (Shearer, 1995). See Fig 1 for the structures of the various vitamin K homologues.

- In addition to these natural types of vitamin K, several synthetic compounds containing the 2-methyl-1,4-naphthoquinone structure but without the side-chain, exhibit vitamin K activity. These include menadione, termed vitamin K₃ and menadiol, vitamin K₄. Menadione, the most potent vitamin K, is available in a water-soluble form, as a sodium bisulphide derivative, while menadiol, a reduced form of menadione, is available as the sodium diphosphate salt. Menadiol, menadiol sodium diphosphate and menadione sodium bisulphate are all converted *in vivo* to menadione (National Research Council 1989).

Occurrence in foods and medicines

Food

- Phylloquinone (vitamin K₁) is present in plant sources such as cabbage, alfalfa and green leafy vegetables. Smaller amounts of phylloquinone are also present in milk and dairy products, meats, eggs, cereals, fruits and vegetables (National Research Council 1989). Vitamin K is also present in UK dietary supplements as phylloquinone or menaquinone derivatives.

Licensed Products for Oral Use

- Medicinal products with vitamin K activity may only be sold under the supervision of a pharmacist. There are five authorised products, of which, three are single constituent products authorised for the treatment or prevention of haemorrhage associated with a low blood level of prothrombin or Factor VII, malabsorption related to obstructive jaundice or as an antidote to coumarin type anti-coagulants. Two multiconstituent products are used as nutritional adjuncts. The forms of vitamin K used are phylloquinone and menadiol.

Intake and Exposure

Food

- Cabbage, alfalfa and green leafy vegetables provide 50-800 µg of phylloquinone per 100g food. It is present in milk and dairy products, meats, eggs, cereals, fruits and vegetables at lower levels (1 to 50 µg/100 g) (National Research Council 1989).
- Booth and Suttie (1998) reviewed 11 studies and concluded that the mean intake of phylloquinone by young adults was approximately 80 µg/day and the intake in older adults approximately 150 µg/day. The majority of the phylloquinone intake was derived from leafy green vegetables and vegetable oils. The age related difference appears to be due to the higher consumption of vegetables by older age groups.

9. Dietary intake from household food in the UK is provisionally estimated to be 68 µg/person/day (see table 1 in Annex 3).
10. Another important source of vitamin K is the menaquinones synthesised by bacterial flora in the jejunum and ileum. The amount synthesised in the gut contributes significantly towards the daily requirement of the vitamin. Thus, in conventional rats, the vitamin K requirement is about 10 µg/kg bw/day, whereas in germ-free rats the requirement is more than doubled, to about 25 µg/kg bw/day (Lefevre *et al* 1982).
11. Human breast milk is relatively low in phylloquinone (approximately 2 µg/l). Thus, breast fed infants may ingest only about 1 µg/day, which amounts to only about 20% of the presumed vitamin K requirement of 5 µg/day. It is thought that gut micro-organisms contribute the remainder of the requirement (Haroon *et al* 1982, Kries *et al* 1987, National Research Council 1989). Infant formula foods (unsupplemented with vitamin K) based on cows milk or vegetable fat or a mixture of both, contain approximately 4 µg/l phylloquinone (Haroon *et al* 1982). The Infant Formula and Follow-on Regulations (1995) recommend a minimum vitamin K content of 4µg/100kcal.
12. Amounts of up to 45 µg vitamin K are present in UK dietary supplements for general consumption; a supplement designed to be taken by women from pre-conception to breast feeding contains 200 µg (OTC, 2000). In the UK, phylloquinone and menaquinone are the form of vitamin K used in dietary supplements.

Recommended amounts

13. Vitamin K is recognised to be essential but it is difficult to establish the precise intake which is necessary. This is because the vitamin K requirement is met, not only by the dietary intake, but also its microbiological synthesis in the gut making the necessary studies difficult to conduct. COMA (DH, 1991) concluded that the data were insufficient to establish accurate Dietary Reference Values for vitamin K but that intakes of 1 µg/kg bw are safe and probably adequate. However, this value only relates to the coagulation function of vitamin K. The US National Research Council (1989) recommended an RDA of 1 µg/kg bw/d. For adults, a dietary intake of 60-80 µg/day is believed to be adequate to maintain plasma thrombin concentrations within a normal range (80-120 µg/ml) (Blanchard *et al* 1981).
14. Only in newborn infants, prior to the establishment of the intestinal flora, does there appear to be any need for special attention to vitamin K intake. It has been proposed, as a guideline, that the dietary intake of infants, especially during their first year of life, should be 10-25 µg/day (Olson 1987).

Tissue Levels and Assessment of Vitamin K status

15. Until relatively recently, only functional tests such as measurement of blood clotting and prothrombin time were available to assess vitamin K status (Basu and Dickerson, 1996). Prothrombin time is generally in the range 11-13 seconds, with a prothrombin time of greater than 25 seconds being associated with severe bleeding. A radioimmunoassay has since been developed which measures the ratio of prothrombin to partially carboxylated prothrombin in the plasma (partially carboxylated prothrombin is secreted into the plasma during vitamin K deficiency). Plasma phylloquinone can also be measured by high-pressure liquid chromatography but this has limited application as a marker since it fluctuates in response to dietary phylloquinone (Booth *et al*, 1999). Serum carboxylation of osteocalcin is also used as a measure of vitamin K status.
16. Sakamoto *et al* (1999) reported that dietary vitamin K (phylloquinone and menaquinone) intake as assessed by a 7 day food questionnaire was well correlated with vitamin K status as measured by plasma vitamin K absence factor II levels, prothrombin time and hepaplastin test.
17. Independent of intake, plasma phylloquinone levels are higher in older adults (Booth and Suttie, 1998). This may be due to the higher triglyceride levels in this group although the precise mechanism is uncertain. When measured as a ratio, plasma phylloquinone:triglyceride is lower suggesting a worse phylloquinone status. The relevance of the ratio to vitamin K status as assessed by functional measures is uncertain.

Interactions

Vitamins A, D and E

18. Experimental evidence suggests that excessive vitamin A has the potential to antagonise vitamin K. In rats, hypervitaminosis A appears to precipitate hypoprothrombinaemia accompanied by haemorrhages, this can be prevented by the administration of phylloquinone (Light *et al*, 1944, Matschiner *et al*, 1967). It should be noted that this effect is only seen when vitamin A is administered orally; no such effect is seen when vitamin A is administered parenterally. It seems therefore that the effect of excessive vitamin A is mediated through interference with the absorption of vitamin K from the intestine. Vitamins A and D have been reported to enhance the reduction in anticoagulant response induced by warfarin (Schrogie, 1975).
19. Excess vitamin E may also antagonise the action of vitamin K. In chicks, excess vitamin E resulted in increased prothrombin time, which was reversed by menaquinone treatment (March *et al*, 1972). It has not yet been determined if the vitamin E-vitamin K interaction is at the level of absorption or metabolism. In a review of this interaction (Anonymous, 1983) a study was discussed in which nine post-myocardial infarction patients were treated with 300 mg/day α -tocopherol. After 18, 44 and 64 weeks a highly significant increase in clotting time was

apparent. There was no change in factor II, VII or X activity; a decrease in platelet factor activity was thought to be responsible for the increased clotting time. This has not been repeated in other studies with different doses and lengths of treatment. It has been suggested that vitamin E could interfere with the oxidation of vitamin K hydroquinone, depriving the system of the energy needed to drive the carboxylation reaction. In addition, α -tocopherolquinone is a metabolic by-product of vitamin E and a structural analogue of vitamin K hydroquinone, which may competitively inhibit the carboxylation reaction (March *et al*, 1976, Rao and Mason, 1975, Bettger and Olson, 1982).

Coumarins

20. Vitamin K antagonises the anticoagulant effects of the coumarins. A large dose of phylloquinone may abolish the anticoagulant effect for some days (Geill *et al* 1954). In patients taking megadoses of vitamin E (up to 26 times the RDA), the coumarin-induced reduction in vitamin K-dependent clotting factors is enhanced (Corrigan and Marcus, 1974, Schrogie, 1975, Olson 1984). The same effect has also been demonstrated in laboratory animals (Schrogie, 1975).

Other Drugs

21. Experimentally, actinomycin D antagonises the prothrombin formation induced by vitamin K₃ (2-methyl-1,4-naphthoquinone) in chicks (Olson 1964). The same doses of actinomycin D also inhibited the synthesis of RNA in the liver suggesting that vitamin K had a genetic action inducing the RNA formation for the synthesis of clotting proteins.

Other chemicals

22. *In vitro*, phylloquinone enhanced the induction of the enzyme aryl hydrocarbon hydroxylase (CYP1A1) by benzo(a)pyrene (Israels, *et al*, 1997). A similar effect was observed when chick embryos were injected with phylloquinone followed by benzo(a)pyrene (Dogra and Israels, 1987). Dietary phylloquinone deficiency decreased the number and growth rate of tumours induced by benzo(a)pyrene, whilst co-incubation increased the number and growth rate. However, co-incubation of benzo(a)pyrene with phylloquinone, did not increase the number of sister chromatid exchanges observed in human leukocytes (Israels *et al*, 1987). In contrast, menadione decreases CYP1A1 activity (Israels, *et al* 1983, Israels *et al*, 1987) A range of vitamin K forms (phylloquinone, menadione, menadiol and 1,4-naphthoquinone) reduced the mutagenic effect of a range of heterocyclic amines *in vitro* (Edenharder *et al*, 1999).

Bioavailability

Phylloquinone

23. Phylloquinone absorption in human volunteers was higher from a 500 μ g supplement than from a portion of raw spinach containing 495 μ g phylloquinone

(Garber *et al*, 1999). Absorption was measured by area under the curve (AUC) and was 27.55 ± 10.08 nmol/l/h for the supplement compared to 4.79 ± 1.11 nmol/l/hr for the spinach.

24. The bioavailability of phylloquinone in vegetables and in fortified oil was compared in younger (mean age 30.7 and 31.2 in females and males) and older (mean age 70.9 and 70.0 in females and males) adult volunteers (Booth *et al*, 1999). In a 3 x 15 day crossover design, younger and older subjects in a metabolic unit were fed a mixed diet containing 100 µg /day phylloquinone. During 2 of the residency periods, the diet was supplemented with either broccoli (377 µg/day phylloquinone) or phylloquinone fortified oil (417 µg/day phylloquinone). The relative bioavailability of phylloquinone was defined by the difference in the plasma phylloquinone, percentage serum undercarboxylated osteocalcin and urinary γ -carboxyglutamic acid. In both younger and older adults, the additional broccoli or fortified oil increased plasma phylloquinone levels and decreased under-carboxylated osteocalcin levels, with no difference in effect from oil or vegetable being found. Urinary γ -carboxyglutamic acid levels did not change in response to supplementation. It was noted that the results were in contrast to those of Garber *et al*, (1999) (see above) and others, possibly due to differences in the protocol used, such as whether volunteers fasted before the food was consumed. Plasma phylloquinone levels were higher in the older adults throughout the experiment.
25. There was no difference in phylloquinone absorption from fresh or cooked broccoli or between fresh romaine lettuce and lettuce consumed with a meal containing 30 or 45% energy as fat (Garber *et al*, 1999). The same authors also reported that more phylloquinone was absorbed from 150g spinach than from 50g spinach (ie that absorption was not being saturated).

Absorption

Phylloquinone and menaquinones

26. As with other fat-soluble vitamins, absorption of vitamin K is enhanced by the presence of bile salts, pancreatic juice or dietary fat (Basu and Dickerson, 1996).
27. According to animal studies, phylloquinone, the predominant form of dietary vitamin K, is absorbed in the proximal small intestine, by a saturable, energy dependent process (Olson 1984).
28. Menaquinones are synthesised by the bacteria of the distal colon. However, the mechanism of absorption and utilisation in humans is uncertain (reviewed Shearer, 1995) since they are highly lipophilic and tightly attached to the bacterial cell membrane in a region where bile salts are not present. Animal studies have shown that both portal and bile salt mediated transport do not occur in the rat colon. The terminal ileum where bile salts do occur may be a more likely site. Absorption undoubtedly occurs since the spectrum of bacterial menaquinones is reflected in the content of human liver.

29. Absorption of dietary vitamin K in humans may vary from 40-70% in the jejunum and ileum but is poor from the colon. (Olson 1987). Enterohepatic circulation also occurs (see below).
30. Healthy human volunteers ingested 1 mg of radiolabelled phylloquinone with a light meal. Total recovery of radioactivity from faeces accounted for 54-60% of the dose administered, of this 15-23% of the dose was excreted unchanged, urinary excretion accounted for 8-26% (Shearer *et al* 1974). However a substantial amount of the faecal radioactivity was derived from phylloquinone that was absorbed, metabolised and re-excreted into the intestinal lumen. In patients with severe fat malabsorptive states, up to 98% of faecal radioactivity was shown to be unchanged phylloquinone. In contrast after i.v. administration only 2-4% of the faecal radioactivity could be attributed to unchanged phylloquinone.

Menadione

31. Menadione and menadiol are commercially available in their water-soluble forms, which are readily absorbed when administered orally. Menadione is believed to be absorbed by passive diffusion in the distal intestine and colon (Basu and Dickerson, 1996)

Distribution

32. Following oral or i.v. administration, phylloquinone was observed to be cleared rapidly from the circulation. After 2 h and 8 h, only 10 and 1% respectively of a 1 mg dose, remained in the plasma of subjects given radiolabelled phylloquinone (Shearer *et al* 1974).
33. Absorbed vitamin K is transported primarily via the lymph in chylomicrons and is carried by chylomicron remnants to the plasma, where it is associated with lipoproteins. It is initially concentrated in the liver and is then distributed widely among body tissues, including other organs such as the adrenal glands, lungs, bone marrow and kidneys (Shearer *et al* 1974). As much as 50% of a parenterally administered dose of phylloquinone may appear in the liver within 1-2 hours of administration (Olson 1984). Some reports suggest that liver vitamin K consists of only 10% phylloquinone and 90% menaquinones, synthesised by intestinal bacteria (National Research Council 1989). However, it has also been reported that liver vitamin K usually exists as half phylloquinone and half bacterial menaquinones (Basu and Dickerson 1996). The total need for vitamin K cannot be supplied from synthesis of menaquinones by intestinal bacteria, however, since simple restriction of dietary vitamin K results in alterations in clotting factors (Suttie *et al* 1988).
34. Transport of vitamin K in the plasma is thought to be via triglyceride rich lipoproteins (Shearer, 1995). This is supported by evidence that fasting plasma phylloquinone concentrations are influenced by the common genetic polymorphism of lipoprotein E (see paragraph 78).

Metabolism

Phylloquinone and menaquinones

35. In humans, the total body pool of vitamin K, is small and its turnover rapid (Olson 1984). Phylloquinone is metabolised to various oxygenated derivatives, yielding carboxylic acids that are conjugated with glucuronic acid. Turnover of phylloquinone is thought to be much more rapid than the long chain menaquinones (Shearer, 1995).
36. In rat liver microsomes, vitamin K (obtained from phylloquinone and menaquinones) exists in three forms; vitamin K, vitamin K-hydroquinone, vitamin K 2,3-epoxide. The vitamin K quinone can be converted to the vitamin K hydroquinone by an NAD(P)H linked reductase (Anon, 1983). It is this reduced form of vitamin K which is involved in the carboxylation reaction (see paragraph 41).
37. In the rat, phylloquinone is converted to menaquinone-4 (MK4). Studies in germ free rats (Ronden *et al*, 1988) suggest that this reaction does not necessarily involve the intestinal flora and is tissue specific. Supplementation with phylloquinone resulted in increased MK-4 levels in extra-hepatic tissues particularly the pancreas, bone, aorta, fat and kidney. The levels of phylloquinone were also increased in these tissues after supplementation but were also increased in the liver and serum. The authors note that it is not known whether the conversion is an intrinsic property of the tissues or occurs at a central source such as the liver.

Menadione

38. Menadione is believed to be rapidly conjugated with sulphate, phosphate and glucuronide (Olson 1984).

Excretion

39. Under normal physiological conditions, 30-40% of absorbed vitamin K is excreted via the bile into faeces as partially degraded, conjugated, water-soluble metabolites. Approximately 15% is excreted as water-soluble metabolites in the urine (Shearer *et al* 1974). Generally, phylloquinone is degraded more slowly than menadione.

Function

Blood Clotting

40. Vitamin K was first identified in 1935 by Dam, who identified it as the fat-soluble factor necessary for the coagulation of blood. The primary function of vitamin K is to catalyse the synthesis of prothrombin by the liver. In the absence of vitamin K, hypoprothrombinaemia occurs in which blood clotting time may be greatly prolonged. Blood coagulation is a highly complex process, the mechanism of which is not fully understood. It involves cells such as thrombocytes, platelets and

erythrocytes, numerous protein factors and Ca^{2+} . Essentially, a cascade of protein factors catalyses the reaction prothrombin to thrombin, the latter protein then converting soluble fibrinogen into insoluble fibrin which forms the basis of the blood clot. Vitamin K is known to be involved in the hepatic synthesis of at least four of the protein factors, which include prothrombin (factor II), proconvertin (factor VII), thromboplastin (factor IX) and the Stuart-Prower factor (factor X) (Committee on Nutrition 1961, Basu and Dickerson 1996).

41. Vitamin K is thought to be necessary for formation of Ca^{2+} binding sites on prothrombin (Gallop *et al* 1980, Olson 1984). These are essential for prothrombin to be bound to phospholipids, for activation to thrombin. In the presence of dicoumarol, a very potent antagonist of vitamin K, the prothrombin produced *in vivo* has a very low Ca^{2+} binding capacity. The Ca^{2+} binding sites of prothrombin are formed by the introduction of a second carboxyl group into the glutamyl side-chains, located in the amino-terminal region of the protein. Once carboxylated, the glutamates are referred to as γ -carboxyglutamic acid (GLA). When the action of vitamin K is blocked by dicoumarol, calcium ions cannot bind to prothrombin because the protein lacks added carboxyl groups. The formation of vitamin K epoxide is an obligatory step in the action of vitamin K in the biosynthesis of prothrombin
42. Like prothrombin, factors VII, IX and X have been found to have a series of glutamic acid residues and vitamin K is also needed for the carboxylation of these residues (Gallop *et al* 1980, Olson 1984). The vitamin K-dependent carboxylation is carried out by a liver microsomal enzyme, through a molecular mechanism that is not fully understood. It is believed to require reduced vitamin K (or its epoxide) and CO_2 . The process appears to be coupled with the simultaneous epoxidation of vitamin K hydroquinone, the active form of the vitamin. There is an epoxide reductase in liver microsomes which reduces vitamin K epoxide, back to the hydroquinone (Suttie *et al* 1988).

Bone

43. Proteins containing GLA have been identified in bone (Price 1988). There appear to be at least two GLA-containing proteins in bone, called bone GLA protein (BGP) or osteocalcin, and matrix GLA protein (MGP). The functions of these proteins have not been clearly defined, but there is an accumulation of evidence suggesting that they may participate in the modulation of bone mineralisation. Experiments with gene knockout mice (discussed Nelsestuen *et al*, 2000) suggest that osteocalcin is involved in the limitation of bone growth.
44. Osteocalcin is one of the most abundant non-collagenous proteins in the extra-cellular matrix of the bone. Its precise function is uncertain but it appears to be a marker of osteoblast activity (Shearer, 1995). Osteocalcin contains three GLA residues spaced at the same interval as calcium ions in the hydroxyapatite lattice. The appearance of osteocalcin in bones has been shown, using embryonic chick bones, to coincide with the beginning of mineralisation. Injection of vitamin K antagonists into eggs containing developing embryos, has been shown to result in a reduction of the GLA content of osteocalcin by 20-50% (Hauschka *et al* 1978).

Undercarboxylated (partially functional) osteocalcin may be associated with low bone mineral density and risk of hip fracture (Shearer, 1995, DH 1998) in older women. Binkley et al (2000) gave 1000µg of phylloquinone a day or placebo for 14 days to 219 healthy adults (aged 18 to 30 and over 65) with normal coagulation variables and serum phylloquinone concentrations. The supplements led to a ten-fold increase in serum phylloquinone concentrations and a fall in percentage under-γ-carboxylated osteocalcin from approximately 7% to 3% in both age groups. There were, however, no changes in other markers of bone turnover. Schaafsma et al (2000) studied the effect of daily vitamin D₃ and phylloquinone supplements in postmenopausal women with normal and low bone mineral density (BMD): in a double-blind randomised trial, 96 women with normal BMD were assigned placebo, 400IU vitamin D and 80µg phylloquinone, or 80µg phylloquinone; in an open trial 45 women with low BMD were randomly assigned 350IU vitamin D or 350IU vitamin D and 80µg phylloquinone. At baseline, women with normal BMD had significantly higher percentage carboxylated osteocalcin (%carbOC) and across the whole group, %carbOC was positively correlated with BMDs of the lumbar spine and femoral neck. After 6 and 12 months women with normal BMD who had received phylloquinone (alone or with vitamin D) had significantly higher %carbOC compared to the placebo group and to baseline. In women with low BMD %carbOC rose significantly from baseline values in both groups but the phylloquinone-vitamin D group were not significantly different to the vitamin D group.

45. The function of MGP is unclear, but it has been related to the action of the active metabolite of vitamin D (1,25-(OH)₂D₃) and therefore the mobilisation and deposition of bone calcium (Price and Baukol 1980). Experiments with gene knockout mice (discussed Nelsestuen *et al*, 2000) suggest that bone matrix protein is needed to prevent the calcification of soft tissues.
46. Although low levels of circulating vitamin K have been associated with an increased risk of hip fractures in older women, it has been pointed out that there is also an association between fractures and poor nutritional status (New, 1999). Thus low vitamin K may be a marker for poor nutrition rather than having an independent effect.

Kidney

47. GLA containing proteins have been found in the kidney. It has been suggested that kidney GLA protein (KGB) is involved in the reabsorption of Ca²⁺ by the kidney tubules, a function related to vitamin D action. It is thought that KGB may solubilise calcium salts in urine. Sakamoto *et al* (1999) reported that urinary calcium excretion was lower in subjects considered to have high dietary vitamin K intakes. KGB has been identified in calcium oxalate renal stones in man (Lian and Prein 1976).

Other Tissues

48. GLA containing proteins have also been found in the placenta, pancreas, spleen and lungs (Shearer, 1995). The majority of these proteins have not yet been fully

characterised. Growth arrest-specific protein (Gas 6) is vitamin K dependent and may be a ligand for tyrosine kinases. In addition, sequence analysis suggests a possible role for vitamin K in cell signalling. It has been suggested (Israels, *et al*, 1997) that the level of vitamin K in the newborn is tightly regulated because of the involvement of vitamin K dependent proteins in tyrosine kinases signalling and thus in growth regulation in the developing foetus. Tight control of vitamin K levels would be necessary to ensure normal embryonic development.

49. A vitamin K deficient diet has been shown to reduce brain sulfatide concentrations in mice (Sundaram *et al*, 1996). Conversely, in rats fed an excess of vitamin K (as menadione) brain sulfatide concentrations were increased, as was galactocerebroside sulfotransferase activity. The authors propose that vitamin K may have a role in the maintenance of normal complex lipid sulfatide activity in rats and mice.

Deficiency

50. Dietary deficiency of vitamin K is not common, since the vitamin is fairly well distributed in foods and intestinal micro-organisms synthesise a significant amount of vitamin K in the intestine. Dietary vitamin K is essentially present in the oxidised hydroquinone form, but an efficient salvage pathway (see fig 2) ensures optimal conversion of the epoxide back to the hydroquinone form, following carboxylation (Olson 1984). The existence of this system also explains the relatively rare occurrence of vitamin K deficiency, despite extremely low body stores of the vitamin in man.
51. Isolated cases of deficiency are seen, but the deficiency is generally secondary, for example, to inadequate absorption or impaired gut synthesis, or as a result of drugs that interfere with vitamin K availability.

Malabsorptive states

52. The absorption of vitamin K from the small intestine requires the presence of bile salts. Hence, any disorder that retards the delivery of bile to the small intestine, such as obstructive jaundice or bile fistula, reduces the absorption of vitamin K from the intestine. Vitamin K deficiency has also been found to occur in other malabsorptive states, such as coeliac disease, Crohn's disease, bowel resection, chronic pancreatic injury and ulcerative colitis (Suttie *et al* 1988). The malabsorptive states leading to hypoprothrombinaemia can be successfully treated with daily oral administration of 10 mg phylloquinone or menadione.

Hepatic insufficiency

53. There may be decreased utilisation of vitamin in the production of the vitamin K-dependent clotting factors, during any form of acute or chronic liver disease. This is as a result of the destruction of the rough endoplasmic reticulum in the hepatocyte. Patients with hypoprothrombinaemia related to hepatic disorders usually respond to daily parenteral doses of 10 mg of vitamin K for three days. If no response to this treatment is noted this suggests serious hepatocellular damage (Basu and Dickerson, 1996).

Newborn infants

54. Both term and pre-term babies have inadequate levels of vitamin K, resulting from a number of factors. Vitamin K does not cross the placental barrier effectively from the maternal circulation (Shearer *et al* 1982) so that levels in the foetus are considerably lower than those in the mother. Some elevation does occur in response to maternal vitamin K administration. In addition, at birth infants do not have menaquinone-producing bacteria in their intestine. However it takes only a few days for the gut flora to become established and to begin supplying menaquinones to the infant (Sann *et al* 1985, Olson 1987). Some infants may also be subject to borderline vitamin K intake as breast milk is generally a poor source of the vitamin.
55. Vitamin K deficiency associated with hypoprothrombinaemia (haemorrhagic disease of the newborn- HDN) usually appears during the first week of life, manifested by ecchymoses, nasal or gastrointestinal bleeding, or excessive bleeding at the umbilical stump. The state of hypoprothrombinaemia can be extremely severe in the presence of factors such as obstructive jaundice, diarrhoea, treatment with antibiotics and prolonged breast-feeding (Shearer *et al* 1982, Lane and Hathaway 1985). The severe signs generally include intracranial haemorrhage, widespread deep ecchymoses, excessive bleeding at puncture sites or surgical incisions and sometimes dysfunction of the central nervous system with vascular collapse. Later onset HDN may also occur 2-12 weeks after birth (Shearer, 1995).

Hospitalised patients

56. Patients in hospital can be at risk of vitamin K deficiency as a result of factors such as antibiotic use, total parenteral nutrition and gastrointestinal surgery (Basu and Dickerson, 1996).

Intakes associated with non-nutritional beneficial effects

57. Vitamin K, especially water-soluble forms, induces radiosensitisation. It can also potentiate the analgesic effects of opiates and salicylates (Jurgens 1958). These interactions have been used in treating cancer patients, the former in amplifying the therapeutic effectiveness of x-rays and the latter, in the relief of pain.

Human Toxicity

58. Relatively few adverse reactions to vitamin K have been reported. This may be due to the fact that it is not available over the counter, unless contained in multivitamin preparations and as it is generally only used for specific indications. In addition, vitamin K is rarely administered for long periods of time therefore chronic toxicity is seldom a factor. Side effects in adults are rare. Cramp-like pains, convulsive movements, tachycardia, cardiac irregularity, chest pain, cyanosis and dulled consciousness have been described after large intravenous doses of vitamin K (form not specified); however this may be related to the

solvent rather than the vitamin (Deutsch 1966). No reports exist of such effects from controlled studies.

59. It has been suggested that vitamin K given i.v. can, in rare cases, cause hypertension (Deutsch 1966). However a review of the literature by the authors does not support this. A single case report does exist, suggesting that i.v. phytanadione (an aqueous colloidal suspension of phylloquinone) may be associated with cardiovascular collapse (Barash *et al* 1976), but this is based on the clinical observation of one patient undergoing numerous therapies for multiple disorders, including carcinoma of the vocal cord, severe nutritional cirrhosis and alcoholic hepatitis.

Menadione and water soluble-forms

60. The basis of vitamin K toxicity has been well established as residing mainly in the water-soluble analogues of the vitamin. In contrast to phylloquinone, the water-soluble synthetic derivatives act as oxidants in the body, causing red blood cell instability and haemolysis (Broberger *et al* 1960, Finkel 1961). The mechanism of interference of 2-methyl-1,4-naphthoquinone with the redox systems of erythrocytes, is presumed to be similar to that of the oxidant metabolites of primaquine, which cause haemolysis in glucose-6-phosphate dehydrogenase deficient individuals. This interference results in the formation of methaemoglobin, reduction of the osmotic resistance of the erythrocytes, haemolysis and haemoglobinuria (Harley and Robin 1962). To prevent the formation of methaemoglobin, NADPH-dependent methaemoglobin reductase is activated in the normal organism; this is possible only if sufficient amounts of glucose and glucose-6-phosphate dehydrogenase are available. Thus, this process occurs more rapidly in individuals with glucose-6-phosphate dehydrogenase deficiency (Deutsch 1966).
61. The above effect is more pronounced in newborn or premature infants, due to their low glucose levels (Allison 1955, Allison 1963). The vitamin was given to treat intercranial and pulmonary haemorrhage even though these complications were due to hypoxia and other perinatal disorders rather than to haemorrhagic diathesis (Zipursky, 1999). Doses of up to 80 mg/kg bw were given compared to the effective prophylactic dose of 1 mg/kg. The subsequent hyperbilirubinemia and overloading of the immature liver in the newborn, resulted in kernicterus and toxicity to the neonatal brain (Allison 1963, Hayes and Hagsted 1973). Laurance (1955) reported an increased incidence of kernicterus in premature infants who had been given menadiol sodium diphosphate (30 mg/day for three days). However, more recently few cases have been reported, as a safe dose level has been established (Zenc and Huxtable 1979).

Adverse skin reactions

62. Where vitamin K has been used to treat conditions such as hypoprothrombinaemia adverse reactions are rare (Bruynzeel *et al*, 1995). Between 1964 and 1994, there were 52 adverse cutaneous effects reported in the literature. The vast majority of these were for phylloquinone. Four different reactions have been reported erythematous plaques surrounding the injection site of parenteral vitamin K;

pseudoscleroderma secondary to vitamin K injections; contact dermatitis on epicutaneously exposed skin, and; localised urticarial lesions. The mechanism of action in many patients is thought to be delayed-type hypersensitivity; no dose response pattern was apparent. In the majority of the reported cases, there was associated liver disease (Barnes and Sarkany 1976, Heydenreich 1977); however, Bruynzeel and colleagues report two cases where liver disease was not apparent.

63. In some cases it has been possible to reproduce the lesions with a test dose. Bullen *et al* (1978) described six patients with chronic liver disease in whom cutaneous reactions developed at the site of injection of oil-soluble phylloquinone. Injections of the water-soluble analogue menadione yielded negative skin results and the patients tolerated treatment without adverse reactions.
64. A patient being treated for alcoholic hepatitis developed a cutaneous reaction at the site of injection of phylloquinone. The patient was tested with the drug and its components by intradermal and epicutaneous application. Sensitivity to the pure phylloquinone was noted but not to the other components of the preparation. Intradermal injections of phylloquinone gave no reaction in four healthy control subjects, as did the components of the preparation (Robison and Odom 1978).
65. In the cases reviewed by Bruynzeel *et al* (1995) it is stated that oral vitamin K is not associated with any hypersensitivity reactions, though they cite a case where the patient's symptoms were exacerbated by vitamin K containing foods such as egg yolk and green vegetables.

Childhood Cancer

66. Studies published by Golding and colleagues (1990, 1992) suggested that treatment of newborns with intra-muscular but not oral vitamin K (form unspecified) increased the risk of developing childhood cancer (reviewed Zipursky, 1996, Zipursky *et al*, 1999). Some reservations were raised about the methodology used in the studies which detected the association *ad hoc*. Subsequent studies analysing existing data sets (Ekelund *et al*, 1993, Klebanoff *et al*, 1993, Olsen *et al*, 1993) did not find the same association. Similarly, no association was found either in a number of case-control studies (Ansell *et al*, 1996, Von Kries *et al*, 1996, Mckinney *et al*, 1998, Passmore *et al*, 1998a) or in an ecologic study (Passmore *et al*, 1998b). A study by Parker *et al*, (1998) suggested that intra-muscular vitamin K could be associated with the development of acute lymphoblastic anaemia in children aged 1-6; a finding also suggested by Passmore *et al*, 1998a). However, it has been suggested (Zipursky *et al*, 1999) that the latter results could be because the investigations considered selected "high risk" infants (due to prematurity or perinatal problems) who had been given the vitamin treatment and that it was that their "high risk" characteristics rather than the treatment *per se* that made them more likely to develop cancer than children who had not been treated. The author (Zipursky *et al*, 1999) considers that this view is supported by the results of studies in areas where it was policy to treat all children with vitamin K. In these (stated to be Ekelund *et al*, 1993, Klebanoff *et al*, 1993, Olsen *et al*, 1993, Passmore *et al*, 1998b and Parker *et al*, (1998)) there was no

difference in the incidence of childhood cancer between babies receiving intramuscular vitamin K, oral vitamin K or no treatment.

Genotoxicity

67. The number of SCEs was measured in the peripheral blood lymphocytes of six newborn babies 24 hrs after intra-muscular injection with phylloquinone and in six control neonates (Cornelissen *et al*, 1991). The mean number of SCEs per metaphase was 8.88 ± 1.22 in the phylloquinone group compared to 9.05 ± 1.14 in the controls. The mean number of chromosome aberrations per 100 mitoses was 3.00 ± 2.61 in the phylloquinone group compared to 2.50 ± 1.87 in the controls. Plasma phylloquinone concentration ranged from 0.0255 to 2.55 μM . The authors concluded that there was no evidence that phylloquinone treatment caused genotoxicity.
68. Sister chromatid exchange (SCE) was measured in human leukocytes taken from adult and placental blood (Israels *et al*, 1987). In the presence of 1 μM phylloquinone the mean number of SCEs per metaphase increased significantly (as determined by Dunnett's many to one test) from 3.32 ± 0.219 in placental blood to 5.76 ± 0.219 and from 5.13 ± 0.273 to 7.81 ± 0.326 in adult blood. Co-incubation with phylloquinone did not affect the number of SCEs caused by benzo(a)pyrene or mitomycin C. The phylloquinone concentration used in these experiments is higher than would be found *in vivo*. Phylloquinone levels in fasting adult plasma are stated to be 0.1 to 0.66 ng/ml (0.2 to 14.6 pmol/ml –or nmol/l) but are noted to be undetectable in cord plasma. Similarly, Booth *et al* (1999) quoted fasting plasma phylloquinone levels of 1-1.43 nmol/l which increased to 2-3 nmol/l after consumption of 400-500 μg phylloquinone.

Human Supplementation studies –Table 1*Phylloquinone*

69. A group of 50 post-menopausal women (aged 55-75) were given 1 mg solubilised phylloquinone daily for 14 days to investigate whether urinary calcium affects vitamin K excretion in (Knapen,1989). The control group was 50 pre-menopausal women. The treatment resulted in a significant decrease in fasting plasma calcium particularly in a subset of the population characterised as post-menopausal and fast losers of calcium. In the same group, the phylloquinone treatment increased the concentration of serum osteocalcin and increased the ability of osteocalcin to bind to hydroxyapatite. It has been noted elsewhere that decarboxylated osteocalcin can bind to hydroxyapatite and therefore the method needs careful evaluation (Shearer, 1995). No adverse effects were reported by Knapen and colleagues.
70. In a trial using a 3 x 15 day crossover design, younger and older subjects (9 males and females each) in a metabolic unit were fed a mixed diet containing 100 µg /day phylloquinone (Booth *et al*, 1999)-see paragraph 24. During 2 of the residency periods, the diet was supplemented with either broccoli (377 µg/day total phylloquinone) or phylloquinone fortified oil (417 µg/day total phylloquinone). No adverse effects were noted.
71. Eight elite female athletes were given 10 mg/day phylloquinone for one month with markers of bone health being assessed before and after treatment (Craciun *et al*, 1998). Four of the eight athletes had been amenorrhoeic for more than 1 year whilst the others had been taking oral contraceptives. The athletes' vitamin K intake was in excess of the 1µg/kg bw/day RDA value. At baseline, the low oestrogen group was biochemically vitamin K deficient as assessed by the calcium binding activity of circulating osteocalcin. In all subjects, supplementation was associated with an increase in the calcium binding capacity of osteocalcin. In the low oestrogen group supplementation was associated with a 15-20 % increase in bone formation markers and a 20-25% decrease in bone resorption markers. No adverse effects were noted.
72. A group of 72 women were divided into 3 groups; pre-menopausal, early post-menopausal and elderly (Plantalech *et al*, 1990) and treated with 1 mg/day phylloquinone (the precise dose regime is unclear). An additional group of 25 elderly women also received a dose of 1mg/day phylloquinone for 30 days or a placebo. A group of patients on chronic warfarin therapy were used as a control to account for undercarboxylation of osteocalcin. Total osteocalcin was increased in menopausal women, however, in early post-menopausal women this was due to increased levels of fully carboxylated osteocalcin, in elderly women, the increase was due to an increase in non-carboxylated osteocalcin. No changes were observed in the placebo group. Although a vitamin K deficiency was not demonstrated, phylloquinone treatment increased the levels of fully carboxylated osteocalcin. No adverse effects were reported.

73. In a single-blind two-week crossover study, 20 post-menopausal, osteoporotic women were treated with 1 mg/day phylloquinone or phylloquinone and 400IU vitamin D (Douglas *et al*, 1995). A three month wash out period allowed them to act as their own controls. Both treatments corrected the undercarboxylation of osteocalcin. The authors state that there were no significant adverse effects, but note that two patients had slightly looser stools. It was thought that the castor oil used as an excipient for vitamin K was responsible for this phenomenon.

Menatetrenone (vitamin K₂)

74. The ability of vitamin K (as menatetrenone) to prevent prednisilone-induced loss of bone mineral density was investigated in patients with chronic glomerulonephritis (Yonemura *et al*, 2000). Twenty patients were treated with 0.8 mg/kg bw (48 mg/day for a 60 kg adult) prednisolone for 4 weeks tapering to 20 mg/day over 6 weeks. Ten of the patients were given 15 mg menatetrenone vitamin K, three times a day during the prednisilone treatment period. The menatetrenone treatment prevented the prednisilone-induced loss of bone mineral density and the reduction of procollagen type I C-peptide (a marker of bone resorption) but did not prevent a decrease in serum intact osteocalcin or urinary excretion of deoxypyridinoline (a biochemical marker of bone resorption). No adverse effects were noted.
75. A dose of 45 mg/day menatetrenone was given to seventeen patients with low parathyroid hormone levels for 1 year (Akiba *et al*, 1991). The treatment significantly increased serum BGP and prevented loss of bone mass. No adverse effects were reported. However the results are presented as an abstract and few details are provided.

Adverse Drug Reactions

76. Suspected adverse reactions to medicinal products are reported to the Committee on the Safety of Medicines/Medicines Control Agency. Many factors influence the number of reports received and there is considerable “under-reporting” of reactions. For oral vitamin K the number of reactions reported is very small with no trends suggesting an association with treatment.

Vulnerable groups

77. As noted in paragraph 61. Infants are susceptible to the induction of oxidative damage by menadione and the formation of methaemaglobinaemia. Subjects with glucose-6-phosphate dehydrogenase deficiency may also be vulnerable to this effect.

Genetic variations

78. Fasting plasma phylloquinone concentrations are strongly influenced by the common genetic polymorphism of apolipoprotein E. Concentrations are highest in subjects with the apoE2 variant with intermediate and low concentrations in subjects with the apoE3 and apoE4 variants respectively (discussed Shearer, 1995). The potential toxicological significance of this is uncertain.

Animal Toxicity

Acute and sub-chronic toxicity

Phylloquinone

79. Molitor and Robinson (1940) conducted toxicity studies in mice, chicks and rats. Oral doses of up to 25 g phylloquinone/kg bw produced no fatalities. Intra-peritoneal doses of phylloquinone as high as 25 g/kg bw again failed to cause death. In sub-chronic studies in rats, they noted that daily feeding of doses as high as 2 g/kg bw phylloquinone over a 30 day period produced no ill effects.
80. The findings of Molitor and Robinson were confirmed by Ansbacher *et al* (1942), who additionally reported that subcutaneous administration of phylloquinone to mice at doses up to 6 g/kg produced no toxicity.
81. No fatalities were reported by Dam *et al* (1954) after intravenous injection of 100 mg/kg bw of phylloquinone in chicks and rats. There is no mention of signs of toxicity but this study was designed primarily to determine concentrations of phylloquinone in various tissues after large doses.

Menadione and water-soluble forms of vitamin K

82. As noted above, Molitor and Robinson (1940) conducted toxicity studies in mice, chicks and rats. The oral LD₅₀ in mice was approximately 0.2 g/kg bw for phthiocol and 0.5 g/kg bw for menadione. Intraperitoneal studies showed that doses of 0.2 g/kg bw of phthiocol or menadione caused 100% mortality in mice (and close to 100% in chicks). In sub-chronic studies in rats, they noted that daily feeding over a 30 day period 0.35 g/kg bw of phthiocol and 0.5 g/kg bw of menadione was fatal and that smaller doses of these two drugs (0.1 and 0.35 g/kg, respectively) resulted in pronounced anaemia. The i.v. LD₅₀ of menadione in mice and rabbits was approximately 250 and 120 mg/kg bw respectively (Richards and Shapiro, 1945).
83. The findings of Molitor and Robinson were confirmed by Ansbacher *et al* (1942), who additionally reported that the subcutaneous LD₅₀ of menadione

was 138 mg/kg. Dogs given three intravenous injections of 5 mg menadione/kg bw developed slight anaemia but showed no pathological changes at autopsy. Larger doses orally (25 or 50 mg/kg/day for 4 to 33 days) also produced anaemia. Parenteral doses of the same magnitude resulted in addition in haemoglobinuria, urobilinuria and urobilinogenuria.

84. Methaemoglobin and cyanosis have been reported in dogs given i.v. doses (25, 50 100 and 150 mg/kg bw) of menadione. The top dose was lethal to all 3 treated dogs, whereas 1/3 of the dog treated with 100 mg/kg bw died. Hepatic damage was revealed upon post mortem examination (Richards and Shapiro 1945). Sub-chronic toxicity studies on dogs given iv doses of 15, 25 or 40 mg/kg bw/d of menadione or menadiol for 15 days showed an increased urinary urobilinogen level, severe anaemia and hepatic and renal damage.
85. The toxicity of the sodium diphosphate salt of menadiol was studied (Wynn 1963) in both adult and newborn rats given as 3 daily i.v doses. Excessive doses caused jaundice by action both as an oxidising hemolysin, and as a competitor of bilirubin, for conjugation with glucuronide. In the adult rat larger doses per unit weight (0.16 mg/g bw) are necessary to cause hyperbilirubinemia, which appears in the adult only at dose levels that cause haemolysis. The newborn develops hyperbilirubinemia at dose levels (0.04 mg/g bw) too low to cause haemolysis. Yellow staining of the brain was noted at lower blood levels of bilirubin in the newborn, but was unaccompanied by microscopic indications of cellular damage, thus the diagnosis of kernicterus could not be made (Wynn 1963).

Carcinogenicity

86. No data identified.

Genotoxicity

In vivo

Phylloquinone

87. Five sheep foetuses were given a 1 mg dose of phylloquinone into the femoral vein via a catheter (Israels *et al*, 1987). The mean number of sister chromatid exchanges (SCEs) per metaphase increased significantly from 3.94 ± 0.15 at 15 minutes pre-injection to 5.38 ± 0.23 at 24 hours post-injection. Before treatment the level of phylloquinone was not detectable, reaching up to $0.3 \mu\text{M}$ after treatment. The increase in SCE was low but was stated to be statistically significant as determined by paired *t* tests using Bonferroni *t* statistics. No positive controls were used.

*In vitro**Phylloquinone*

88. A range of 16 naphthoquinones were tested for mutagenicity in the Ames bacterial *Salmonella* strains TA98, TA100 or TA2637 (Tikkanen, 1983). All three strains are stated to contain plasmid pKM101. Phylloquinone (concentration not stated) was not mutagenic with or without metabolic activation.
89. Israels (1987) investigated the dose response curves for SCE induction *in vitro* in both fetal and adult sheep leukocytes incubated with phylloquinone. The doses tested ranged from 0.1 nM to 1 µM. Using the Kruskal-Wallis test, it was determined that at 0.1nM the number of SCEs in the adult cells (from the dam) were not significantly different from the solvent control. However, the number of SCEs in the fetal cells was significantly increased compared to the controls. The increase in SCEs in the adult cells became significant at 10 nM. The actual number of SCEs at different dose levels are not given but the figure indicates that SCEs in adults increased from approximately 6 to 9 per metaphase and in the foetus from 4.3 to 7.5 per metaphase. The protocol is unusual as all individual metaphases were counted (rather than at least 25) but the full results are not provided and the reasons for the method used are not explained.

Menadione

90. In the study by Tikkanen *et al* (1983) sixteen naphthoquinones (including menadione) were tested in *Salmonella* strains TA98, TA100 and TA2637 (which all contain plasmid pKM101). Six of the sixteen naphthoquinones were mutagenic in strain TA2637; menadione and 3 others were also slightly mutagenic in TA 98 in the presence of metabolic activation. The mutagenic activity of the compounds was attributed to the presence of one or two methyl and/or hydroxyl substituents.

*Modification of mutagenic response**Phylloquinone*

91. Phylloquinone may be able to modify the effects of the carcinogenic compound benzpyrene. In chick embryos injected with 0.1 µmol phylloquinone 24 hours prior to injection with 0.8 µmol benzo(a)pyrene, phylloquinone augmented the aryl hydrocarbon hydroxylase response to benzo(a)pyrene and reduced glutathione-S-transferase activity compared to controls (Dogra and Israels, 1987). P450 content was also increased. Phylloquinone increased the production of benzo(a)pyrene metabolites (measured by HPLC) and the derived proximate carcinogen by rat liver microsomes *in vitro* (Israels, 1985). Conversely, phylloquinone deficiency reduced the number of benzo(a)pyrene-DNA adducts in the livers of mice injected with benzo(a)pyrene. Tumour development is slower and lifespan

longer in benzo(a)pyrene treated mice fed a diet deficient in phyloquinone for 2 weeks before and after benzo(a)pyrene treatment compared with benzo(a)pyrene treated mice given a phyloquinone replete diet; similar effects are observed when warfarin is given (Israels *et al*, 1983). Conversely, concomitant supplementation with i.p. phyloquinone accelerates the onset of tumour appearance and the increased the number of tumour deaths caused by intra-peritoneal benzo(a)pyrene. This may result from the proposed role that vitamin K has in tyrosine kinase mediated cell signalling and thus in mitogenesis and cell regulation (discussed Israels *et al*, 1997).

92. Phyloquinone caused a concentration-related reduction in the mutagenicity of six heterocyclic amines in strains TA98 and TA100 in the Ames *Salmonella* assay (Edenharder *et al*, 1999). The mutagenicity of these compounds involves CYP1A1 and 2 metabolism. However the mechanism of the phyloquinone anti-mutagenic effects was not investigated so it is uncertain whether it would have demonstrated enzyme inhibition in contrast to the activation reported by Israels *et al* (1983, 1987).

Menadione

93. In contrast to phyloquinone, menadione decreased CYP1A1 activity and reduced the rate of tumour appearance and tumour deaths in mice treated with benz(a)pyrene (Israels *et al*, 1983, 1987). The authors speculate that the site of phyloquinone and menadione action in the metabolism of benzo(a) pyrene must differ or must involve different mechanisms.
94. Menadione, menadiol and 1,4 naphthoquinone caused a concentration-related reduction in the mutagenicity of six heterocyclic amines in strains TA 98 and TA100 in the Ames *salmonella* assay (Edenharder *et al*, 1999). The mechanism for the anti-mutagenic effect was investigated and it was reported that menadione reduced the activities of 7-ethoxyresorufin-*O*-deethylase (EROD) and 7-methoxyresorufin-*O*-demethylase (MROD), markers for cytochrome 1A1 and 1A2 activity. In further enzyme kinetic experiments, menadione and menadiol behaved as competitive inhibitors of 2-amino-3-methyl-imidazo[4,5-*f*] quinoline (IQ)- induced mutagenesis.

Reproductive Toxicity.

95. No data identified.

Mechanism of Toxicity.

96. Menadione causes oxidative damage possibly as a result of its unsaturated sidechain (DH, 1991). Some reports indicate that phyloquinone increases cytochrome P4501A1 activity, enhancing the effect of benz(a)pyrene, whereas others report a reduction in the mutagenicity of heterocyclic amines

(Edenharder *et al*, 1999). Phylloquinone may be involved in cell signalling and cell regulation (Israels, 1997).

Regulatory considerations.

97. The Infant Formula and Follow-on Formula Regulations (1995) recommend a minimum vitamin K content of 4 µg/100kcal.

Recommendations on maximum intakes

98. COMA (DH, 1991) considered that a vitamin K dose of 1 µg/kg bw/day was probably safe. However, they further noted that synthetic preparations of menadione were best avoided for nutritional purposes since, besides lacking intrinsic biological activity, the high reactivity of its unsubstituted 3-position has been linked to haemolysis and liver damage in the newborn.

Recommendations on maximum supplementation levels.

99. The Consumers for Health Choice (CHC, 1998) state that an upper safe level of 30mg/day vitamin K (form unspecified) is safe.

Summary

100. Vitamin K is a group of homologous fat-soluble compounds derived from 2-methyl-1,4-naphthoquinone. Phylloquinone, vitamin K₁, is synthesised by plants. Vitamin K₂, menaquinones, are synthesised by various Gram-positive bacteria. Several synthetic compounds containing the 2-methyl-1,4-naphthoquinone structure also exist, these include menadione (vitamin K₃) and menadiol (vitamin K₄).
101. Dietary vitamin K is largely obtained from green leafy vegetables and vegetable oils, with lesser amounts present in dairy products meat and eggs. It is also present in multi-vitamin food supplements and licensed medicines. Precise dietary requirements for vitamin K are uncertain.
102. A number of interactions have been reported. Vitamin K interacts with vitamins A and E, drugs such as coumarins and actinomycin D, and carcinogenic chemicals such as benzpyrene.
103. Vitamin K is readily absorbed but there are conflicting reports on whether this is higher from supplements or from a food matrix. Absorption of phylloquinone takes place in the proximal small intestine and absorption of bacterial menaquinones in the terminal ileum. Vitamin K is transported via the

lymph in chylomicrons, initially concentrated in the liver prior to distribution. Metabolism of vitamin K involves the formation of an epoxide and a quinone, which can then be reduced. Vitamin K is largely excreted in the faeces via bile.

104. Vitamin K is involved in blood clotting, bone and kidney metabolism. Roles in cell signalling and brain lipid metabolism have also been proposed. Because vitamin K is widespread in the diet and provided by bacteria, deficiency is generally secondary to conditions such as malabsorption. However, newborn babies have low levels of vitamin K, which may result in haemorrhagic disease of the newborn.
105. There are relatively few reports of human toxicity. High doses of water-soluble forms of vitamin K (menadione and menadiol) may result in oxidative damage, red cell fragility and the formation of methaemoglobin. Premature infants given high doses of menadione and menadiol resulting in hyperbilirubinaemia, resulting in kernicterus and toxicity to the neonatal brain. Local hypersensitivity reactions to injections of all forms of vitamin K have been reported.
106. In animal studies, administration of menadione and menadiol has resulted in anaemia, haemoglobinaemia, urobilinuria and urobilinogenuria. High doses have also been reported to cause liver damage. Phylloquinone, however, is well tolerated at high doses. No data on reproductive toxicity have been identified. Phylloquinone is negative in the Ames bacterial mutagenicity test but there are conflicting reports of its ability to induce sister chromatid exchange in human and animal leukocytes. Phylloquinone may enhance the carcinogenic activity of benzpyrene possibly by enhancing cytochrome P450 1A1 activity or affecting tyrosine kinase cell signalling. In contrast, menadione and menadiol are positive in the Ames test and reduce the activity of CYP1A1 enzymes. Carcinogenicity data for either natural or synthetic forms of vitamin K are not available.

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ANNEX 1 TO EVM/01/09

Tables and figures referred to in the review

Figure 1. Structural formulas of (A) phylloquinone, K₁; (B) menaquinone K₂ and (C) menadione, K₃. (*from* Basu and Dickerson, 1996)

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Figure 2. Vitamin K reaction cycle (adapted *from* Nelsestuen *et al*, 2000)

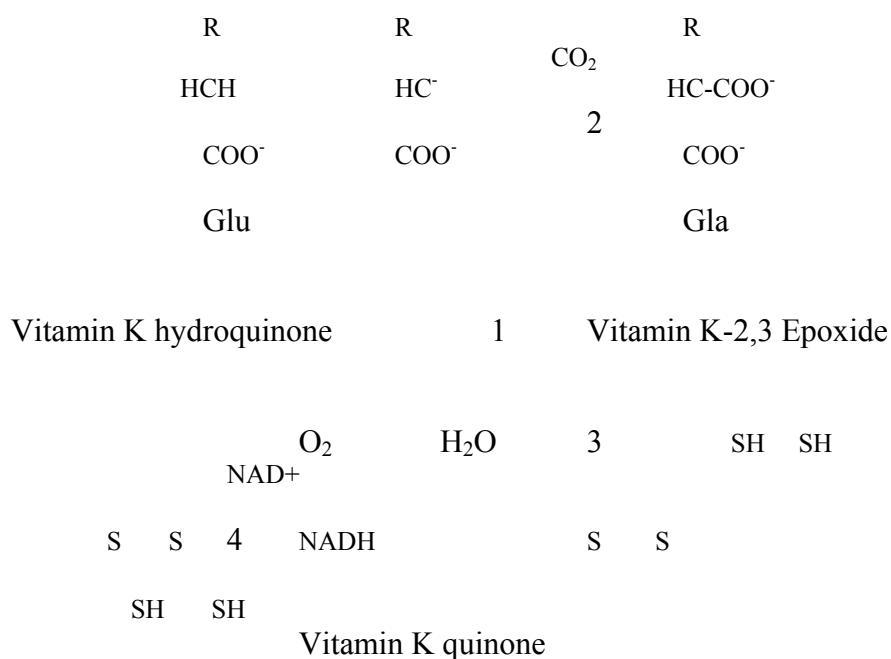


Figure 3. Vitamin K salvage pathway (XH₂:NAD(P)H⁺ or RSH-HSR (dithiol) (*from* Basu and Dickerson, 1996)

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Table 1. Human supplementation studies

Exposure	Subjects	Dose	Duration	Endpoint & Effects	NOAEL	Comment	Reference
Vitamin K (form not specified)	Post-menopausal women (55-75 y) vs pre-menopausal controls	1 mg/day	2 weeks	Decreased plasma calcium, increased serum osteocalcin and calcium binding ability of osteocalcin particularly in fast losers of calcium sub group	1mg =LOAEL		[Knapen <i>et al</i> , 1989]
Phylloquinone as fortified oil and as broccoli	Groups of 18 younger (mean age 30.7 and 31.2 females and males) and Older (mean age 70.9 and 30.0 females and males) adults	Three diets: 100 µg/day control; 77 µg/day + broccoli; 417µg/day + fortified oil	15 days for each treatment	The study investigated the bioavailability of phylloquinone. Increases in plasma phylloquinone and decreased undercarboxylated osteocalcin occurred. Urinary γ-carboxyglutamic acid unchanged. No adverse effects noted.			Booth <i>et al</i> (1999)
Vitamin K as menatetrenone	20 adults with chronic glomerular nephritis	15 mg/day	10 weeks	The study investigated whether menatetrenone reduced the loss of bone mineral density induced by prednisolone. No adverse effects noted			Yonemura <i>et al</i> (2000)
Phylloquinone	8 Elite female athletes; 4 low-oestrogen.	10 mg/day	1 month	Increased bone formation and decreased bone loss markers in low oestrogen group. Increased calcium binding of osteocalcin in all subjects.			Craciun <i>et al</i> (2000)

Table 2. Oral Toxicity of Vitamin K in animals

Species	Endpoint	Dose	Duration	NOAEL/LOAEL	Comment	Reference
Acute Toxicity						
White Mice	LD50 test symptoms not reported.	Vitamin K as pthiocol, menadione (2-methyl-1,4 naphthoquinone) or phylloquinone	Single dose	LD50 = 0.2 g/kg pthiocol and 0.5g/kg menadione. No effects with phylloquinone at doses up to 25g/kg.		Molitor and Robinson, 1940
White Mice	LD50 test	0.1 and 0.35 g/kg bw/day Pthiocol, 0.25, 0.35 and 0.5 g/kg bw/day menadione and 0.35 and 2g/kg bw/day phylloquinone	Single dose	LD50 = 0.62 g/kg menadione, approximately 0.3g/kg menadiol. Vitamin K esters less toxic. No effects with phylloquinone at doses up to 25g/kg.	Results given in own scale (Ansbacher units) of potency rather than as conventional LD50	Ansbacher <i>et al</i> (1942)
Sub-Chronic toxicity						
White Mice	No effects on growth. 0.35 g/kg Pthiocol and 0.5 g/kg menadione lethal. Reduced red cell and haemoglobin in animals treated with 0.1 g pthiocol or	0.1 and 0.35 g/kg bw/day Pthiocol, 0.25, 0.35 and 0.5 g/kg bw/day menadione and 0.35 and 2g/kg bw/day phylloquinone	30 days	NOAEL= 0.25 g/kg menadione or 2g/kg phylloquinone. LOAEL = 0.1g/kg pthiocol	Very limited study. No gross or microscopic pathology reported.	Molitor and Robinson, 1940

	and 0.5 g/kg menadione					
Cats	2-50 mg/kg Menadione and menadiol diproponate	Decreased red cell count			Limited study. Gross pathology conducted but not reported.	Ansbacher <i>et al</i> (1942)
Rabbits			28-36 days		Limited study. Gross pathology conducted but not reported.	Ansbacher <i>et al</i> (1942)
Monkeys	1 or 2 mg/kg Menadione and menadiol diproponate	Anaemia and other toxic signs not observed.	50-57 days		Limited study. Gross pathology conducted but not reported.	Ansbacher <i>et al</i> (1942)
Dogs	Anaemia	25-50 mg/kg bw/day	4-33 days		Limited study. Gross pathology conducted but not reported.	Ansbacher <i>et al</i> (1942)

ANNEX 2 TO EVM/01/09

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Provisional intakes of Vitamin K₁ (phylloquinone) from food

ANNEX 3 TO EVM/01/09

Vitamin K Summary table of selected nutrient related information and existing guidance on intakes

Unit of usage	$\mu\text{g}/\text{kg}/\text{d}$	$\mu\text{g} /100 \text{ kcal}$
<i>UK DRV² for adults (19-50+)</i> <i>Safe intake</i>	Adults 1.0 Infants 10.0 1 $\mu\text{g}/\text{kg}/\text{d}$ is considered safe and adequate for adults since it maintains vitamin K dependent clotting factors at normal concentrations and in their fully carboxylated form.	
Regulations Infant formula ³		4.0
<i>Guidance on high intakes</i> COMA 1991 ¹	Natural K vitamins seem remarkably free from toxic side effects when taken orally even in milligram quantities. On the other hand, synthetic preparations of menadione are best avoided for nutritional purposes.	

² Committee on Medical Aspects of Food and Nutrition Policy (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects 41. London: HMSO.

³ The Infant Formula and Follow-on Formula Regulations 1995

Summary table of selected nutrient related information and existing guidance on intakes

Unit of usage	$\mu\text{g}/\text{kg}/\text{d}$	$\mu\text{g}/100\text{ kcal}$
<i>UK DRV⁴ for adults (19-50+)</i> <i>Safe intake</i>	Adults 1.0 Infants 10.0 1 $\mu\text{g}/\text{kg}/\text{d}$ is considered safe and adequate for adults since it maintains vitamin K dependent clotting factors at normal concentrations and in their fully carboxylated form.	
Regulations Infant formula ⁵		4.0
<i>Maximum total safe daily intake</i> COMA 1991 ¹	Natural K vitamins seem remarkably free from toxic side effects when taken orally even in milligram quantities. On the other hand, synthetic preparations of menadione are best avoided for nutritional purposes.	

⁴ Committee on Medical Aspects of Food and Nutrition Policy (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects 41. London: HMSO.

⁵ The Infant Formula and Follow-on Formula Regulations 1995

