

Risk Assessment

Vitamin K

General Information

Chemistry

Vitamin K is not a single compound but a group of homologous fat-soluble compounds derived from 2-methyl-1,4-naphthoquinone. Phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) is designated as vitamin K₁. The menaquinones, collectively known as vitamin K₂, are a large series of compounds containing an unsaturated side chain with differing numbers of isoprenyl units at the 3 position in the methyl-1,4-naphthoquinone nucleus. Depending on the number of isoprenyl units the individual compounds are designated as menaquinone-*n*-(MK-*n*). Several synthetic water-soluble compounds containing the 2-methyl-1,4-naphthoquinone structure also exist. These include menadione (vitamin K₃) and menadiol (vitamin K₄) (see table).

Table: Summary of the nomenclature of the various forms of vitamin K

	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

Natural occurrence

Vitamin K₁ is synthesised by plants. The vitamin K₂ series is synthesised by various Gram-positive bacteria present in the jejunum and ileum.

Occurrence in food, food supplements and medicines

Dietary vitamin K is largely obtained from green leafy vegetables and vegetable oils, with lesser amounts present in dairy products, meat and eggs. Vitamin K is also present as K₁ and K₂ in multi-vitamin food supplements and as K₁ and K₄ in licensed medicines. Vitamins K₁ and K₄ are used in the treatment or prophylaxis of haemorrhage as a result of low blood level of prothrombin or Factor VII, malabsorption or use of coumarin anticoagulants.

UK food supplements may contain up to 0.045 mg vitamin K (either as K₁ or K₂) for general consumption and 0.20 mg in supplements intended for women from pre-conception to nursing.

Other sources of exposure

Vitamin K (as the K₂ series) is also provided by the bacterial flora in the intestine, and this contributes significantly towards the daily requirement of the vitamin.

Recommended amounts

Although vitamin K is known to be essential, recommendations on adequate nutritional intakes have not been precisely established, because of the unquantified contribution made by the intestinal bacteria. COMA considered that an intake of 0.001 mg/kg bw/day was probably adequate with regard to the coagulation function of vitamin K and noted that synthetic preparations of K₃ were best avoided for nutritional purposes, due to their link with haemolysis and liver damage in the newborn (COMA, 1991).

The Infant Formula and Follow-on Formula regulations (1995) recommend a minimum vitamin K intake of 0.004 mg/100 kcal.

Analysis of tissue levels and vitamin K status

Functional tests of blood clotting were initially used to assess vitamin K status. However, a radioimmunoassay which measures the ratio of prothrombin to partially carboxylated prothrombin in plasma (the latter is formed during vitamin K deficiency) is now available. Measurement of the carboxylation of serum osteocalcin, and plasma vitamin K₁ are also used to determine vitamin K status. However, the measurement of plasma vitamin K₁ is of limited value because it reflects recent dietary intake.

Brief overview of non-nutritional effects

Vitamin K (in particular, the water-soluble form) has been reported to potentiate the analgesic effect of opiates and salicylates, and induce radiosensitisation.

Function

Vitamin K catalyses the carboxylation of a number of protein factors involved in blood clotting including prothrombin, forming the calcium binding sites on glutamyl side chains in the protein. Once carboxylated, the glutamates are referred to as gamma-carboxyglutamic acid (GLA).

GLA-containing proteins are also found in the bone. For example, bone GLA protein (or osteocalcin) is thought to be involved in the limitation of bone growth, and matrix GLA protein may be involved in mobilisation and deposition of bone calcium.

Kidney GLA proteins may be involved in reabsorption of calcium by the kidney tubules and solubilisation of calcium salts in urine.

GLA-containing proteins have also been found in the placenta, pancreas, spleen and lungs, but the majority of these have not yet been characterised.

Vitamin K-dependent proteins are also thought to have roles in cell signalling and brain lipid metabolism.

Deficiency

Vitamin K is widely available from the diet and is also provided by gut bacteria. Thus, deficiency is generally secondary to conditions such as malabsorption or impaired gut synthesis. Hospitalised patients can be at risk of vitamin K deficiency following the use of antibiotics or gastrointestinal surgery. Newborn babies have low levels of vitamin K, which may result in haemorrhagic disease of the newborn.

Interactions

It has been shown experimentally that excessive vitamin E exposure results in increased prothrombin times which are normalised by vitamin K₂. The effects of vitamin K₁ are also antagonised by excessive oral intakes of vitamin A.

Vitamin K₁ interacts with coumarin anticoagulants. The effect of 2-methyl-1,4-naphthoquinone (vitamin K₃) is antagonised by actinomycin D. The carcinogenic activity of benzo(a)pyrene has been shown to be increased by vitamin K₁, as a result of induction of cytochrome P450 1A1 (CYP1A1). In contrast, vitamin K₃ decreased CYP1A1 activity.

Absorption and bioavailability

Vitamin K is readily absorbed, but there are conflicting reports on whether this is higher from supplements or from a food matrix. The bioavailability of vitamin K₁ is the same from raw and cooked broccoli and is unaffected by the fat content of the meal.

Absorption of vitamin K₁ takes place in the proximal small intestine by a saturable, energy dependent process. Absorption of bacterial menaquinones (K₂) occurs in the terminal ileum by passive diffusion. Absorption of vitamin K is enhanced by bile salts and pancreatic juice. Overall, 40-70% of vitamin K may be absorbed in the jejunum, but absorption is lower elsewhere in the digestive system.

Vitamin K₃ is believed to be absorbed by passive diffusion in the distal intestine and colon.

Distribution and metabolism

Vitamin K is transported via the lymph in chylomicrons and concentrated in the liver prior to wide distribution. The body pool of vitamin K is low. The metabolism of vitamins K₁ and K₂ involves the formation of an epoxide and a quinone, which can then be reduced by NAD(P)H reductases. The reduced form is involved in carboxylation reactions. The carboxylic acid derivatives of vitamin K₁ are conjugated with glucuronic acid. In rats, vitamin K₁ can be converted to K₂ in the tissues.

Vitamin K₃ is thought to be metabolised by conjugation with sulphate, phosphate and glucuronide.

Excretion

Approximately 30-40% of vitamin K is excreted, via the bile, in the faeces as partially degraded, conjugated water-soluble metabolites. Smaller quantities (approximately 15%) are excreted in the urine as water-soluble metabolites.

Toxicity

Human data

There are relatively few reports of human toxicity following ingestion.

High doses of water-soluble vitamin K₃ (menadione) may result in oxidative damage, red cell fragility and the formation of methaemoglobin. High doses of vitamin K₃ given to premature infants to treat intracranial and pulmonary haemorrhage have been reported to have caused hyperbilirubinaemia and overloading of the immature liver, resulting in kernicterus and brain toxicity. Fewer cases have been reported recently as a safer level has been established.

Local hypersensitivity reactions to dermal injections of vitamin K (mostly of the K₁ form) have been reported. They are thought to be delayed-type hypersensitivity reactions.

Animal data

Acute doses of vitamin K₁ (phylloquinone) up to 25,000 mg/kg bw caused no fatalities in rats, mice or chicks.

In contrast, oral vitamin K₃ (menadione) administration to rats, chicks and mice resulted in anaemia and death. Parenteral administration of high doses of K₃ to dogs caused methaemoglobinaemia, urobilinuria and urobilinogenuria, and fatal liver damage.

No data on reproductive toxicity have been identified.

Carcinogenicity and genotoxicity

No data on carcinogenicity have been identified.

Vitamin K₁ was negative in the Ames bacterial mutagenicity test, but there are conflicting reports of its ability to cause sister chromatid exchange in human and animal leukocytes.

Mechanisms of toxicity

Menadione causes oxidative damage and lipid peroxidation.

Dose response characterisation

No data have been identified.

Vulnerable groups

Individuals with glucose-6-phosphate dehydrogenase deficiency are more susceptible to the development of methaemoglobinemia, and this would include oxidative damage caused by menadione (vitamin K₃). Infants are vulnerable to vitamin K₃ toxicity. An initial suggestion that intramuscular administration of vitamin K was associated with childhood cancer has not been supported in further studies.

Genetic variations

No data have been identified.

Studies of particular importance in the risk assessment

(For full review see <http://www.food.gov.uk/science/ouradvisors/vitandmin/evmpapers> or the enclosed CD).

Human data

Israels et al., 1987

Sister chromatid exchange (SCE) was measured in human leukocytes taken from adult and placental blood. In the presence of 1 μ M vitamin K₁, the mean number of SCEs per metaphase increased significantly 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 vitamin K₁ did not affect the number of SCEs caused by benzpyrene or mitomycin C.

Cornelissen et al., 1991

The numbers of SCEs were measured in the peripheral blood lymphocytes of six newborn babies 24 hrs after intra-muscular injection with 1 mg vitamin K₁ (as the drug Konakion) and in six control neonates. The mean number of SCEs per metaphase was 8.88 ± 1.22 in the vitamin K₁ 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 vitamin K group compared to 2.50 ± 1.87 in the controls. Plasma vitamin K₁ concentration ranged from 0.0255 to 2.55 μ M. The authors concluded that there was no evidence that vitamin K₁ treatment caused genotoxicity. However, it is possible that taking the sample 24 hours after treatment may have been too early to detect SCEs.

Craciun et al., 1998

Eight elite female athletes were given 10 mg/day vitamin K₁ for one month and markers of bone health were assessed before and after treatment. Four of the eight athletes had been amenorrhoeic for more than 1 year whilst the others had been taking oral contraceptives. The athletes' dietary vitamin K intake was stated to be in excess of the 0.001 mg/kg bw/day RDA value. At baseline, the amenorrhoeic 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, indicating correction of a deficiency. In the amenorrhoeic 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 at 10 mg/day.

Booth et al., 1999

In a trial in a metabolic unit using a 3 x 15 day crossover design, both younger and older male and female subjects were fed a mixed diet containing 0.10 mg vitamin K₁ per day. During two of the 15-day residency periods, the diet was supplemented with either broccoli (0.377 mg total K₁ per day) or K₁ fortified oil (0.417 mg total K₁ per day). No adverse effects were noted at 0.377-0.417 mg total K₁ per day.

Animal data

Molitor and Robinson, 1940

Toxicity studies were conducted in mice, chicks and rats. Vitamin K₁ in single oral doses up to 25,000 mg/kg bw, produced no fatalities in mice, whereas vitamin K₃ was highly toxic. Intraperitoneal studies showed that doses of 200 mg/kg bw of vitamin K₃ caused 100% mortality in mice but intraperitoneal doses of vitamin K₁ as high as 25,000 mg/kg bw failed to cause death. In sub-chronic studies in rats, daily feeding over a 30 day period of 500 mg/kg bw of vitamin K₃ was fatal and smaller doses resulted in pronounced anaemia. Daily doses as high as 2000 mg/kg bw vitamin K₁ produced no ill effects.

Israels et al., 1987

Five foetal sheep were given a 1 mg dose of vitamin K₁ into the femoral vein via a catheter. The mean number of SCEs per metaphase increased 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 vitamin K₁ was not detectable, but increased to 0.3 μ M after treatment. The increase in SCE was low but was stated to be statistically significant. No positive controls were used and it is difficult to assess the biological importance of the result.

The *in vitro* dose response curve for SCE induction was investigated in both foetal and adult sheep leukocytes incubated with vitamin K₁. The doses tested ranged from 0.1 nM to 1 μ M. At 0.1 nM 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 foetal cells was significantly increased compared to the controls. The increase in SCEs in the adult cells became significant at 10 nM. The actual numbers of SCEs at different dose levels were 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.

Edenharder et al., 1999

Vitamins K₁, K₃, K₄ and 1,4 naphthoquinone caused a concentration-related reduction in the mutagenicity of six heterocyclic amines in the Ames *Salmonella* assay. The mechanism for the anti-mutagenic effect was related to reduced activities of 7-ethoxyresorufin-O-deethylase (EROD) and 7-methoxyresorufin-O-demethylase (MROD), markers for cytochrome P450 1A1 and 1A2 activity. In further enzyme kinetic experiments, vitamins K₃ and K₄ behaved as competitive inhibitors of 2-amino-3-methyl-imidazo[4,5-f]quinoline induced mutagenesis. The mechanism of the anti-mutagenic effect of vitamin K₁ was not investigated.

Exposure assessment

Total Exposure/intake:

Food Mean: 0.068 mg/day (based on National Food Survey 2000, DEFRA, 2001).
Data for 97.5th percentile not available.

Supplements up to 0.20 mg/day (Annex 4; OTC, 2001)

Estimated maximum intake: $0.068 + 0.20 = 0.27$ mg/day

No potential high intake groups have been identified.

Risk assessment

The data are sparse but the different forms of vitamin K are associated with different adverse effects. There are relatively few reports of human toxicity for vitamin K₁ (phylloquinone) and it is stated to be well tolerated in animal studies. Although there are some reports of genotoxicity *in vitro* and *in vivo*, no effect was found in cells taken from babies given prophylactic intramuscular vitamin K₁. The significance of the positive genotoxicity findings is uncertain.

High doses of vitamin K₃ (menadione) may result in oxidative damage, red cell fragility and the formation of methaemoglobin. Hyperbilirubinaemia, resulting in kernicterus and toxicity to the neonatal brain occurred in premature infants given high doses of vitamin K₃. Local hypersensitivity reactions to injections have been reported. In animal studies, vitamin K₃ administration has resulted in anaemia, haemoglobinaemia, urobilinuria and urobilinogenuria. High doses have also been reported to cause liver damage.

Vitamin K₃ has demonstrated some mutagenic activity in the Ames test, possibly as a result of the structure of the side chain.

Few data are available relating to vitamins K₂ and K₄.

ESTABLISHMENT OF GUIDANCE LEVEL

There are insufficient data from studies in humans or animals to establish a Safe Upper Level for vitamin K. There are clear differences in the toxicity of different forms of vitamin K.

Vitamin K₁ (phylloquinone) the form occurring naturally in food is not associated with adverse effects at high doses in animal studies. Acute doses up to 25,000 mg/kg bw did not cause fatalities in rats, mice or chicks. Human supplementation studies in small numbers of subjects suggest that, although having biological activity, doses of up to 10 mg/day for 1 month are not associated with adverse effects. Applying an uncertainty factor of 10 for inter-individual variation because of the very limited human database, indicates that for guidance purposes, a daily supplementary intake of 1 mg/day would be

unlikely to result in adverse effects. This is equivalent to 0.017 mg/kg bw in a 60 kg adult. There are insufficient data available to provide guidance on total intakes.

The water soluble forms of vitamin K, particularly K₃ (menadione) are more active and can be toxic at high doses in both humans and animals. Use of menadione in food supplements is therefore undesirable.

References

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