VITAMIN K SUBSTANCES

Vitamin K comprises a group of substances, which are widespread in nature and are an essential co-factor in humans in the synthesis of several proteins that play a role in haemostasis and others that may be important in calcium homeostasis. The K vitamins all contain the 2-methyl-1,4-naphthoquinone (menadione) moiety, and the various naturally occurring forms differ in the alkyl substituent at the 3-position. Phylloquinone (vitamin K_1) is 2-methyl-3-phytyl-1,4-naphthoquinone and is widely found in higher plants, including green leafy vegetables, and in green and blue algae. The menaquinones (formerly vitamin K_2) have polyisoprenyl substituents at the 3-position and are produced by bacteria. The compound menadione (formerly vitamin K_3) lacks an alkyl group at the 3-position but can be alkylated *in vivo* in some species. Several synthetic water-soluble derivatives, such as the sodium diphosphate ester of menadiol and the addition product of menadione with sodium bisulfite, also have commercial applications (National Research Council, 1989; Gennaro, 1995; Weber & Rüttimann, 1996).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature, structural and molecular formulae and relative molecular masses*

Vitamin K (generic)

Chem. Abstr. Serv. Reg. No.: 12001-79-5
Chem. Abstr. Name: Vitamin K

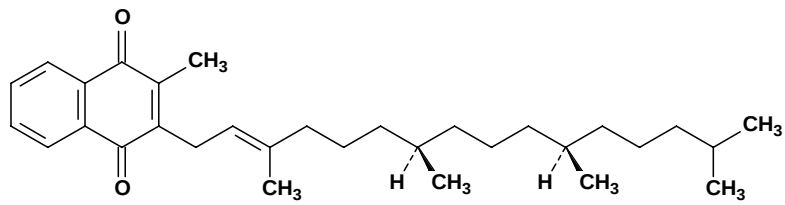
Vitamin K_1 (generic)

Chem. Abstr. Serv. Reg. No.: 11104-38-4
Chem. Abstr. Name: Vitamin K_1

Phylloquinone

Chem. Abstr. Serv. Reg. No.: 84-80-0
Deleted CAS Reg. Nos.: 10485-69-5; 15973-57-6; 50926-17-5

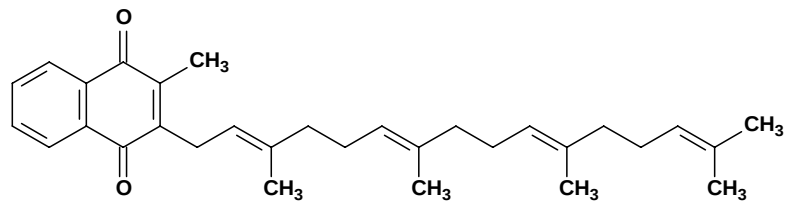
Chem. Abstr. Name: 2-Methyl-3-[(2*E*,7*R*,11*R*)-3,7,11,15-tetramethyl-2-hexadecenyl]-1,4-naphthalenedione
IUPAC Systematic Name: [*R*-[*R**,*R**-(*E*)]]-2-Methyl-3-(3,7,11,15-tetramethyl-2-hexadecenyl)-1,4-naphthalenedione
Synonyms: Antihaemorrhagic vitamin; 2-methyl-3-phytyl-1,4-naphthoquinone; 2-methyl-3-(3,7,11,15-tetramethyl-2-hexadecenyl)-1,4-naphthalenedione; α-phylloquinone; *trans*-phylloquinone; phylloquinone K_1; phytomenadione; phytonadione; phytylmenadione; 3-phytylmenadione; phytylmenaquinone; vitamin K_1; vitamin $K_{1(20)}$; 2′,3′-*trans*-vitamin K_1 [Note: The IUPAC recommends use of the name 'phylloquinone' and the abbreviation 'K' (rather than 'K_1'). Both phylloquinone and vitamin K_1 are in common use. The *United States Pharmacopeia* uses the name 'phytonadione'; *The European Pharmacopoeia* uses the name 'phytomenadione', which is a synonym occasionally found in the pharmaceutical and pharmacological literature.]



$C_{31}H_{46}O_2$ Relative molecular mass: 450.71

Menaquinone-4

Chem. Abstr. Serv. Reg. No.: 863-61-6
Deleted CAS Reg. Nos.: 15261-37-7; 20977-31-5; 39776-41-5
Chem. Abstr. Name: 2-Methyl-3-[(2*E*,6*E*,10*E*)-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl]-1,4-naphthalenedione
IUPAC Systematic Name: 2-Methyl-3-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl)-1,4-naphthoquinone
Synonyms: Menaquinone-K4; menatetrenone; (*E*,*E*,*E*)-2-methyl-3-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl]-1,4-naphthalenedione; MK4; vitamin $K_{2(20)}$; vitamin MK4



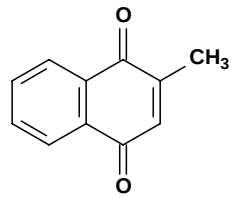
$C_{31}H_{40}O_2$ Relative molecular mass: 444.66

Vitamin K_2 (generic)

Chem. Abstr. Serv. Reg. No.: 11032-49-8
Chem. Abstr. Name: Vitamin K_2

Menadione

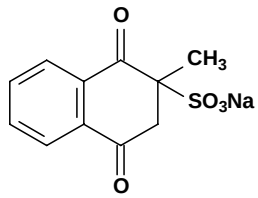
Chem. Abstr. Serv. Reg. No.: 58-27-5
Chem. Abstr. Name: 1,4-Naphthalenedione, 2-methyl-
IUPAC Systematic Name: 1,4-Naphthoquinone, 2-methyl-
Synonyms: 1,4-Dihydro-1,4-dioxo-2-methylnaphthalene; 2-methyl-1,4-naphthalenedione; 2-methylnaphthoquinone; β-methyl-1,4-naphthoquinone; 2-methyl-1,4-naphthoquinone; 3-methyl-1,4-naphthoquinone; MK-0; vitamin K_0; vitamin $K_{2(0)}$; vitamin K_3 [Note: 'Menadione' is the common name preferred by IUPAC for the chemical, previously called vitamin K_3]



$C_{11}H_8O_2$ Relative molecular mass: 172.18

Menadione sodium bisulfite

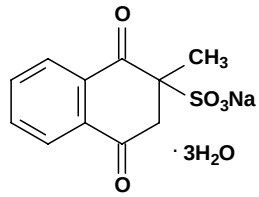
Chem. Abstr. Serv. Reg. No.: 130-37-0
Alternate CAS Reg. No.: 57414-02-5
Deleted CAS Reg. Nos.: 8012-53-1; 8017-97-8; 8028-24-8; 8053-08-5
Chem. Abstr. Name: 1,2,3,4-Tetrahydro-2-methyl-1,4-dioxo-2-naphthalenesulfonic acid, sodium salt
IUPAC Systematic Name: 1,2,3,4-Tetrahydro-2-methyl-1,4-dioxo-2-naphthalenesulfonic acid, sodium salt
Synonyms: 3,3-Dihydro-2-methyl-1,4-naphthoquinone-2-sulfonate sodium; menadione sodium hydrogen sulfite; menaphthone sodium bisulfite; menaphthone sodium bisulphite; 2-methyl-1,4-naphthalenedione, sodium bisulfite deriv.; 2-methyl-1,4-naphthoquinone sodium bisulfite; 2-methylnaphthoquinone sodium hydrogen sulfite; 2-methyl-1,4-naphthoquinone sodium hydrogen sulfite; MSBC; sodium menadione bisulfite; vitamin K injection; vitamin K_3 sodium bisulfite



$C_{11}H_9NaO_5S$ Relative molecular mass: 276.24

Menadione sodium bisulfite trihydrate

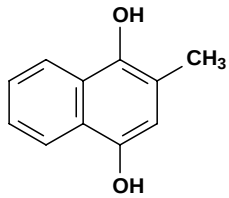
Chem. Abstr. Serv. Reg. No.: 6147-37-1
Chem. Abstr. Name: 1,2,3,4-Tetrahydro-2-methyl-1,4-dioxo-2-naphthalenesulfonic acid, sodium salt, trihydrate



$C_{11}H_9NaO_5S.3H_2O$ Relative molecular mass: 330.28

Menadiol

Chem. Abstr. Serv. Reg. No.: 481-85-6
Chem. Abstr. Name: 2-Methyl-1,4-naphthalenediol
IUPAC Systematic Name: 2-Methyl-1,4-naphthalenediol
Synonyms: Dihydrovitamin K_3; menaquinol; 2-methyl-1,4-dihydroxynaphthalene; 2-methylhydronaphthoquinone; 2-methylnaphthalene-1,4-diol; 2-methyl-1,4-naphthohydroquinone; 2-methyl-1,4-naphthoquinol; reduced menadione; reduced vitamin K_3; vitamin K_3H_2

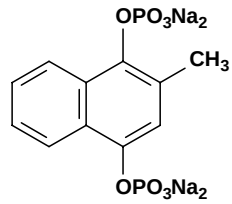


$C_{11}H_{10}O_2$ Relative molecular mass: 174.19

Menadiol sodium phosphate

Chem. Abstr. Serv. Reg. No.: 131-13-5
Chem. Abstr. Name: 2-Methyl-1,4-naphthalenediol, bis(dihydrogen phosphate), tetra-sodium salt

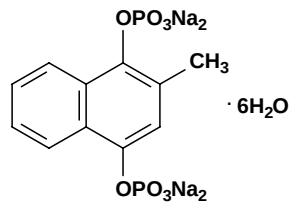
IUPAC Systematic Name: 2-Methyl-1,4-naphthalenediol, diphosphate, tetrasodium salt
Synonyms: Menadiol diphosphate tetrasodium salt; menadiol sodium diphosphate; menadiol tetrasodium diphosphate; menadione diphosphate tetrasodium salt; 2-methyl-1,4-naphthoquinol bis(disodium phosphate); tetrasodium 2-methyl-1,4-naphthalenediol bis(dihydrogen phosphate)



$C_{11}H_8Na_4O_8P_2$ Relative molecular mass: 422.09

Menadiol sodium phosphate hexahydrate

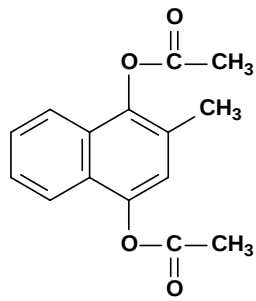
Chem. Abstr. Serv. Reg. No.: 6700-42-1
Chem. Abstr. Name: 2-Methyl-1,4-naphthalenediol, bis(dihydrogen phosphate), tetrasodium salt, hexahydrate
IUPAC Systematic Name: 2-Methyl-1,4-naphthalenediol, diphosphate, tetrasodium salt, hexahydrate
Synonyms: Menadiol sodium diphosphate hexahydrate



$C_{11}H_8Na_4O_8P_2.6H_2O$ Relative molecular mass: 530.18

Acetomenaphthone

Chem. Abstr. Serv. Reg. No.: 573-20-6
Chem. Abstr. Name: 2-Methyl-1,4-naphthalenediol, diacetate
IUPAC Systematic Name: 2-Methyl-1,4-naphthalenediol, diacetate
Synonyms: 1,4-Diacetoxy-2-methylnaphthalene; menadiol diacetate; 2-methyl-1,4-naphthohydroquinone diacetate; 2-methyl-1,4-naphthoquinol diacetate; 2-methyl-1,4-naphthylene diacetate; vitamin K diacetate; vitamin K_4



$C_{15}H_{14}O_4$ Relative molecular mass: 258.27

IUPAC recommends that 2-methyl-3-polyprenyl-1,4-naphthoquinone be referred to as menaquinone-*n*, previously vitamin K_2, *n* being the number of prenyl residues. Vitamin $K_{2(20)}$ is so named because it contains 20 carbon atoms in the chain. In the biological literature, vitamin K_2 is frequently referred to as menaquinone and is further designated by the number of isoprene units in the side-chain. For example, vitamin $K_{2(20)}$ is also called menaquinone-4 for the four isoprene units in the side-chain. The compound originally isolated from rotting fish meal and named vitamin K_2 was later identified as menaquinone-7 (2-methyl-3-farnesylgeranyl-geranyl-1,4-naphthoquinone). In the older literature, the designation vitamin $K_{2(35)}$ is used for menaquinone-7, but this is no longer used. Menaquinones found in nature have side-chains of 4–13 isoprenoid residues and are usually in the all-*trans* configuration; however, menaquinones with the *cis* configuration and partially saturated side-chains also exist (Suttie, 1985, 1991; Weber & Rüttimann, 1996; Van Arnum, 1998).

1.1.2 *Chemical and physical properties of the pure substances*

Phylloquinone

(*a*) *Description*: Clear, yellow to amber, very viscous, odourless liquid (Gennaro, 1995; Budavari, 1996)

(*b*) *Spectroscopy data*: Ultraviolet, infrared, nuclear magnetic resonance (proton and ^{13}C) and mass spectral data have been reported (Hassan *et al*., 1988).

(*c*) *Solubility*: Insoluble in water; sparingly soluble in methanol; soluble in acetone, benzene, chloroform, diethyl ether, dioxane, ethanol, hexane, petroleum ether and other fat solvents and vegetable oils (Budavari, 1996)

(*d*) *Stability*: Stable to air and moisture; decomposes in sunlight; unaffected by dilute acids; destroyed by solutions of alkali hydroxides and by reducing agents (Gennaro, 1995; Budavari, 1996)

(*e*) *Optical rotation*: $[\alpha]_D^{25}$, –28° (Budavari, 1996)

Menaquinone-4

From Japan Medical Products Trade Association (1996)

(a) *Description*: Yellow crystals or an oily substance

(b) *Melting-point*: 34–38 °C

(c) *Solubility*: Practically insoluble in water; very soluble in diethyl ether, chloroform and hexane; freely soluble in isooctane; sparingly soluble in ethanol and isopropanol; slightly soluble in methanol

(d) *Stability*: Decomposed by light or alkalis

Menadione

(*a*) *Description*: Bright-yellow crystals with a very faint acrid odour (Budavari, 1996)

(*b*) *Melting-point*: 105–107 °C (Budavari, 1996)

(*c*) *Spectroscopy data*: Infrared (prism [8077]; grating [8522]), ultraviolet [2183] and nuclear magnetic resonance (proton [3217]; ^{13}C [6002]) spectral data have been reported (Sadtler Research Laboratories, 1980; British Pharmacopoeial Commission, 1993).

(*d*) *Solubility*: Insoluble in water; soluble in benzene (1 g/10 mL), ethanol (1 g/60 mL), and vegetable oils (1 g/50 mL); moderately soluble in carbon tetrachloride and chloroform (Budavari, 1996)

(*e*) *Stability*: Stable in air; decomposed by sunlight; destroyed by alkalis and reducing agents (Budavari, 1996)

Menadione sodium bisulfite (trihydrate)

(*a*) *Description*: White, crystalline, odourless, hygroscopic powder (Gennaro, 1985; Budavari, 1996)

(*b*) *Solubility*: Soluble in water (~0.5 g/mL); slightly soluble in chloroform and ethanol; practically insoluble in benzene and diethyl ether (Gennaro, 1985; Budavari, 1996)

(*c*) *Stability*: Discolours and may turn purple under light (Budavari, 1996)

Menadiol

(*a*) *Description*: White needles (Budavari, 1996)

(*b*) *Melting-point*: 168–170 °C (Budavari, 1996)

(*c*) *Solubility*: Very soluble in acetone and ethanol; slightly soluble in benzene and chloroform (Budavari, 1996)

Menadiol sodium phosphate (hexahydrate)

(*a*) *Description*: White to pinkish, hygroscopic powder with a salty taste (Gennaro, 1995; Budavari, 1996)
(*b*) *Spectroscopy data*: Infrared spectral data have been reported (British Pharmacopoeial Commission, 1993).
(*c*) *Solubility*: Very soluble in water; practically insoluble in acetone, diethyl ether, ethanol and methanol (Budavari, 1996)

Acetomenaphthone

(*a*) *Description*: Crystalline solid (Budavari, 1996)
(*b*) *Melting-point*: 112–114 °C (Budavari, 1996)
(*c*) *Solubility*: Practically insoluble in water; slightly soluble in ethanol; soluble in acetic acid (Budavari, 1996)
(d) *Spectroscopy data*: Infrared (prism [20206]; grating [32489]), ultraviolet [6761] and nuclear magnetic resonance (proton [2298]; ^{13}C [2451]) spectral data have been reported (Sadtler Research Laboratories, 1980).

1.1.3 *Technical products and impurities*

Commercially available phylloquinone is prepared synthetically and may contain not only 2′,3′-*trans*-phylloquinone (not less than 75%) but also 2′,3′-*cis*-phylloquinone and *trans*-epoxyphylloquinone (not more than 4.0%). Phylloquinone occurs in nature only as the 2′,3′-*trans*-phylloquinone stereoisomer (Weber & Rüttimann, 1996; American Hospital Formulary Service, 1997; Council of Europe, 1997).

Phylloquinone is available as a 5- and 10-mg tablet (chewable), a 2- and 10 mg/mL injection solution, a 10- and 20-mg/mL oral solution and a 20-mg/mL emulsion. The tablet may also contain carmellose, carob bean flour, carob gum, cocoa butter, cocoa powder, ethyl cellulose, ethyl vanillin, glucose, glycerol, gum arabic, hard and viscous paraffin, lactose, rice starch, sugar, silicic acid, silicon dioxide, skim-milk powder, sodium cyclamate, talc and titanium dioxide. The injection solution may also contain benzyl alcohol, dextrose, glacial acetic acid, glucose, glycocholic acid, hydrochloric acid, macrogol ricinoleate, phenol, phosphatidylcholine from soya beans, polyethoxylated fatty acid derivative (castor oil), polysorbate 80, propylene glycol, sodium acetate, sodium hydroxide and water. A widely used injectable formulation, Konakion®, formerly contained a polyethoxylated castor oil as an emulsifying agent, but has been reformulated as a mixed micellar preparation, Konakion MM®, containing glycocholic acid, lecithin and buffered to pH 6. The oral solution may also contain benzoic acid, glycocholic acid, hydrochloric acid, lecithin, macrogol ricinoleate, methyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate, sodium hydroxide and water. The emulsion may also contain polysorbate 80, purified water and sorbic acid.

Phylloquinone is also available as a component (200 μg) of a multivitamin lyophilized, sterile powder intended for reconstitution and dilution in intravenous infusions, as a component (0.075 mg) of an effervescent multivitamin tablet, and as a component (5.5 μg) of a multivitamin infant formula (Gennaro, 1995; American Hospital Formulary Service, 1997; Canadian Pharmaceutical Association, 1997; British Medical Association/Royal Pharmaceutical Society of Great Britain, 1998; Editions du Vidal, 1998; LINFO Läkemedelsinformation AB, 1998; Rote Liste Sekretariat, 1998; Thomas, 1998; US Pharmacopeial Convention, 1998).

Trade names for phylloquinone include AquaMEPHYTON, AquaMephyton, AquaMephyton R, Combinal K_1, Hymeron, Kanakion, Kanavit, Kaywan, Kephton, Kinadion, K1 Delagrange, Konakion, Konakion MM, Menadion 'Dak', Mephyton, Monodion, Synthex P, Vitacon, Vita-K1, Vitamina K1 Biol, Vitamine K1 Roche and Vitamin K_1 (CIS Information Services, 1998; Royal Pharmaceutical Society of Great Britain, 1999; Swiss Pharmaceutical Society, 1999).

Menaquinone-4 is available in Japan as 5- and 15-mg capsules and as a 2-mg/mL syrup. The capsules may also contain ethyl parahydroxybenzoate, propyl paraoxyhydroxybenzoate, sodium lauryl sulfate and FD&C Yellow No. 6 (Sunset Yellow). The syrup may also contain polyoxyethylene hydrogenated castor oil 60, propylene glycol, ethyl parahydroxybenzoate, sodium benzoate and flavouring (Japan Medical Products Trade Association, 1996).

Trade names for menaquinone-4 include Glakay and Kaytwo (Japan Medical Products Trade Association, 1996).

Menadione is available as a 2-, 5- and 10-mg tablet and as a 2- and 10-mg/mL injection (in oil). Menadione sodium bisulfite is available as a 10-mg tablet and as a 5- and 10-mg/mL and 72-mg/10 mL injection (Gennaro, 1985).

Trade names for menadione include Aquakay, Aquinone, Austrovit-K Depot, Hemodal, K-Thrombyl, K-Vitan, Kaergona, Kanone, Kaom Belgarum, Kappaxan, Kappaxin, Karanum, Karcon, Kareon, Kativ-G, Kavitamin, Kayklot, Kaykot, Kayquinone, Kipca, Kipca-Oil Soluble, Klottone, Koaxin, Kolklot, Menadion, Menaphthon, Menaphthone, Menaquinone 0, Mitenon, Mitenone, MNQ, Neo-Zimema-K, Panosine, Prokayvit, Synkay, Thyloquinone, Vikaman, Vita-Noxi K and Vitavel-K (Swiss Pharmaceutical Society, 1999).

Trade names for menadione sodium bisulfite include Austrovit-K, Golagen K, Hemoklot, Hetrogen K, Hetrogen K Premix, Hykinone, Ido-K, K-Thrombin, K-Trombina, Kalzon, Kareon, Kavitamin, Kavitan, Kavitol, Kawitan, Klotogen, Libavit K, Nuvit K, Vikaman, Vikasol, Vitaminum K and Zimema K (Swiss Pharmaceutical Society, 1999).

Menadiol sodium phosphate (as the hexahydrate) is available as a 5-mg and 10 mg (equivalent of menadiol phosphate) tablet and as 5- and 10-mg/mL and 75-mg/2 mL injections (Gennaro, 1995; British Medical Association/Royal Pharmaceutical Society of Great Britain, 1998; US Pharmacopeial Convention, 1998).

Acetomenaphthone is available in a chilblain formula tablet containing 30 mg nicotinamide and 5 mg acetomenaphthone and as a component (10 mg) of a multivitamin injection solution, which may also contain butyl hydroxyanisole, butyl hydroxytoluene, peanut oil, medium-chain triglycerides and olive oil (Rote Liste Sekretariat, 1998; Thomas, 1998).

Trade names for menadiol sodium phosphate hexahydrate include Kappadione, Kativ (injection), Kipca water soluble, Naphthidone, Procoagulo, Synkavit, Synka-Vit, Synkavite, Synkayvite and Thylokay (Swiss Pharmaceutical Society, 1999).

Trade names for acetomenaphthone include Adaprin, Davitamon-K, Davitamon-K-oral, Kapathrom, Kapilin, Kapilon, Kappaxan, Kativ powder, Kayvite, Pafavit, Prokayvit Oral and Vitavel K.

1.1.4 *Analysis*

Several international pharmacopoeias specify infrared (IR) and ultraviolet (UV) absorption spectrophotometry with comparison to standards as the methods for identifying phylloquinone; UV absorption spectrophotometry and liquid chromatography are used to assay its purity. Phylloquinone is identified in pharmaceutical preparations by IR and UV absorption spectrophotometry and liquid chromatography; liquid chromatography is used to assay for its content (British Pharmacopoeial Commission, 1993; US Pharmacopeial Convention, 1994; Society of Japanese Pharmacopoeia, 1996; Council of Europe, 1997). AOAC International (1996) has developed a liquid chromatographic method with UV detection for the determination of phylloquinone in ready-to-feed milk-based infant formulae.

As a result of its high selectivity and sensitivity, high-performance liquid chromatography (HPLC) is the method of choice for the determination of phylloquinone and menaquinones in blood, tissues, milk and foods. Various procedures for extraction and preliminary purification, normal or reversed-phase HPLC and UV, electrochemical and fluorescence detection (both after electrochemical or chemical reduction and after photochemical decomposition) of the various vitamin K substances have been described. The limit of detection of phylloquinone is 25–500 pg, depending on the detection method used. Similar values, which vary according to the length of the side-chain, apply to the menaquinones. HPLC methods are also available for the determination of menadione and water-soluble derivatives in feedstuffs, premixes and vitamin concentrates (Weber & Rüttimann, 1996).

Alternative methods are thin-layer chromatography, high-performance thin-layer chromatography and gas chromatography. The spectrophotometric, fluorimetric and colorimetric methods previously used without chromatographic purification of the samples to be analysed are frequently less sensitive and less specific than HPLC, for instance allowing no distinction between phylloquinone and menaquinones (Weber & Rüttimann, 1996).

Several international pharmacopoeias specify IR absorption spectrophotometry with comparison to standards and colorimetry as the methods for identifying menadiol sodium phosphate hexahydrate; potentiometric titration with ceric sulfate is used to assay its purity. In pharmaceutical preparations, menadiol sodium phosphate is identified by IR absorption spectrophotometry and colorimetry; potentiometric titration with ceric sulfate and UV absorption spectrophotometry are used to assay for its content (British Pharmacopoeial Commission, 1993; US Pharmacopeial Convention, 1994; Council of Europe, 1997).

Several international pharmacopoeias specify IR and UV absorption spectrophotometry with comparison to standards as the methods for identifying menadione; titration with ammonium and cerium nitrate or ceric sulfate is used to assay its purity. Visible (635 nm) absorption spectrophotometry is used to assay for its content in pharmaceutical preparations (British Pharmacopoeial Commission, 1993; US Pharmacopeial Convention, 1994; Council of Europe, 1997).

1.2 Production

Although the predominant commercial form of phylloquinone is the synthetic racemate, natural phylloquinone is accessible either by extraction from a natural source or from condensation of menadione with natural phytol. The stability of phylloquinone to heat made possible the use of commercially dehydrated alfalfa meal, for example, as a natural source (Hassan *et al*., 1988). The synthesis and spectral properties of all four stereoisomers of (*E*)-phylloquinone have been described and their biological potencies determined. When natural phylloquinone was used as a standard in bioassays, it was concluded that all four stereoisomers have essentially identical activity (Van Arnum, 1998).

The first syntheses and structural elucidation of phylloquinone were published in 1939 almost simultaneously by four groups. The starting materials were menadione or menadiol as the aromatic component and natural phytol or one of its derivatives. A breakthrough in commercial synthesis was achieved in the 1950s, when it was found that monoacylated menadiols (e.g. the monoacetate or the monobenzoate) could be used advantageously in the alkylation step and that natural phytol could be replaced by isophytol, which is easy to synthesize (Weber & Rüttimann, 1996).

In the Isler-Lindlar method, excess menadiol monobenzoate is condensed with isophytol in the presence of boron trifluoride etherate as a catalyst. The alkylation product is obtained as a 70:30 *trans*/*cis* mixture. The *trans* form can be enriched by recrystallization. The *trans*-enriched alkylation product (*trans*:*cis* 9:1) is saponified with potassium hydroxide and oxidized to phylloquinone with oxygen (Weber & Rüttimann, 1996).

The industrial synthesis of menaquinones parallels that of phylloquinone and involves as a key step alkylation of monosubstituted menadione with an appropriate (all-*trans*) polyisoprenyl derivative. Considerably more work has been done on

fermentative approaches to menaquinones than for phylloquinone. Menaquinones of varying chain lengths, from C_5 to C_{65}, have been produced and isolated from bacteria. Menaquinone-4 is produced and used extensively in Japan (Van Arnum, 1998).

Menadione can be prepared by oxidizing 2-methylnaphthalene with chromic acid or hydrogen peroxide (Weber & Rüttimann, 1996). A process based on biotechnological techniques has been reported in Japan (Van Arnum, 1998).

Menadione sodium bisulfite can be prepared by reacting menadione with sodium bisulfite. The reaction may be visualized as consisting of the typical addition of sodium bisulfite to a ketone, forming the $R(OH)(SO_3Na)$ compound, which then rearranges at the expense of one degree of unsaturation of the quinoid nucleus. The compound readily regenerates menadione on treatment with mild alkali and behaves as a typical ketone–sodium bisulfite addition compound (Gennaro, 1985; Van Arnum, 1998).

Menadiol sodium phosphate can be prepared by reducing menadione to the diol, followed by double esterification with hydriodic acid, metathesis of the resulting 1,4-diiodo compound with silver phosphate and neutralization of the bis(dihydrogen phosphate) ester with sodium hydroxide (Gennaro, 1995).

Information available in 1999 indicated that phylloquinone was manufactured and/or formulated in 41 countries, menadione in 26 countries, menadione sodium bisulfite in 21 countries, menadiol and menadiol sodium phosphate (as the hexahydrate) in two countries each and acetomenaphthone in seven countries (CIS Information Services, 1998; Royal Pharmaceutical Society of Great Britain; 1999; Swiss Pharmaceutical Society, 1999).

1.3 Use

1.3.1 *Physiological function*

The only established biochemical role for vitamin K is as a cofactor in a unique post-translational chemical modification in which selective glutamate (Glu) residues on certain specialized calcium-binding proteins are transformed to γ-carboxyglutamate (Gla) residues (Suttie, 1991; Shearer, 1997). The modification is catalysed by a microsomal enzyme called γ-glutamyl or vitamin K-dependent carboxylase, which is present in most tissues. The best-known vitamin K-dependent proteins are those synthesized in the liver, which play a role in the maintenance of normal haemostasis. They comprise four proteins (II, VII, IX and X) that promote coagulation and two proteins (C and S) that act in the regulatory feedback control of coagulation. Vitamin K-dependent proteins, of uncertain function, are also known to occur in a variety of other tissues such as bone, kidney, pancreas, placenta, spleen and lungs. They include the bone protein osteocalcin (also called bone Gla protein) and matrix Gla protein; there is growing evidence that these proteins may be important for bone health and other regulatory functions in calcium metabolism. In those proteins with well-established functions, such as

coagulation proteins, the Gla groups are essential for the biological activity (Thijssen & Drittij-Reijnders, 1996; Shearer, 1997).

Naturally occurring phylloquinone and menaquinones all γ-carboxylate the vitamin K-dependent coagulation proteins. Synthetic forms of menadione (and related water-soluble salts) that lack a side-chain at the 3-position have biological activity *in vivo* only after side-chain alkylation, which results in the specific synthesis of menaquinone-4 (Suttie, 1991; see also section 4).

1.3.2 *Supplementation and therapy*

Vitamin K is given as a supplement to prevent or cure vitamin K deficiency when the endogenous vitamin K supply from the diet is likely to become or has proven to be insufficient. Neonates are born with very limited vitamin K stores, but most infants do not show relevant hypoprothrombinaemia at birth (von Kries *et al*., 1987a, 1988; von Kries, 1991). Biochemical signs of vitamin K deficiency are common during the first week of life, however, unless sufficient amounts of vitamin K are ingested. The natural diet of newborns is human milk, which contains vitamin K at concentrations of 0.69–9.2 ng/mL (see Table 1). [The Working Group noted that some of the high values in the Table may reflect methodological problems with analysis and milk collection.] Bleeding, the classical clinical manifestation of vitamin K deficiency, is extremely rare on the first day of life, and the typical time of onset is during the first week, with bleeding from mucous membranes, the umbilicus, following circumcision, and rarely, into the central nervous system (von Kries *et al*., 1988; von Kries, 1991). This condition was originally called ‘classical haemorrhagic disease of the newborn’; the present nomenclature is ‘classical vitamin K deficiency bleeding’ (Sutor *et al*., 1999).

During the first three months of life, exclusively breast-fed infants remain at risk for vitamin K deficiency bleeding. In many of these infants, the bleeding episode, which is often intracranial haemorrhage, is the first perceived symptom of an underlying cholestatic disease. In 10–30% of the cases, however, no underlying disease can be found (von Kries *et al*., 1988).

After the first three months of life, vitamin K deficiency is almost completely confined to patients with cholestatic diseases (congenital or acquired obstruction of the bile duct), malabsorption syndromes or cystic fibrosis (Houwen *et al*., 1987; van den Anker & Sinaasappel, 1993; O’Brien *et al*., 1994; Kowdley *et al*., 1997; Nowak *et al*., 1997; see also section 1.3.4).

1.3.3 *For prevention of vitamin K deficiency in newborns and early infancy*

The use of vitamin K prophylaxis since the 1950s has varied widely over time, between countries and within countries between institutions. The predominant patterns were to give either selective intramuscular prophylaxis only to infants presumed to be at special risk for bleeding (mainly premature and low-birth-weight

Table 1. Concentrations of phylloquinone in human and cow's milk, infant formulae and various oils

Sample	Concentration of phylloquinone	Comments	Reference
Human milk	2.1 ng/mL (range, 1.1–6.5) 2.3 ng/mL (range, 0.7–4.2)	Mature milk Colostrum	Haroon *et al*. (1982)
Human milk	3.8 ng/mL (range, 1.1–8.3)	Mature milk	Motohara *et al*. (1984)
Human milk	1.2 ng/mL 1.8 ng/mL	Mature milk Colostrum	von Kries *et al*. (1987b)
Human milk	Median, 5.2 ng/mL (range, 3.1–11) Median, 8.9 ng/mL (range, 6.3–16) Median, 9.2 ng/mL (range, 4.8–13)	Day 3 of lactation (colostrum) Day 8 of lactation Day 10 of lactation (mature milk)	Fournier *et al*. (1987)
Human milk	1.6 ng/mL 0.9 ng/mL	Mature milk Colostrum	Canfield *et al*. (1988)
Human milk	Mean, 0.64 ng/mL Mean, 0.86 ng/mL Mean, 1.14 ng/mL Mean, 0.87 ng/mL	Week 1 of lactation (colostrum) Week 6 of lactation (mature milk) Week 12 of lactation Week 26 of lactation	Greer *et al*. (1991)
Human milk	0.69 ng/mL 76 ng/mL 75 ng/mL 82 ng/mL	Before supplements At 2 weeks with 5 mg/day supplement At 6 weeks with 5 mg/day supplement At 12 weeks with 5 mg/day supplement	Greer *et al*. (1997)
Human milk	0.11 μg/100 g milk		Indyk & Woollard (1997)
Cow’s milk	Mean, 4.9 ng/mL (range, 3.6–8.9) Mean, 8.7 ng/mL (range, 3.8–18)	Holstein cows Jersey or Guernsey cows	Haroon *et al*. (1982)
Cow’s milk	7.5 and 37 ng/mL	Measurements in January and July	Fournier *et al*. (1987)
Cow’s milk	0.54 μg/100 g milk		Indyk & Woollard (1997)
Goat’s milk	1.18 μg/100 g milk		Indyk & Woollard (1997)

Table 1 (contd)

Sample	Concentration of phylloquinone	Comments	Reference
Formula	79–118 ng/mL	Milk-substituted formulae with soya oil but without added vitamin K_1	Schneider *et al*. (1974)
	118–256 ng/mL	Milk-substituted formulae with various vegetable oils and with added vitamin K_1	
	19–69 ng/mL	Milk-based formulae with various vegetable oils but without added vitamin K_1	
Formula	Mean, 4.4 ng/mL	Unsupplemented infant formula containing only milkfat	Haroon *et al*. (1982)
	Mean, 11.5 ng/mL	Unsupplemented infant formula containing only vegetable oils	
Formula	~72–166 ng/mL	Ready-to-feed	Bueno & Villalobos (1983)
	~125–146 ng/mL	Concentrate	
	~129–175 ng/mL	Powder	
Formula	30–225 ng/mL (*trans* isomer); 2.8–25 ng/mL (*cis* isomer; 9.3–11% of total)	Ready-to-feed liquids	Hwang (1985)
	120–211 ng/mL (*trans* isomer); 7.2–31 ng/mL (*cis* isomer; 6.0–15% of total)	Concentrated liquids	
	90–195 ng/mL (*trans* isomer)	Powders	
Formula	0.87 µg/g	Powder (milk-based)	Schneiderman *et al*. (1988)
	0.95 µg/g	Powder (soya protein-based)	

Table 1 (contd)

Sample	Concentration of phylloquinone	Comments	Reference
Formula	37–130 µg/100 g	Powder (milk-based, predominantly milkfat containing < 5% corn oil)	Indyk & Woollard (1997)
	46–140 µg/100 g	Powder (milk-based, predominantly vegetable oil containing < 2% milkfat)	
	67–77 µg/100 g	Powder (goat milk-based, containing equivalent goat milkfat and vegetable oils)	
	72 µg/100 g	Powder (soya protein-based, containing exclusively vegetable oils)	
	110 µg/100 g	Powder (NIST SRM 1846 (standard reference milk))	
Soya bean oil	1.8 mg/kg (*trans* isomer)		Hwang (1985)
Corn oil	0.13 mg/kg (*trans* isomer)		
Coconut oil	< 0.06 mg/kg (*trans* isomer)		
Soya bean oil	1.9 µg/g		Haroon *et al*. (1982)
Palm oil	0.08 µg/g		
Oleo oil	0.06 µg/g		
Oleic oil	0.03 µg/g		
Corn oil	0.03 µg/g		
Coconut oil	< 0.01 µg/g		

Table 1 (contd)

Sample	Concentration of phylloquinone	Comments	Reference
Peanut oil	0.65 µg/100 g (range, 0.30–1.19)	Combined average	Ferland & Sadowski (1992)
Corn oil	2.91 µg/100 g (range, 1.63–4.18)	Combined average	
Almond oil	6.70 µg/100 g		
Sunflower oil	9.03 µg/100 g (range, 8.86–9.19)	Combined average	
Safflower oil	9.13 µg/100 g (range, 6.49–11.77)	Combined average	
Walnut oil	15.0 µg/100 g		
Sesame oil	15.5 µg/100 g (range, 12.1–18.7)	Combined average	
Olive oil	55.5 µg/100 g (range, 37.2–82.1)	Combined average	
Rapeseed oil	141 µg/100 g (range, 114–188)	Combined average	
Soya bean oil	193 µg/100 g (range, 139–290)	Combined average	

infants and those delivered surgically) or general prophylaxis for all infants. In the latter case, vitamin K was given either intramuscularly or orally.

Several preparations of fat-soluble vitamin K have been in use. In the early 1950s, water-soluble menadiol sodium phosphate was widely used, until haemolysis due to high doses of this preparation in neonates was identified (Meyer & Angus, 1956). In most countries, phylloquinone has been used since that time, although in some third-world countries water-soluble menadione sodium bisulfite still seems to be used (Sharma *et al*., 1995). Because it is technically difficult to dissolve phylloquinone, only a limited number of preparations became available. The Roche preparation (Konakion®) in which Cremophor (polyethoxylated castor oil) is used as an emulsifying vehicle has been widely available in Europe and North America. The manufacturer has recently replaced the Cremophor preparation by a new mixed micellar preparation Konakion–MM® (British Medical Association/Royal Pharmaceutical Society of Great Britain, 1998). In Japan, an oral preparation of menaquinone-4 is used instead of phylloquinone (Hanawa, 1992).

Almost all cases of vitamin K deficiency bleeding can be prevented by intramuscular administration of 1 mg of vitamin K at birth (von Kries & Hanawa, 1993). Clinical observations and laboratory investigations have also clearly shown that a single oral dose of vitamin K protects against classical vitamin K deficiency bleeding (Clark & James, 1995) but is less effective for prevention of this condition later in life (Tönz & Schubiger, 1988; Ekelund, 1991). Without vitamin K prophylaxis, the incidence of late vitamin K deficiency bleeding in Europe was estimated to be 40–100 per million livebirths, whereas in Asia the condition appears to be considerably more common (Hanawa, 1992; Choo *et al*., 1994).

Since intramuscular vitamin K prophylaxis has proven effective against late deficiency bleeding, 1 mg of vitamin K at birth was recommended in most western countries (von Kries, 1991). After reports of a potential association between vitamin K prophylaxis and the risk for childhood cancer (Golding *et al*., 1990, 1992), several countries switched to oral prophylaxis regimens with repeated doses of phylloquinone (Hill, 1994; Doran *et al*., 1995; Hansen & Ebbesen, 1996; Cornelissen *et al*., 1997). The optimal oral dose regimen remains to be established (von Kries, 1999).

1.3.4 *Cholestatic and malabsorption syndromes*

Vitamin K deficiency is observed in patients with cholestatic jaundice, cystic fibrosis, primary biliary cirrhosis and other diseases. In most cases, however, vitamin K deficiency is detectable only by measuring the plasma concentrations of vitamin K or with sensitive biochemical markers of vitamin K deficiency (Cornelissen *et al*., 1992; O'Brien *et al*., 1994; Kowdley *et al*., 1997). Bleeding is observed only rarely. Additional risk factors, such as therapy with antibiotics that interfere with vitamin K metabolism, may cause bleeding in patients with cystic fibrosis (Nowak *et al*., 1997). Some patients with this disease are given vitamin K supplements, although there are no uniform recommendations (Durie, 1994).

1.3.5 *Vitamin supplementation to overcome side-effects of drugs that interfere with vitamin K metabolism*

An important indication for vitamin K supplementation is the side-effects of drugs that interfere with its metabolism. Mothers on antiepileptic drugs, for example, are at high risk of delivering an infant with manifest vitamin K deficiency (Cornelissen *et al*., 1993a) and intracranial bleeding (Renzulli *et al*., 1998).

Hypoprothrombinaemia may be caused by some cephalosporins, especially those containing an *N*-methylthiotetrazole side-chain, and may require vitamin K supplementation (Breen & St Peter, 1997).

1.3.6 *Vitamin K therapy*

(*a*) *Overdosage of vitamin K antagonists*

The coumarin derivatives acenocoumarol, phenprocoumon and warfarin are among the most commonly used oral anticoagulants (Keller *et al*., 1999). The clinical symptom of overdosage of these drugs is bleeding. A tendency to bleed is also increased by individual susceptibility to one of these anticoagulants, interference with other drugs or poor dietary intake of vitamin K. The biochemical indicator for overdosage is an excessive prolongation of the prothrombin time. Minor bleeding is most commonly managed by temporarily discontinuing treatment and by giving vitamin K to counteract the effects of the coumarin derivative. In the case of major bleeding, especially intracranial haemorrhages, higher doses of vitamin K and use of prothrombin complex concentrates are recommended to induce immediate reversal of anticoagulation (Pindur *et al.*, 1999). In the past, the oral or intravenous dose of phylloquinone used to counteract supratherapeutic anticoagulation was 10–50 mg (Fetrow *et al*., 1997). Much lower doses have been proposed recently. In asymptomatic patients, a 1-mg oral dose of vitamin K was shown to reduce the international normalized ratio effectively (Crowther *et al*., 1998). Low subcutaneous doses of phylloquinone are an effective alternative to intravenous administration of phylloquinone in the treatment of warfarin-induced hypoprothrombinaemia (Fetrow *et al*., 1997).

(b) Prevention of intracranial haemorrhage in very-low-birth-weight, premature infants

The effect of high doses of vitamin K given to women at imminent risk of early preterm parturition has been studied with the primary aim of preventing periventricular haemorrhage and the associated neurological injury in the infant. A first meta-analysis of the trials came to the conclusion, however, that it is ineffective (Thorp *et al*., 1995).

1.3.7 *Other uses*

Menadione is of industrial importance as an intermediate in the synthesis of phylloquinone, and salts of its bisulfite adduct are used as stabilized forms in the animal feed

industry. Commercially significant forms are menadione sodium bisulfite and menadione dimethyl pyrimidinol (Van Arnum, 1998).

Menaquinone-4 has been used in Japan at high doses for the treatment of osteoporosis (Shearer, 1997).

1.4 Occurrence

Phylloquinone is widely distributed in higher plants and in some blue–green algae. It is present in many foods, especially leafy green vegetables and some vegetable oils. Table 2 shows the concentrations in some common foods (Booth *et al.*, 1995; Shearer *et al.*, 1996; Booth & Suttie, 1998).

The Total Diet Study of the US Food and Drug Administration is conducted periodically to monitor the safety and nutritional quality of the US food supply by assessing the levels of nutrients and contaminants in daily diets. It is based on the collection and analysis of 265 core foods. Intakes are estimated from the concentrations of individual nutrients and contaminants in the core foods and the mean consumption of the foods in each population group. The quantitative contributions of specific foods to the phylloquinone intake of the total population are presented in Table 3. Table 4 gives the estimated daily intake in 1990 for 14 categories of age and sex (Booth *et al.*, 1996).

Phylloquinone has been determined by several analytical methods in human milk, in cow's milk, in many brands of infant formula and in the oils that have been added to infant formulas for many years. Some of the concentrations found in each of these sources are presented in Table 1.

Menaquinones are synthesized by bacteria. They have a more restricted distribution in the diet than phylloquinone, and nutritionally significant amounts probably occur only in animal liver and some fermented foods, including cheese. Menaquinones are also synthesized by specific inhabitants of the human gut microflora. The major intestinal forms are MK-10 and MK-11 produced by *Bacteroides*, MK-8 by *Enterobacteria*, MK-7 by *Veillonella* genus and MK-6 by *Eubacterium lentum* (Shearer *et al.*, 1996). The total concentration of menaquinones in human distal colonic contents is about 20 μg/g dry weight, with MK-10 predominating (Conly & Stein, 1992; Shearer, 1995). It seems likely that menaquinones synthesized by the gut microflora make a significant contribution to human tissue stores and are used by the hepatic vitamin K-dependent carboxylase, but the extent of this contribution remains uncertain (Shearer, 1995; Suttie, 1995).

1.5 Regulations and guidelines

Phylloquinone is listed (as phytomenadione or phytonadione) in the British, Chinese, Czech Republic, European, French, German, International, Japanese, Swiss and US pharmacopoeias (Royal Pharmaceutical Society of Great Britain, 1999; Swiss Pharmaceutical Society, 1999).

Table 2. Phylloquinone content of common foods[a]

0.1–1.0 μg/100 g	1–10 μg/100 g	10–100 μg/100 g	100–1000 μg/100 g
Avocado [1]	Apple pie [11]	Asparagus [60]	Broccoli [179–180]
Banana [0.1]	Apples [6]	Beans, runner [26]	Brussels sprouts [147–177]
Beef, steak [0.8]	Aubergines [6]	Beans, French [39]	Cabbage [145–339]
Bread, white [0.4]	Baked beans [3]	Beans, broad [19]	Canola oil [127]
Chicken, thigh [0.1]	Barley [7]	Beef chow mein [31]	Collards [440]
Coconut oil [0.5]	Beef, corned [7]	Cabbage, red [19]	Kale [618]
Cod, fresh, fillet [< 0.1]	Beef, minced [2]	Cauliflower [20–31]	Lettuce [122–129]
Cornflakes [< 0.1]	Bilberries [4]	Chick peas [21]	Rapeseed oil [123]
Flour, white [0.8]	Bran, wheat [10]	Coleslaw [80]	Salad greens [315]
Grapefruit [< 0.1]	Bread [3]	Cottonseed oil [60]	Soya bean oil [173–193]
Ham, tinned [0.1]	Bread, wholemeal [2]	Cucumbers [20–21]	Spinach [380]
Maize [0.3]	Butter [7]	Dry lentils [22]	Watercress [315]
Mangoes [0.5]	Carrots [6–10]	Dry soya beans [47]	
Melon, yellow [0.1]	Cheeses, various [2–6]	Greengages [15]	
Melon, water [0.3]	Chocolate, plain [2]	Green beans [33]	
Milk, cows [0.6]	Corn oil [3]	Green peas [24]	
Mushrooms [0.3]	Courgettes [3]	Iceberg lettuce [35]	
Oranges [< 0.1]	Cranberries [2]	Margarine [42]	
Parsnips [< 0.1]	Cream, double [6]	Mayonnaise [41]	
Peanuts, roasted [0.4]	Dates, fresh [6]	Muffins [25]	
Pilchards, in brine [0.6]	Doughnuts [10]	Mustard greens, cress [88]	
Pineapple [0.2]	Egg yolk [2]	Okra [40]	
Pork, chop, lean [< 0.1]	Eggs [2]	Olive oil [55–80]	
Potatoes [0.9]	Figs, fresh [3]	Peas [34]	
Rice, white [0.1]	French fries [5]	Potato chips [15]	
Rice, brown [0.8]	Grapes, black [8]	Salad dressings [100]	
Salmon, tinned, in brine [0.1]	Grapes, green [9]	Tuna in oil [24]	
Sausage, pork or beef [0.2]	Hamburger and bun [4]		
Spaghetti [0.2]	Hot dog and bun [3]		
Tuna, tinned, in brine [0.3]	Lasagna [5]		
Turnips [0.2]	Leeks [10]		
Yoghurt [0.8]	Liver, lamb [7]		
	Liver, ox [4]		
	Macaroni with cheese [5]		
	Nectarines [3]		
	Oats [10]		
	Palm oil [8]		
	Peaches, fresh [4]		
	Pears [6]		
	Peppers, green [6]		
	Peppers, red [2]		
	Pizza [4]		
	Plums, red [8]		
	Potatoes [1]		
	Raisins [4]		
	Rhubarb [4]		
	Safflower oil [3]		
	Strawberries [3]		
	Sunflower oil [6]		
	Swedes [2]		
	Tomatoes [6]		
	Wheat [8]		

From Shearer *et al*. (1996); Booth & Suttie (1998)

[a] Numbers in brackets are actual levels measured. The phylloquinone content of oil-based preparations varies widely depending on the source of the oil used.

Table 3. Contribution of certain food groups to total adult intake (%) of phylloquinone in the USA, stratified by age and sex

Food group	Age group							
	25–30		40–45		60–65		≥ 70	
	Men	Women	Men	Women	Men	Women	Men	Women
Milk and cheese	1.7[a]	1.4	0.9	0.9	1.0	0.9	0.8	0.8
Eggs	3.6	2.5	2.1	1.7	2.4	1.3	1.7	1.4
Meat, poultry, fish	4.8	4.2	4.6	4.0	5.7	4.3	4.8	2.8
Legumes and nuts	1.3	0.5	0.5	0.6	0.7	0.4	0.7	0.6
Grain products	4.4	4.3	3.8	3.4	4.6	3.3	4.6	3.1
Fruits	1.3	1.4	1.4	1.6	1.7	2.0	2.5	2.2
Vegetables	51	56	60	61	59	67	63	73
Mixed dishes and meals	16	14	13	12	9.1	7.1	7.2	4.8
Desserts	4.9	4.2	4.3	3.8	4.6	5.1	5.2	3.9
Snacks	2.6	1.7	1.4	1.5	1.0	0.7	0.7	0.2
Condiments, sweeteners	1.3	1.0	0.6	1.0	1.0	1.0	1.2	0.9
Fats, dressings	6.8	8.7	7.7	8.1	9.1	6.3	7.3	5.1
Beverages	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2

From Booth *et al*. (1996)

[a] Percentages in columns may not add up to 100% as values were rounded to the nearest 0.1.

The Food and Drug Administration (1999) requires that all infant formulae sold in the USA contain a minimum of 4 μg/100 kcal (0.2 mg/kg) vitamin K; and that any vitamin K added should be in the form of phylloquinone.

Menadione is listed in the Austrian, Belgian, British, Dutch, European, French, German, International, Italian, Portuguese, Swiss and US pharmacopoieas, and menadione sodium bisulfite is listed in the Belgian, International, Swiss and US pharmacopoeias (Royal Pharmaceutical Society of Great Britain, 1999; Swiss Pharmaceutical Society, 1999). Menadiol sodium phosphate is listed in the British, Czech Republic and US pharmacopoeias (Swiss Pharmaceutical Society, 1999).

2. Studies of Cancer in Humans

The association between childhood cancer and vitamin K administered during the perinatal period with a view to preventing haemorrhagic disease of the newborn has been investigated in a number of studies (summarized in Table 5). The prophylactic use of vitamin K in newborns has varied with time, geographical location and among hospitals within countries. Some hospitals during some periods have had a selective policy based on the indications low birth weight, prematurity and operative delivery.

Table 4. Estimated and recommended mean dietary intakes of phylloquinone in the USA, stratified by age and sex

Population group	Phylloquinone intake (μg/day)	
	Estimate[a]	Recommended
Infants		
6-month-old infants	77	10
Children		
2-year-old children	24	15
6-year-old children	46	20
10-year-old children	45	30
14–16-year-old girls	52	45–55
14–16-year-old boys	64	45–65
Younger adults		
25–30-year-old women	59	65
25–30-year-old men	66	80
40–45-year-old women	71	65
40–45-year-old men	86	80
Older adults		
60–65-year-old women	76	65
60–65-year-old men	80	80
> 70-year-old women	82	65
> 70-year-old men	80	80

From Booth *et al.* (1996)
[a] From Total Diet Study

The hypothesis that vitamin K might be a risk factor for childhood cancer was generated on the basis of the results of a cohort study of 16 193 infants delivered in Great Britain in one week of April 1970, who were followed up at ages five and 10. The 33 cases included in the study were in patients who had died from cancer or were identified through cancer registration as having a cancer diagnosed before the age of 10. An unexpected statistically significant association was found between childhood cancer and administration of any drug during the first week of life (Golding *et al.*, 1990), and 16 of the 18 patients who had received drugs during the first week of life had received vitamin K. Within the cohort, a comparison was made between the 33 cases and 99 controls matched with the cases for the age of the mother at the time of the birth of the child, parity, social class, marital status at delivery and whether the birth was single or multiple. Statistically significant associations were identified not only with drug administration during the first week of life, but also with antenatal X-rays, antenatal smoking, non-term delivery and use of pethidine or pethilorfan (a pethidine-containing drug) during labour. Only two of the 33 cases had fewer than two of these risk factors, whereas

Table 5. Studies on childhood cancer and vitamin K administered during the perinatal period

Area and period of birth of children, period of diagnosis, reference	Age group	Type of preparation containing vitamin K	Method of determining route of administration	Route of administration	Prevalence of exposure to vitamin K in controls (%)	Group or subgroup	Total no. of cases	Total no. of controls	RR (95% CI)	Matching variables	Adjustment variables
Great Britain; birth, 1970; diagnosis, 1970–80 (Golding *et al.*, 1990)	5 and 10 years	NR	NR	Oral, intravenous, intramuscular	31.2[a] (for drug to neonate) (28.1 for vitamin K to neonate)	All cancers	33	99 (96 with data on drug intake)	2.6 (1.3–5.2)[a] (drug to neonate)	Maternal age, parity, social class, marital status, multiplicity	Social class, smoking during pregnancy, X-ray in pregnancy, term delivery and pethidine in labour
Case–control studies											
United Kingdom, Bristol; birth, 1965–87; diagnosis, 1971–91 (Golding *et al.*, 1992)	0–14 years[b]	Konakion[c]	Recorded in medical records or imputed on the basis of year of birth, type of delivery and whether or not infant admitted to special care Recorded in medical records	Intramuscular[d] Oral Intramuscular[d] Intramuscular[d]	40.6[e] 35.1[e] NR	All cancers Leukaemia Cancers other than leukaemia All cancers	180 [g] [i] NR	544 544 544 NR	2.2 (1.1–4.4)[f] 1.2 (0.5–2.7)[f] 2.7 (1.3–5.2)[h] 1.7 (1.0–2.8)[h] 2.0 (1.2–3.3)[h] (route of administration clearly stated)		Hospital and year of delivery
USA, multicentre; birth, 1959–66; diagnosis, 1959–66 (Klebanoff *et al.*, 1993)	1 day–8 years	Aquamephyton Konakion[k]	Review of records prospectively completed by labour and delivery room observers	Intramuscular[j]	71.2	All cancers Leukaemia Cancers other than leukaemia	44 15 29	226 NR NR	0.84 (0.41–1.7)[f] 0.47 (0.14–1.6)[f] 1.1 (0.45–2.6)[f]	Follow-up time	

Table 5 (contd)

Area and period of birth of children, period of diagnosis, reference	Age group	Type of preparation containing vitamin K	Method of determining route of administration	Route of administration	Prevalence of exposure to vitamin K in controls (%)	Group or subgroup	Total no. of cases	Total no. of controls	RR (95% CI)	Matching variables	Adjustment variables
Germany, Lower Saxony; birth, 1975–93; diagnosis, 1988–93 (von Kries *et al.*, 1996)	30 days–15 years	Konakion[c]	Determined from medical records	Intramuscular or subcutaneous	61.4	Leukaemia, brain tumours, nephroblastoma, neuroblastoma and rhabdomyosarcoma	272	334	1.0 (0.74–1.5)[h]	Sex, date of birth, locality or state	Type of region (urban or rural), social class and prematurity
						Leukaemia	136	334	1.0 (0.64–1.5)[h]		
						Nephroblastoma, neuroblastoma, rhabdomyosarcoma, CNS tumours	136	334	1.2 (0.77–1.8)[h]		
Northern England; birth, 1960–91; diagnosis, 1968–92 (Parker *et al.*, 1998)	3 months–14 years	Konakion[c]	Determined from medical records (obtained from case notes)	Intramuscular	NR	All cancers	438	NR	0.96 (0.67–1.4)		
						All cancers except ALL	306	NR	0.83 (0.54–1.3)		
						All ALL	132	NR	1.4 (0.71–1.7)		
						ALL diagnosed at 1–6 years	94	NR	2.3 (0.98–5.2)		
			Determined from medical records, route imputed from hospital records if not recorded	Intramuscular	NR	All cancers	664	3442	0.89 (0.69–1.15)		
						All cancers except ALL	457	NR	0.79 (0.59–1.1)		
						All ALL	207	NR	1.2 (0.75–1.9)		
						ALL diagnosed at 1–6 years	144	NR	1.8 (1.0–3.2)		

Table 5 (contd)

Area and period of birth of children, period of diagnosis, reference	Age group	Type of preparation containing vitamin K	Method of determining route of administration	Route of administration	Prevalence of exposure to vitamin K in controls (%)	Group or subgroup	Total no. of cases	Total no. of controls	RR (95% CI)	Matching variables	Adjustment variables
United Kingdom, Scotland; birth, 1976–94; diagnosis, 1991–94 (McKinney *et al.*, 1998)	0–14 years	Konakion[c]	Determined from medical records, route imputed from hospital records if not recorded	Intramuscular (recorded) Intramuscular (imputed)	48.9 51.0 36.0 48.2 51.5 59.5 62.4 50.0 59.6 59.4	Leukaemia ALL ALL diagnosed at 1–6 years Lymphoma CNS tumours Other solid tumours Leukaemia ALL ALL diagnosed at 1–6 years Lymphoma CNS tumours Other solid tumours	150 129 90 46 79 142 150 129 NR 46 79 142	284 247 174 86 141 266 284 247 NR 86 141 266	1.2 (0.77–2.0) 1.2 (0.70–2.0) 1.2 (0.62–2.2) 1.7 (0.59–5.0) 1.1 (0.55–2.1) 0.6 (0.36–1.0) 1.3 (0.78–2.1) 1.1 (0.65–1.9) 1.3 (0.70–2.5) 1.6 (0.49–4.9) 1.0 (0.49–2.2) 1.0 (0.61–1.8)	Sex, date of birth, health board of residence	Social class and type of delivery
United Kingdom, 16 hospitals with large maternity units, 1969–86; born 1968 onwards; Cardiff births survey (3 more hospitals) (Passmore *et al.*, 1998a)	1–14 years	Konakion[c]	Determined from medical records, route imputed from hospital records if not recorded	Intramuscular	NR	All cancers Leukaemia All cancers except leukaemia ALL ALL diagnosed at 1–5 years	597	NR	1.4 (1.0–2.1) 1.5 (0.82–2.9) 1.4 (0.88–2.2) 1.7 (0.89–3.3) 1.0 (0.48–2.2)	Sex, month of birth, hospital of birth	

Table 5 (contd)

RR, relative risk; CI, confidence interval; NR, not reported; ALL, acute lymphoblastic leukaemia; CNS, central nervous system

[a] Drug given to neonate; 16 of 18 case patients and 27 of 30 controls who received drugs were given vitamin K.

[b] Passmore *et al.* (1998a)

[c] Konakion contains phenol, Cremophor EL (polyoxyl 35 castor oil), propylene glycol and phytomenadione (vitamin K_1) (see Table 6).

[d] The authors reported a few instances of intravenous administration among those who received vitamin K intramuscularly.

[e] Calculated for 507 controls with information also on type of delivery and admission to special care. The frequency of intramuscularly administered vitamin K in one hospital was 59.4%, in the other 23.9%; that of oral administration was 13.8% and 54.1% respectively.

[f] Reference category is no vitamin K.

[g] 74 cases of leukaemia were ascertained; it is not stated how many were included in the analysis.

[h] Reference category is either no vitamin K or vitamin K administered only by the oral route.

[i] 143 cases of childhood cancer other than leukaemia were ascertained; it is not stated how many were included in the analysis.

[j] One child in the sample received vitamin K orally.

[k] When Konakion was used, the preparation contained polysorbate-80 as emulsifier (rather than Cremophor EL), phenol and propylene glycol and phytomenadione (Rennie & Kelsall, 1994) (see Table 6).

45/99 (47%) of the controls had either no or only one risk factor. All but four of the mothers of the 16 cases who had received vitamin K had received pethidine or pethilorfan during labour. In a logistic regression analysis carried out on the whole cohort, in which social class was included with the other variables already mentioned, the relative risk associated with drug administration during the first week of life was 2.6 (95% confidence interval [CI], 1.3–5.2). [The Working Group noted that Cremophor EL was the only emulsifier used in Great Britain for vitamin K injection at the time (Draper & Stiller, 1992; Rennie & Kelsall, 1994; see Table 6.]

Table 6. Brands of vitamin K and vehicle used in different countries

	Konakion[a]		Aquamephyton[a] (USA)
	Germany/United Kingdom/Sweden	USA	
Antimicrobial agent			
Phenol	+	+	
Emulsifier			
Cremophor EL	+		
Polysorbate-80		+	+
Propylene glycol	+	+	
Benzyl alcohol			+

From Rennie & Kelsall (1994)
[a] Trade name for phytomenadione

2.1 Case–control studies

In most of the case–control studies, the reference group comprised infants who had not received vitamin K and/or those who had received it orally. This combination is justified because the plasma concentrations after intramuscular administration are more than 10 times higher than those after oral administration (McNinch *et al.*, 1985).

In a second study (Golding *et al.*, 1992), 195 children with cancer diagnosed in the period 1971–91 who had been born at two major maternity hospitals in Bristol, England, in the period 1965–87 were compared with 558 controls identified from the delivery books of these hospitals. The cases were ascertained from the oncology register of the regional paediatric oncology unit and from the National Registry of Children's Tumours. The basic method of control selection was to select every 300th birth in each year in each hospital. In view of the observation that the immediate effects of identical oral and intramuscular doses of vitamin K are different, the investigators sought to distinguish the effects of administration by the two routes. When the route of vitamin K administration

was not recorded in the neonatal notes, a route was imputed on the basis of year of birth, the type of delivery and whether or not the infant was admitted to special care; the imputed route was identified in the absence of knowledge of case or control status. On the basis of 180 cases (92% of those for which notes were available) and 544 controls (98% of those for which notes were available), the relative risk (adjusted for hospital and year of delivery) for childhood cancer associated with intramuscular vitamin K was 2.2 (95% CI, 1.1–4.4) when compared with no vitamin K and 1.2 (95% CI, 0.5–2.7) for oral vitamin K. In view of the absence of an association with oral vitamin K in these data, the authors conducted a subsequent analysis in which the reference group was defined to include infants who had not received vitamin K or who had received it orally. The relative risk for leukaemia associated with intramuscular vitamin K was 2.7 (95% CI, 1.3–5.2) and that for other types of childhood cancer was 1.7 (95% CI, 1.0–2.8). Thus, there was no clear difference in the association by type of childhood cancer. When the analysis was confined to records in which the route was clearly stated, the odds ratio for all childhood cancer was 2.0 (95% CI, 1.2–3.3). These results could not be accounted for by other factors associated with the administration of intramuscular vitamin K, such as type of delivery or admission to a special care unit. Data were collected on 319 variables for all controls and for 111 cases of cancer ascertained from the oncology register of the regional paediatric oncology unit; these data were not obtained for the remaining 84 cancer cases. Of these variables, the presence of rubella antibody, resuscitation by intermittent positive pressure and paediatric estimate of gestation were statistically significant at the 1% level, which is what would be expected by chance. Adjustment for these and other variables reported to be associated with childhood cancer or known to be indicators for administering intramuscular vitamin K had little effect on the odds ratio for childhood cancer associated with vitamin K. Nineteen of the cases were diagnosed in the first year of life, and the possibility was considered that these cancers might have been present before the child was born and could therefore not have been initiated by an injection of vitamin K; however, the association persisted after exclusion of these 19 cases from the analysis. When the analysis was restricted to subjects who would have been followed for at least 10 years, by considering only those born in the period 1971–80, the relative risk for all childhood cancer associated with intramuscular vitamin K was 1.9 (95% CI, 1.1–3.4), similar to that assessed for all subjects. [The Working Group noted, as acknowledged by the authors, a large number of instances in which the information on potentially confounding variables was not available, for example on smoking in pregnancy. Medical records are not necessarily reliable sources of information about pregnancy and childbirth (Hewson & Bennett, 1987; Oakley *et al.*, 1990), and this, together with the fact that potential confounding was assessed only for a subset of cases, constitutes a limitation of the study. The relationship between the type of delivery and intramuscular administration of vitamin K differed markedly between the two maternity hospitals in Bristol in which the case and control subjects in the study had been born (Carstensen, 1992; Draper & Stiller, 1992). The association with childhood cancer is largely accounted for by data from one of the hospitals in which virtually

all of the control infants who received intramuscular vitamin K had been born by an assisted delivery. This raises the issue as to whether bias arose in control selection in that hospital.]

A study in the USA was reported by Klebanoff *et al.* (1993) which was based on follow-up to the age of seven or eight years of 54 795 liveborn children of women enrolled between 1959 and 1966 in 12 centres contributing to the National Collaborative Perinatal Project. Neonates whose cancer was diagnosed or strongly suspected during the first day of life were excluded because vitamin K could not have been a factor in those cases. Vitamin K was administered in the delivery room or the nursery, and information about the administration was recorded with other events during and after delivery by observers who were not involved in the clinical care of the mother or the infant. Cancer was diagnosed in 48 of 54 795 liveborn children after the first day of life. For each case, five controls were selected and matched with the index case on length of follow-up. In spite of the prospective recording by the observers, the data on vitamin K administration were not recorded unambiguously for 43 infants; a review of hospital records without knowledge of case or control status resulted in data for 25 (58%) of these. The exposure status was unknown for four case children. The relative risk for all childhood cancer associated with vitamin K was 0.84 (95% CI, 0.41–1.7), and that for leukaemia was 0.47 (95% CI, 0.14–1.6; based on 15 cases). In the USA, only two brands, Aquamephyton and Konakion, have been approved for use (see Table 6). Konakion in the USA contains polysorbate-80 rather than Cremophor EL as an emulsifier and phenol as an antrimicrobial agent. In the study of Klebanoff *et al.* (1993), the relative risk for total childhood cancer associated with the two brands together was 0.6, whereas that for children who had received the phenol-containing preparation alone was 0.7. In this study, only one child had received vitamin K orally.

von Kries *et al.* (1996) carried out a case–control study of children born in 162 obstetric hospitals in Lower Saxony (Germany) during the period 1975–93 when only one vitamin K preparation, Konakion, the same as that used in the United Kingdom, was licensed for neonatal vitamin K prophylaxis. Of a total of 218 children with leukaemia identified as eligible, information on vitamin K prophylaxis was obtained for 136 (62%). For each leukaemia case, one control was selected from the municipality where the patient lived at the time of diagnosis (local control), and a second one (state control) from a municipality selected at random in Lower Saxony by means of a population-weighted sampling scheme. These controls were matched with cases by sex and date of birth. Case and control families were contacted initially by being sent a questionnaire. If a control family refused to collaborate in the study or did not return the questionnaire within three months, another control family was invited; control families that returned the questionnaire after more than three months were also included. Thus, a total of 305 local and 308 state controls were invited to participate. Information on vitamin K prophylaxis was obtained for 174 (57%) of the local controls and 160 (52%) of the state controls. As the study was performed as part of a population-based case–control study to explore possible causes of childhood leukaemia in Lower

Saxony, a third control group for the leukaemia study was identified which comprised cases of brain tumours, nephroblastoma, neuroblastoma and rhabdomyosarcoma. No population-based controls were selected for these cases, but they were used as additional cases in the study of vitamin K. Of a total of 246 potentially eligible cases of this type, information on vitamin K prophylaxis was obtained for 136 (55%). Data on vitamin K prophylaxis were abstracted from the birth report with no knowledge of the case or control status of each child. Information on the dose and route of vitamin K prophylaxis was obtained from the birth record or in the delivery book for 72% of the 272 cases of leukaemia and other cancers and 64% of the 334 controls. When this information was not available, the index child was assumed to have had the same exposure to vitamin K as the child nearest to the index infant in the delivery book with the same route of delivery and same perinatal morbidity (nine cases and six controls). When this could not be established, staff who worked in the delivery unit at the time when the index child was born were asked what kind of vitamin K prophylaxis the index infant would have received, given the birth weight and route of delivery (63 cases and 109 controls). Finally, similar information was sought from medical staff who did not work in the delivery unit at the time the index child was born (four cases and four controls). In the comparison with local controls (n = 107), the risk for leukaemia (n = 107) associated with intramuscular or subcutaneous administration of vitamin K relative to that for oral or no vitamin K prophylaxis was 1.2 (95% CI, 0.68–2.25). In the comparison with state controls (n = 160; leukaemia cases = 136), the relative risk was 0.82 (95% CI, 0.50–1.4). When the control groups were pooled (n = 334), the relative risk was close to unity (136 leukaemia cases), and the relative risk for brain tumours, nephroblastoma, neuroblastoma and rhabdomyosarcoma combined (n = 136) associated with vitamin K prophylaxis was 1.2 (95% CI, 0.77–1.8). When the analyses were repeated for subjects for whom vitamin K prophylaxis had been documented in birth records or delivery books, the results were almost unchanged, except in the comparison of leukaemia cases with local controls, which gave a relative risk of 2.0 (95% CI, 0.69–6.0). When the analyses were repeated for parenteral prophylaxis versus no prophylaxis, most of the relative risks were slightly decreased. The risk of the subgroup of cases of leukaemia in children aged 1–6 years was analysed as this was considered to be a relatively homogeneous subgroup, most of the cases having common acute lymphoblastic leukaemia. [The Working Group noted that it is not clear whether the decision to make this subgroup analysis was specified in the original study protocol or was made *post hoc*.] The risk relative to both control groups combined was 1.2 (95% CI, 0.69–2.15), in the comparison with state controls it was 0.99 (95% CI, 0.52–1.9) and in the comparison with local controls it was 2.3 (95% CI, 0.94–5.5). There was no difference between cases and controls in the source of information on vitamin K prophylaxis. The increased relative risk in the comparison with local controls could not be explained by any of the potential confounders. It would be expected that the policy of administration of vitamin K would be more likely to be similar for cases and local controls than for cases and state controls. Therefore, the

relative risk would be expected to be closer to unity in the comparison between cases and local controls than in the other comparison, whereas the opposite was observed. The non-significantly increased risk relative to local controls may be a chance result in subgroup analysis with multiple testing, as acknowledged by the authors.

In a case–control study of childhood leukaemia based on births in three hospitals in England (Cambridge, Oxford and Reading), no association with intramuscular vitamin K, either as determined from hospital records (91 cases, 171 controls) or as imputed from hospital policy (132 cases, 264 controls), was found. In addition, no association was found specifically for acute lymphoblastic leukaemia (Ansell *et al.* 1996). Subsequently, Roman *et al.* (1997) reported a more detailed analysis of data on leukaemia and non-Hodgkin lymphoma diagnosed before the age of 30 years in subjects whose obstetric records were stored in the same three hospitals. Ninety-two per cent (132/143) of the cases of leukaemia were diagnosed at age 14 or less; these cases and their controls were included in the report of Ansell *et al.* (1996). There was no association between leukaemia and intramuscular vitamin K administration either recorded in the notes (relative risk, 1.2; 95% CI, 0.7–2.1) or imputed from information about hospital policy (relative risk, 1.2; 95% CI, 0.5–2.4). In view of the finding of von Kries *et al.* (1996), acute lymphoblastic leukaemia diagnosed between the ages of 1–6 years was considered; the relative risk associated with recorded administration (based on hospital notes) was 0.6 (95% CI, 0.3–1.4), and that based on hospital policy was again 0.6 (95% CI, 0.2–1.7).

Parker *et al.* (1998) identified 1432 children born in northern England between 1960 and 1991 from the regional Children's Malignant Disease Registry, in whom cancer was diagnosed in 1968–92 when they were aged between three months and 14 years while still resident in the region. The birth records of 701 of these children could not be traced, usually because the maternity unit had retained only its most recent records or because the unit had closed and the records could not be located. Thirty children who had been given vitamin K orally at birth and 16 cases in multiple births were excluded. The controls were selected by taking the fourth, eighth and 12th birth before and after the index birth from birth or admission registers for the hospital of birth of the index child. Towards the end of the study, the number of controls per case was reduced from six to three because of time constraints. When the birth notes for control children could not be located, or when the child selected was found to be on the Malignant Disease Register, the next possible control was selected. The fact of intramuscular administration of vitamin K or non-administration of vitamin K was recorded in the maternity unit records for 438 of 685 cases (case notes). [The Working Group noted that the corresponding proportion for controls was not specified.] There was no association between intramuscular vitamin K administration and either all cancers or all cancers other than acute lymphoblastic leukaemia. The relative risk for acute lymphoblastic leukaemia associated with vitamin K administration based on case notes was 1.4 (95% CI, 0.71–1.7; 132 cases). Two secondary analyses were conducted to consider cases typical of the peak incidence of leukaemia in early childhood. When the 51 children in the case note

analysis who had T-cell leukaemia or for whom subtype characterization was not available were excluded, the relative risk for the 81 cases of non-T-cell lymphoblastic leukaemia was 1.8 (95% CI, 0.82–3.9). In an analysis of 94 children aged 1–6 years at diagnosis, the relative risk was 2.3 (95% CI, 0.98–5.2). In all of these analyses, adjusted relative risks were calculated separately for the specified potential confounding factors—sex, gestation, birth weight, opiates during labour, assisted delivery, signs of asphyxia at birth, admission to special care or neonatal blood transfusions. Except for adjustment for assisted delivery, admission to special care or opiate exposure in labour, none of these changed any of the relative risks by more than 10%. Adjustment for assisted delivery or admission to special care caused a larger rise in the relative risk. The relative risk for acute lymphoblastic leukaemia diagnosed at ages 1–6 was 2.4 (95% CI, 1.0–5.7) after adjustment for exposure to opiates and 3.6 (95% CI, 1.3–9.7) after adjustment for assisted delivery based on case note analysis. As in many of the other studies, information on hospital policy was obtained in order to impute exposure when this was unclear from medical records. This information was obtained by a research midwife and neonatal staff in each unit in the region and by a paediatrician from current and recently retired medical staff, and this independently obtained information was then cross-validated. When inconsistencies were identified, the case notes were sampled to determine what policy had actually been followed. This enabled a further 226 cases to be included at the analysis; 21 cases were excluded because the policy of the local unit could not be ascertained. The relative risks were similar to but somewhat lower than those in the analysis based exclusively on subjects for whom data on vitamin K exposure was obtained only from medical records. [The Working Group noted that it was unclear which hypotheses about subgroups had been pre-specified. Bias may have arisen from the fact that while a large proportion of cases had to be excluded there was a mechanism for adding controls when a control record was unobtainable. Availability of records might have associations with both perinatal health problems and subsequent development of childhood cancer.]

McKinney *et al.* (1998) carried out a case–control study on childhood cancer in Scotland using data abstracted from 76 hospital records. A total of 500 cases of cancer diagnosed in children aged 0–14 years during the period 1991–94 while resident in Scotland were identified. Controls matched on age, sex and health board of residence were randomly selected from among all eligible children registered for primary care within each health board. A total of 1338 eligible controls was identified. A total of 460 mothers of cases (92%) and 861 mothers of controls (64%) were interviewed, and medical notes were abstracted for 440 cases and 802 controls. The data set for statistical analysis was restricted to matched sets, and information was lost for 23 cases without matched controls and 25 controls without a matched case. Therefore, 417 cases and 777 controls were included in the matched case–control analysis. Vitamin K was recorded as given or definitely not given only when this was mentioned in the notes. Similarly, the route of administration was classified as intramuscular, oral or not recorded. None of the relative risks reported for leukaemias, acute lymphoblastic leukaemia, lymphomas,

central nervous system tumours or other solid tumours, either crude or adjusted for social class and type of delivery, was statistically significantly different from unity. The adjusted relative risk for leukaemia associated with vitamin K given intramuscularly (recorded) in the neonatal period was 1.2 (95% CI, 0.77–2.0) and that for acute lymphoblastic leukaemia was 1.2 (95% CI, 0.70–2.0). In view of the findings of Parker *et al.* (1998, see above), the subset of acute lymphoblastic leukaemia diagnosed in children aged 1–6 years (90 cases, 174 controls) was also analysed, and the adjusted relative risk was found to be 1.2 (95% CI, 0.62–2.2). As nothing about vitamin K had been written in the medical records for a substantial proportion of children (37% of cases and 35% of controls), the authors also sought to impute exposure on the basis of hospital policies. Information on the vitamin K policies of hospitals in which over 500 infants were delivered annually was validated by abstraction of a sample of medical records and through consultations with hospital pharmacies and senior labour room midwives. For 100 (24%) cases and 191 (25%) controls, no hospital policy was available for any imputation. The relative risks for the specific diagnostic categories associated with intramuscular vitamin K administration in the neonatal period either as recorded in medical records or imputed from hospital policy were very similar to those calculated for subjects for whom only data from medical records were included. The adjusted relative risk for leukaemia was 1.3 (95% CI, 0.78–2.1), that for acute lymphoblastic leukaemia was 1.1 (95% CI, 0.65–1.9) and that for acute lymphoblastic leukaemia in children aged 1–6 years was 1.3 (95% CI, 0.70–2.5). Very few subjects were recorded as having or imputed to have been given vitamin K orally in the neonatal period (12 cases, 2.9%; and 33 controls, 4.3%).

Passmore *et al.* (1998a) identified cases of childhood cancer diagnosed at ages up to 14 years in persons who were resident in Great Britain and had been born in 16 hospitals with large maternity units in 1968 or later and diagnosed by the end of 1986 from the National Registry of Childhood Tumours (excluding retinoblastoma, Down syndrome or neurofibromatosis). The 16 hospitals were selected on the basis of a survey which showed that they had a selective policy for the use of vitamin K prophylaxis. Of 1092 cases initially identified as born in these hospitals, 523 were born in the years for which a policy was known and for whom the medical records were found. Four controls matched on sex, month of birth and hospital of birth were selected randomly from these registers. Medical records departments were asked to locate the records for each case and for one control. Initially, two out of each of the four potentially eligible controls were selected randomly for location by the medical records department. If the records department was unable to locate the notes of either of these, details were supplied of the other two. Controls with illegible records, twins, stillbirths and neonatal deaths were excluded. In addition, infants with severe neural tube defects or a birth weight of less than 1000 g were excluded, as they were unlikely to have survived to the age at which the case patient developed cancer. For these, an alternative control was selected by using the next suitable birth in the hospital birth register. [The numbers of control replacements were not specified.] A second group of cases from the same period was chosen from

records of the National Registry of Childhood Tumours in order to identify cases of cancer among children included in a survey of more than 100 000 births in South Glamorgan, Wales. For each case, two controls matched for sex, month of birth and hospital were selected, applying the same set of exclusions. Medical records were sought for all cases and controls, and information on vitamin K administration taken from these records was supplemented by data from the birth survey, which was available for most but not all of the period of study. This added three further hospitals to the study, all of which had selective policies of vitamin K administration, and 74 cases. In the combined data (16 maternity units in England and Wales and the three hospitals included in the survey in South Glamorgan), the relative risk for childhood cancer of all types associated with intramuscular vitamin K administration was 1.4 (95% CI, 1.0–2.1). In the data for the 16 maternity units in England and Wales, the relative risk was 1.2 (95% CI, 0.77–1.9), while in the data from South Glamorgan, the relative risk was 2.1 (95% CI, 1.1–4.1). For the combined data and for the data from South Glamorgan, mode of delivery (forceps, vacuum extraction, breech or caesarean) was a statistically significant confounding variable, and adjustment for this reduced the relative risks to 1.1 for the combined data and 1.3 for the South Glamorgan data. In the combined data, the relative risk for leukaemia was 1.5 (95% CI, 0.82–2.85), that for acute lymphoblastic leukaemia was 1.7 (95% CI, 0.89–3.3) and that for acute lymphoblastic leukaemia diagnosed at ages 1–5 years was 1.0 (95% CI, 0.48–2.2). Again, adjustment for mode of delivery reduced the relative risks. [The Working Group noted that the substantially lower relative risk for the 1–5 year-old group than for all ages combined implies that the effect for children of other ages is higher than that for this group, in contrast to the observations of von Kries *et al.* (1996) and Parker *et al.* (1998).] The relative risk for non-leukaemia cancers was 1.4 (95% CI, 0.88–2.2) in the combined data and 2.4 (95% CI, 1.1–5.4) in the data from South Glamorgan. In the South Glamorgan data, none of the potential confounders that were adjusted for reduced the magnitude of the relative risk. [The Working Group noted that in the absence of an effect in the data from the 16 maternity units in England and Wales, the South Glamorgan finding may reflect an unidentified bias or be a chance finding.]

[The Working Group noted that in the subgroup analyses of acute lymphoblastic leukaemia diagnosed at 1–6 years carried out by Parker *et al.* (1998) and 1–5 years by Passmore *et al.* (1998a), adjustment for mode of delivery had contrasting effects. In the study of Passmore *et al.* it attenuated the relative risk associated with vitamin K, while in the study of Parker *et al.* the relative risk was increased.]

2.2 Ecological studies

These studies are summarized in Table 7.

Ekelund *et al.* (1993) investigated the association between childhood cancer and intramuscular administration of vitamin K in a study in Sweden based on linkage of the medical birth registry to the national cancer registry. The study was restricted to full-

Table 7. Ecological studies on childhood cancer and vitamin K administered intramuscularly during the perinatal period as Konakian[a]

Area and period of birth of children, period of diagnosis, reference	Age group	Method of determining route of administration	Prevalence of exposure in all children (%)	Group or subgroup	Total no. of cases	No. of patients	RR (95% CI)	Reference category
Sweden; full-term non-instrumental deliveries;		Imputed on the basis of hospital policy				Nos of patients given vitamin K intramuscularly and orally		Vitamin K orally
birth, 1973-89 (follow-up, 1992);	30 days–17 years		78.4	All cancers	2287	1 357 734	1.0 (0.88–1.2)[b]	
				Leukaemia	708	1 357 734	0.90 (0.70–1.2)[b]	
birth, 1982–89 (Ekelund *et al.*, 1993)	30 days–9 years		66.2	All cancers	722	655 454	1.1 (0.88–1.4)	
				Leukaemia	250	655 454	1.2 (0.69–2.1)	
Denmark, 1945–54, 1975–84; (Olsen *et al.*, 1994)	1–12 years	Imputed from recommended practice as: no vitamin K for births 1945–54; intramuscular administration for births 1975–84	NR		NR	No. of patients given vitamin K intramuscularly and not given vitamin K		No vitamin K
				All cancers		1 421 808	1.3 (1.2–1.4)	
				Leukaemia		1 421 808	1.0 (0.9–1.1) at age 13	

RR, relative risk; CI, confidence interval; NR, not reported

[a] Konakion contains phenol, Cremophor EL (polyoxyl 35 castor oil), propylene glycol and phytomenadione (see Table 6).

[b] Adjusted for year of birth

term infants (gestation, 37–42 weeks) who had survived and who were born in 1973–89 after a delivery without use of forceps or vacuum extraction. The infants were followed up to 1 January 1992. Cancers diagnosed within 30 days of birth were regarded as congenital and were excluded from the analysis. Routines for administration of vitamin K were obtained from all 95 maternity hospitals and validated for a subset of 102 children with cancer and 100 control children randomly selected from among those who, according to the information on routine exposure, received intramuscular vitamin K, and 94 children with cancer and 100 control children from among those who should have received oral vitamin K. The doses of vitamin K given in Sweden were similar to those given in the United Kingdom, and the same preparation was used (phylloquinone, Konakion, see Table 6). When the method of administration of vitamin K was recorded, it agreed with the stated routine method of administration in 92% of the 235 cases for which individual information could be found. The relative risk for all childhood cancer associated with a hospital policy of intramuscular administration of vitamin K as compared with oral administration was 1.0 (95% CI, 0.88–1.2, after stratification for year of birth). The relative risk for leukaemia was 0.90 (95% CI, 0.70–1.2).

Olsen *et al.* (1994) compared the cumulative risk of childhood cancer among children aged 1–15 years who were born during the period 1945–54 (n = 835 430), in which no vitamin K was administered, those aged 1–15 years born during the period 1960–69 (n = 797 472), in which pregnant women received oral vitamin K, and those aged 1–13 years born during the period 1975–84 (n = 586 378), in which virtually all newborns received vitamin K intramuscularly. There was a small increase in risk for all tumour types combined, due mainly to lymphoma in boys and neuroblastoma in boys and girls. There was no trend for childhood leukaemia. The preparation was the same as that used in the United Kingdom (Draper & McNinch, 1994).

In addition to the case–control study in northern England described above, Parker *et al.* (1998) compared the incidence of acute lymphoblastic leukaemia diagnosed in children aged up to 14 years who were born in hospital units in which all infants received vitamin K, with those born in units where less than a third received this prophylaxis. As described above, information on hospital policy was obtained separately and independently by two people and then cross-validated. In units with a policy of selective prophylaxis, less than 30% of infants received intramuscular vitamin K at birth, while in units offering universal prophylaxis, sampling of case notes showed that more than 95% of babies received vitamin K. The risk for acute lymphoblastic leukaemia in children born in hospitals with a policy of universal prophylaxis relative to those born in hospitals with a policy of selective prophylaxis was 0.95 (95% CI, 0.78–1.2). The relative risk of the subgroup diagnosed at 1–6 years was 1.05 (95% CI, 0.82–1.35). [The Working Group noted that the cases included in this analysis overlapped with those included in the case–control study, so that the results are not independent].

Passmore *et al.* (1998b) carried out a similar comparison of cancers of all types other than retinoblastoma or associated with Down syndrome or neurofibromatosis diagnosed in children aged 1–14 years who were born in 94 hospital units in Great

Britain. Information on hospital policy for neonatal vitamin K was obtained during the case–control studies of Passmore *et. al.* (1998a) and Ansell *et al.* (1996), described above, for 30 hospitals in Scotland from members of the Scottish Neonatal Network and from paediatricians for 41 of a further 80 hospitals in England and Wales in which more than 25 children who subsequently developed cancer had been born in the period 1968–85. The observed numbers of cases in hospitals with universal and selective policies were compared with the numbers expected on the basis of national rates. Separate analyses were carried out for births in hospitals that followed one policy throughout the period of study and births in hospitals in which the policy changed during the period of study. A large number of observed:expected ratios were calculated. The ratio for all cancers was 0.97, that for leukaemia at 1–14 years was 1.03, and that for acute lymphoblastic leukaemia at 1–5 years was 1.01 for hospitals with a consistent, non-selective policy. The ratio tended to be smaller in hospitals with a selective policy than in those offering universal prophylaxis. The only statistically significant ($p < 0.05$, two-tailed test) departure from unity indicated a lower risk for cancer other than leukaemia among children born in hospitals offering universal prophylaxis that those born in hospitals consistently offering selective prophylaxis in Scotland. [The Working Group noted that the cases included in this analysis overlapped with those in the case–control studies of Parker *et al.* (1998) and Ansell *et al.* (1996), so that the results are not independent.]

3. Studies of Cancer in Experimental Animals

No reports of studies specifically designed to investigate the carcinogenicity of vitamin K substances were available to the Working Group. One study on the initiating effects of menadione in an assay of liver foci in rats was available (Denda *et al*., 1991) but could not be evaluated owing to methodological limitations.

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

The studies summarized in this section should be considered in the light of the differences between naturally occurring forms of vitamin K that have a lipophilic side-chain at the 3-position of the 2-methyl-1,4-naphthoquinone (menadione) ring structure (phylloquinone and menaquinones) and the synthetic forms which lack this side-chain (menadione and its water-soluble derivatives). Lack of this side-chain results in profound differences in the absorption, tissue distribution and metabolism of natural K vitamins. Importantly, the lack of a lipophilic side-chain is the reason for the increased chemical reactivity and greater toxicity of menadione when compared with

phylloquinone and menaquinones. In the strict sense, menadione is a provitamin K, because it is biologically active for the synthesis of vitamin K-dependent proteins only after conversion to the naturally occurring menaquinone-4 (four prenyl units) *in vivo*.

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

(*a*) *Intestinal absorption and plasma transport in adults*

The major dietary form of vitamin K is phylloquinone (Shearer *et al.*, 1996). It is absorbed chemically unchanged from the proximal intestine after solubilization into mixed micelles composed of bile salts and the products of pancreatic lipolysis. In healthy adults, the efficiency of absorption of phylloquinone in its free form is about 80% (Shearer *et al.*, 1974), but the efficiency of absorption from green leafy vegetables such as spinach is < 10% (Gijsbers *et al.*, 1996).

Within the intestinal mucosa, phylloquinone is incorporated into chylomicrons, is secreted into the lymph and enters the blood via the lacteals (Shearer *et al.*, 1970, 1974). After a phylloquinone-containing meal, the plasma concentration peaks between 3 and 6 h (Shearer *et al.*, 1970; Lamon-Fava *et al.*, 1998). Once in the circulation, phylloquinone is rapidly cleared at a rate consistent with its continuing association with chylomicrons and the chylomicron remnants that are produced by lipoprotein lipase hydrolysis at the surface of capillary endothelial cells. During the postprandial phase and after an overnight fast, more than half of the circulating phylloquinone is associated with triglyceride-rich lipoproteins, and the remainder is carried by low-density and high-density lipoproteins (Kohlmeier *et al.*, 1996; Lamon-Fava *et al.*, 1998). Although phylloquinone is the major circulating form of vitamin K, menaquinone-7 is present in plasma at lower concentrations and has a similar lipoprotein distribution to phylloquinone. While phylloquinone in blood is derived exclusively from the diet, it is not known what proportion of circulating menaquinones such as menaquinone-7 derives from the diet or the intestinal flora (Shearer *et al.*, 1996).

(*b*) *Plasma pharmacokinetics of phylloquinone in adults*

The plasma clearance of an intravenous dose of 1 mg [^{3}H]phylloquinone during the first 6 h resolved approximately into two exponential functions, the first with a half-time of 20–24 min and the second with a half-time of 121–150 min (Shearer *et al.*, 1972). The curves for clearance up to 12 h after an intravenous injection of a 10-mg dose of phylloquinone (Konakion MM) were similar to those after 1 mg and were consistent with a two-compartment (sometimes three-compartment) model in which the log-linear terminal phase over 3–12 h had a half-time of about 3 h (Soedirman *et al.*, 1996). A gradual slowing of the clearance rate was seen after the first 6 h (Shearer *et al.*, 1972, 1974), as was also found in a study of the clearance of pharmacological doses of 10–60 mg by Øie *et al.* (1988), who reported that the log-

linear terminal elimination phase was not reached before 8–12 h and that the average half-time was 14 h (range, 8–22 h). This slowing of the clearance rate may be explained by the complexity of the plasma transport of phylloquinone, in which the proportion of phylloquinone associated with low-density and high-density lipoproteins increases progressively (Lamon-Fava *et al.*, 1998).

The plasma disposition of oral doses of 5–60 mg phylloquinone (Konakion or AquaMephyton) is similar to that found after a more physiological dose (≤ 1 mg), with peak plasma concentrations at 4–6 h followed by a rapid clearance phase (Shearer *et al.*, 1974; Park *et al.*, 1984; Øie *et al.*, 1988; Hagstrom *et al.*, 1995). After an oral dose of 10 or 50 mg Konakion, the plasma concentration declined from the peak absorptive level at a similar log-linear rate as that seen after intravenous administration, with a terminal half-time of about 2 h for measurements up to 9–12 h (Park *et al.*, 1984). The absorption of oral preparations of phylloquinone shows inter- and intra-individual variation and, for doses of Konakion ranging from 10 to 60 mg, the bioavailability was 10–63% (Park *et al.*, 1984) and 3.5–60% (Øie *et al.*, 1988).

The pharmacokinetics of phylloquinone after an intramuscular dose is completely different, showing sustained, slow release from the muscle site over many hours and marked inter-individual variation (Hagstrom *et al.*, 1995; Soedirman *et al.*, 1996). The pharmacokinetics may also be influenced by the solubilizing agent. The systemic availability of intramuscularly injected Konakion MM, which is a mixed-micellar solution of phylloquinone in natural solubilizers, the bile acid glycocholic acid and the phospholipid lecithin (Schubiger *et al.*, 1997; see Table 6), was irregular and < 65% in 20% of subjects (Soedirman *et al.*, 1996). After intramuscular injection of phylloquinone (AquaMephyton R), most of the substance was carried by low-density and high-density lipoproteins instead of by triglyceride-rich (very-low-density) lipoproteins as found after oral administration (Hagstrom *et al.*, 1995).

(*c*) *Plasma pharmacokinetics of phylloquinone in neonates*

The pharmacokinetics of phylloquinone during the early clearance phase up to 6 h in neonates (of low birth weight) after intravenous injection was very similar to that of adults (Shearer *et al.*, 1972), declining bi-exponentially with median half-times of 23 and 109 min (Sann *et al.*, 1985).

An early study of the plasma disposition of 1 mg Konakion given orally or intramuscularly at birth showed wide inter-individual differences during the first 24 h, especially after oral administration (McNinch *et al.*, 1985). The peak plasma concentration after an oral dose occurred after 4 h; the median concentration was 73 ng/mL, which fell to 23 ng/mL after 24 h. The plasma concentration after administration of 1 mg of Konakion intramuscularly exceeded those after oral administration at all times, and after 24 h the median was 444 ng/mL. Physiologically, these concentrations compare with adult endogenous levels of about 0.5 ng/mL (Shearer, 1992).

In a comparison of the plasma concentrations of Konakion and Konakion MM in exclusively breast-fed infants at 24 h and 4 and 24 days after a single oral dose of 2 mg

at birth (Schubiger *et al.*, 1997), the mixed-micellar Konakion MM preparation resulted in higher median concentrations at all times, suggesting greater bioavailability. The largest difference was seen after four days, with median concentrations of 41 ng/mL Konakion MM and 12 ng/mL Konakion. By 24 days, the concentrations in both groups were mainly within the adult physiological range (0.3–0.4 ng/mL). An earlier study by the same group (Schubiger *et al.*, 1993) had shown that a single oral dose of 3 mg Konakion MM resulted in higher plasma concentrations than a single dose of 1.5 mg of the same preparation given intramuscularly after four days. In this study, however, the plasma concentrations after 24 days were significantly higher after intramuscular injection, consistent with the hypothesis of the depot effect of intramuscular phylloquinone (Loughnan & McDougall, 1996; see also section 4.1.1(f)).

Stoeckel *et al*. (1996) pointed out that the terminal elimination plasma half-time of phylloquinone in neonates is probably longer than that in adults. They calculated from published studies that a realistic estimate of the terminal plasma half-time in neonates was 26–193 h (median, 76 h), as compared with 8–22 h (median, 14 h) in adults after intravenous administration (Øie *et al.*, 1988). This longer terminal half-time may reflect the poorly developed organ systems of neonates and a reduced capacity to metabolize and excrete vitamin K (Stoeckel *et al.*, 1996).

(*d*) *Plasma pharmacokinetics of menaquinone-4*

Oral preparations of menaquinone-4 are used in Japan for the prophylaxis of vitamin K deficiency bleeding. The plasma profile of an oral dose of this preparation in five-day-old infants appeared to be similar to that of phylloquinone; after a 4-mg dose, a peak concentration of about 100 ng/mL was achieved after 3–4 h, before declining to about 30 ng/mL by 12 h (Shinzawa *et al.*, 1989). The half-time of menaquinone-4 was not calculated.

(*e*) *Adult tissue reserves and distribution of vitamin K*

Dietary vitamin K is delivered to the liver and possibly other tissues, including bone marrow, in the form of chylomicron remnants (Kohlmeier *et al.*, 1996). The liver has often been assumed to be a major depot for vitamin K because it is the site of synthesis of the vitamin K-dependent coagulation proteins. Measurements of phylloquinone in livers obtained at autopsy from 32 adults in the United Kingdom revealed hepatic concentrations ranging from 1.1 to 21 ng/g wet tissue [2.4–47 pmol/g], with a median concentration of 5.5 ng/g [12 pmol/g]. The corresponding total liver stores of phylloquinone were 1.7–38 μg [3.8–85 pmol/g], with a median total store of 7.8 μg [17 pmol/g] (Shearer *et al.*, 1988). Similar hepatic concentrations of phylloquinone were found in a smaller number of analyses of post-mortem samples from adults in Japan (10 ng/g) (Uchida & Komeno, 1988) and in The Netherlands (11 ng/g) (Thijssen & Drittij-Reijnders, 1996). The limited ability of the liver to store vitamin K is illustrated by the observation that the phylloquinone reserves are about 40 000-fold lower than those of vitamin A despite a daily dietary intake of vitamin K (~100 μg) which is

only about 10-fold lower than that of vitamin A (~1000 μg). The distribution of the various forms of vitamin K in the liver is quite different from that in plasma in that the major transport form, phylloquinone, represents the minority of total hepatic stores (about 10%); the remainder comprises bacterial menaquinones, mainly menaquinones-6–13 (Shearer *et al.*, 1988; Shearer, 1992; Shearer *et al.*, 1996). The pattern of individual menaquinones in the liver varies considerably between individuals (Shearer *et al.*, 1988; Uchida & Komeno, 1988; Thijssen & Drittij-Reijnders, 1996), perhaps reflecting their origin from the intestinal microflora (Shearer *et al.*, 1996). This proposal is supported by the finding that two menaquinones, -10 and -11, which are major forms in most liver samples (Uchida & Komeno, 1988; Thijssen & Drittij-Reijnders, 1996), are known to be synthesized by *Bacteroides* species which are predominant members of the human intestinal flora (Conly & Stein, 1992); yet menaquinone-10 and menaquinone-11 do not make appreciable contributions to normal diets (Shearer *et al.*, 1996).

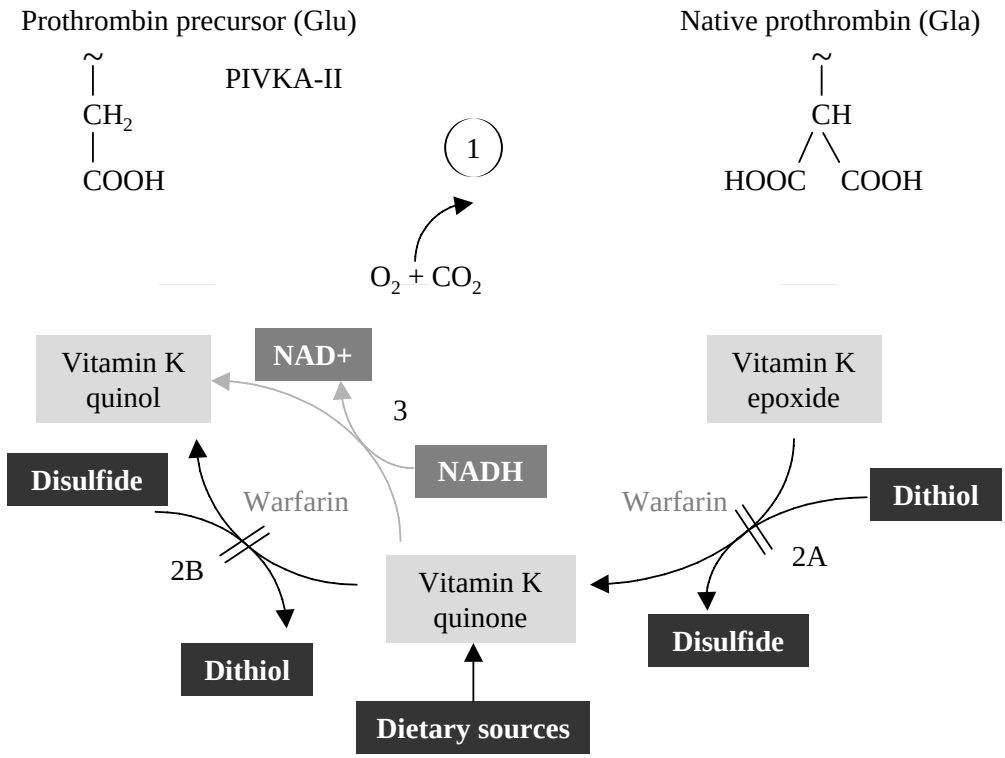
Phylloquinone is also present in other human tissues. The concentration in the heart (~5 ng/g) [~10 pmol/g] is comparable to those in the liver, and even higher concentrations (~13 ng/g) [~25 pmol/g] are found in the pancreas, but lower concentrations (< 1 ng/g) [< 2 pmol/g] were detected in brain, kidney and lung. These tissues do not appear to contain appreciable concentrations of menaquinones except for the short-chain menaquinone-4. Particularly high concentrations of menaquinone-4 relative to phylloquinone are present in the kidney, brain and pancreas. Although these and other tissues contain the enzymes of the vitamin K epoxide cycle (see Figure 1) and carry out vitamin K-dependent carboxylation of protein precursors, this would not appear to account for the tissue-specific accumulation of menaquinone-4 and may suggest a hitherto unrecognized physiological role for menaquinone-4 in certain tissues (Shearer, 1992; Thijssen & Drittij-Reijnders, 1996). Indeed, menaquinone-4 may arise by tissue synthesis from phylloquinone itself (Davidson *et al.*, 1998).

Osteocalcin is a major vitamin K-dependent bone protein synthesized by osteoblasts and therefore requires a source of vitamin K for γ-glutamyl carboxylation. Both trabecular and cortical bone contain ample reserves of vitamin K, with phylloquinone predominating and smaller amounts of shorter-chain menaquinones (Hodges *et al.*, 1993; Shearer, 1997). With the absence of the typical hepatic forms menaquinones-10–13, the vitamin K content of bone resembles that of other extrahepatic tissues.

(*f*) *Tissue stores and blood concentrations in neonates and infants*

Information on liver stores (the site of synthesis of vitamin K-dependent clotting proteins) in infants and their response to vitamin K prophylaxis is limited (Shearer *et al.*, 1988; Guillaumont *et al.*, 1993). The endogenous stores of vitamin K in the liver of the newborn differ both quantitatively and qualitatively from those of adults because the concentrations and total reserves of phylloquinone are lower than those of adults (Shearer *et al.*, 1988) and because bacterial menaquinones are undetectable (Shearer *et al.*, 1988; Guillaumont *et al.*, 1993). The endogenous hepatic concentrations of

Figure 1. Cyclic metabolism of vitamin K for conversion of glutamate (Glu) residues to γ-carboxy glutamate (Gla) residues in vitamin K-dependent proteins



Adapted from Shearer (1992) and Suttie (1987)
PIVKA-II, protein induced by vitamin K absence factor II
The active form of vitamin K needed for carboxylation is the reduced form, vitamin K quinol. The carboxylation reaction is driven by a vitamin K-dependent carboxylase activity (1) coupled to vitamin K-epoxidase activity (1) which simultaneously converts vitamin K quinol to vitamin K 2,3-epoxide. Vitamin K 2,3-epoxide is reduced back to the quinone by vitamin K epoxide reductase (2A). The cycle is completed by the reduction of recycled vitamin K quinone by vitamin K reductase activity (2B). The activities of both vitamin K epoxide (2A) and vitamin K reductase (2B) are dithiol-dependent (dithiol and disulfide denote reduced and oxidized dithiols) and are inhibited by coumarin anticoagulants such as warfarin. Exogenous vitamin K may enter the cycle via an NAD(P)H-dependent vitamin K reductase activity (3) which is not inhibited by warfarin.

phylloquinone ranged from 0.3 to 6.0 ng/g (median, 1.4 ng/g) in preterm infants and from 0.1 to 8.8 ng/g (median, 1.0 ng/g) in term infants. The median hepatic concentration of 1 ng/g in term infants is equivalent to a total liver pool of about 0.1 μg phylloquinone, whereas the concentration is 5.5 ng/g and the pool 7.8 μg in adult liver. In infants who had received 0.5 or 1 mg phylloquinone at birth by intramuscular injection, these liver reserves were raised by some two to three orders of magnitude

within 24 h. Hepatic phylloquinone concentrations may remain elevated for several weeks after injection: in two infants known to have received 1 mg phylloquinone by the intramuscular route and who survived 13 and 28 days, the total hepatic stores were 24 and 15 μg, respectively (Shearer *et al.*, 1988). Guillaumont *et al.* (1993) measured hepatic concentrations in post-mortem liver samples obtained within the first 48 h of death from infants who had received 2 mg phylloquinone intravenously or orally (in some cases combined with extra intravenous or oral doses of 1, 5 or 10 mg). In three newborns who survived < 24 h, the hepatic concentrations of phylloquinone ranged from 63 to 94 μg/g (total liver stores, 2800–7300 μg), which were four orders of magnitude higher than the endogenous concentrations of 0.002–0.008 μg/g (total liver stores, 0.1–0.9 μg). Between 24 and 48 h, the hepatic concentrations in 10 infants had fallen to a median of 8.4 μg/g (total liver stores, 550 μg), and in one infant who survived for five days it was 2.9 μg/g (110 μg). The quite rapid fall in hepatic stores presumably reflects the relatively rapid metabolism and excretion of vitamin K via the urine and bile (Shearer *et al.*, 1974). The lower hepatic concentration after intramuscular injection (Shearer *et al.*, 1988) compared with intravenous injection (Guillaumont *et al.*, 1993) is consistent with the idea that phylloquinone injected intramuscularly is released relatively slowly from the injection site (Loughnan & McDougall, 1996).

The reduced hepatic reserves of vitamin K in the human neonate are best explained by the existence of a barrier to placental uptake or transfer. This suggestion was originally made on the basis of the large concentration gradient of physiological concentrations of phylloquinone between maternal and cord blood plasma and the inefficient maternal–fetal transfer of pharmacological doses administered as an intravenous injection to the mother just before delivery (Shearer *et al.*, 1982). The poor placental transport of phylloquinone has been confirmed by others (Mandelbrot *et al.*, 1988; Yang *et al.*, 1989). There is now general agreement that the cord plasma concentration of phylloquinone is < 50 pg/mL [110 pmol/L] and that the average maternal–fetal concentration gradient is within the range 20:1 to 40:1 (Shearer, 1992).

Few longitudinal studies have been conducted of plasma concentrations in infants who were not given vitamin K prophylaxis. In one such study, cord plasma concentrations were compared for breast-fed and formula-fed infants and in blood on days 3, 7 and 28 after birth (Pietersma-de Bruyn *et al.*, 1990). In entirely breast-fed infants, the blood concentration rose from undetectable (< 20 pg/mL) at birth to mean values of 0.76, 0.49 and 0.49 ng/mL [1.7, 1.1 and 1.1 pmol/mL] on days 3, 7 and 28, respectively. In infants fed a milk formula containing 68 ng/mL phylloquinone, the plasma concentration rose steadily, with mean values of 1.4, 3.1 and 4.4 ng/mL [3.2, 6.8, and 9.9 pmol/mL] on days 3, 7 and 28, respectively. In another group of infants, Pietersma-de Bruyn *et al.* (1990) found that phylloquinone was undetectable in cord blood and in venous blood taken at 30 min but became measurable in venous blood after 12 h in 30% of infants (range, 0.04–0.40 ng/mL) and after 24 h in 60% of infants (range, 0.04–0.63 ng/mL).

A more detailed longitudinal comparison of plasma concentrations in breast-fed and formula-fed infants at 6, 12 and 26 weeks was made by Greer *et al.* (1991). This study

is of special interest because the intakes of phylloquinone were also estimated at each time by measuring the vitamin K content of the milk and the volume of milk ingested (by weighing the infant). Such an assessment of the intake of phylloquinone depends on both the analytical accuracy of the measurements in breast milk and validation of the milk collection and sampling technique; both have proved problematical. The study of Greer *et al.* (1991) seems to have met the requisite criteria, and, although the concentrations were at the lower end of published values, they were in the same range as those in a carefully designed longitudinal study of the phylloquinone content of breast milk over the first five weeks of lactation (1–2 ng/mL) (von Kries *et al.*, 1987b). The results, summarized in Table 8, illustrate the extreme differences in intakes between breast-fed and formula-fed infants, which are also reflected in the plasma concentrations. The plasma concentrations in the formula-fed infants agree with those found by Pietersma-de Bruyn *et al.* (1990) after 28 days (4.5 ng/mL), and suggest that they plateau at around one month. The concentrations in entirely breast-fed infants aged one month and beyond tend, as in this study, to be at the lower end of the normal range in adults (~0.15–1.0 ng/mL; mean, ~0.5 ng/mL), even when the infants have received prophylaxis during the first week of life (Cornelissen *et al.*, 1992; Schubiger *et al.*, 1993, 1997). In contrast, the plasma concentrations in formula-fed infants are about 10-fold higher than the average values in adults (Pietersma-de Bruyn *et al.*, 1990; Greer *et al.*, 1991).

Table 8. Dietary intakes and plasma concentrations of phylloquinone in breast-fed and formula-fed infants aged 0–6 months in the USA

Age (weeks)	Phylloquine intake (μg/day)		Plasma phylloquinone (μg/L)	
	Breast-fed[a]	Formula-fed[b]	Breast-fed	Formula-fed
6	0.55	45	0.13	6.0
12	0.74	56	0.20	5.6
26	0.56	52	0.24	4.4

From Greer *et al*. (1991)

[a] The average breast-milk concentrations were 0.86, 1.1 and 0.87 μg/L (ng/mL) at 6, 12 and 26 weeks, respectively.

[b] All infants were fed a formula containing 55 μg/L (ng/mL) phylloquinone.

(*g*) *Hepatic catabolism*

The liver plays an exclusive role in the metabolic transformations leading to the elimination of vitamin K from the body. After intravenous doses of 45 μg to 1 mg [^{3}H]phylloquinone, about 20% of the radiolabel was excreted in the urine within three days, and 35–50% was excreted as metabolites in the faeces via the bile (Shearer

et al., 1974). Rapid depletion of hepatic reserves of phylloquinone was also seen in surgical patients placed on a low-phylloquinone diet (Usui *et al.*, 1990). These results suggest that the body stores of vitamin K are replenished constantly.

The route of hepatic catabolism leading to urinary excretion of vitamin K proceeds by oxidative degradation of the phytyl side-chain, probably involving the same enzymes used for ω-methyl and β-oxidation of fatty acids, steroids and prostaglandins. Two major metabolites or aglycones have been identified, which are carboxylic acids with five- and seven-carbon atom side-chains and are excreted in the urine as glucuronide conjugates (McBurney *et al.*, 1980). The biliary metabolites have not been clearly identified but are initially excreted as water-soluble conjugates and become lipid-soluble during their passage through the gut, probably through deconjugation by the gut flora. There is no evidence that the body stores of vitamin K are conserved by enterohepatic circulation. Vitamin K itself is too lipophilic to be excreted in the bile, and the side-chain-shortened carboxylic acid metabolites are not biologically active.

(*h*) *Vitamin K-epoxide cycle*

In all tissues and cells found to carry out vitamin K-dependent carboxylation, the reaction has been shown to be intimately linked to a metabolic sequence known as the vitamin K-epoxide cycle. This cycle and the associated enzyme activities are shown in Figure 1. Its function seems to be to serve as a salvage pathway to conserve tissue reserves of vitamin K. In the course of γ-glutamyl carboxylation, vitamin K quinol is transformed into vitamin K epoxide, and the epoxide product is recycled in two steps; firstly by vitamin K epoxide reductase activity to produce vitamin K quinone and secondly by quinone reductase activity to produce the co-enzyme vitamin K quinol. Both these activities are thiol-dependent and are probably effected by the same enzyme (Suttie, 1987).

An important property of the dithiol-dependent epoxide and quinone reductase is their sensitivity to certain antagonists, especially those based on 4-hydroxycoumarin (e.g. warfarin) or indandione structures, which have long been used as oral anti-coagulants. It is now clear that their anticoagulant action is based on their ability to inhibit epoxide reductase activity and block the recycling of the vitamin. The dithiol-dependent quinone reductase is also sensitive to warfarin, but the activity of a second quinone reductase catalysed by an NAD(P)H-dependent enzyme is less sensitive to warfarin inhibition and provides an alternative pathway for the reduction of vitamin K quinone to quinol in the presence of warfarin and other oral anticoagulant drugs (Shearer, 1992).

(i) *Menadione and related water-soluble derivatives*

No studies appear to have been conducted on the absorption, distribution, metabolism or excretion of menadione and related compounds in humans. Water-soluble salts of menadione (vitamin K_3) were introduced for vitamin K prophylaxis in newborns in the early 1940s and, until their use was almost entirely superseded by phylloquinone in

the early 1960s, there were no suitable techniques for measuring menadione, its salts or their metabolites other than by radioisotopic techniques.

4.1.2 *Experimental systems*

(*a*) *Absorption*

The route and mechanism of absorption of menadione is different from that of natural K vitamins such as phylloquinone. Jaques *et al*. (1954) fed [^{14}C]menadione to rats and measured the radiolabel in faeces, bile, lymph and urine. They deduced that all the absorbed menadione was transported exclusively via the portal vein to the liver, unlike phylloquinone which is transported by the lymphatic pathway. Also unlike phylloquinone, menadione participated in rapid entero-hepatic circulation after excretion in the bile. Mezick *et al*. (1968) suggested that, while the portal route is important in rats, menadione could also be transported via the lymphatic system. Direct evidence for some lymphatic transport was found by experiments in dogs, showing that about 10% of the absorbed menadione was recovered in thoracic duct lymph. In studies with bile exclusion, the absorption of menadione in rats was found not to be dependent on bile, as would be expected if menadione is absorbed predominantly via the portal vein.

(*b*) *Tissue distribution, metabolism and excretion*

Early experiments with [^{14}C]menadione in mice showed rapid clearance from the intramuscular injection site of doses of 0.1 and 1.0 mg (about 4–40 mg/kg bw) within the first hour and excretion in the urine. Radiolabel was initially detectable in blood, but the concentrations later declined. No significant accumulation was seen in tissues. Small amounts of activity were sometimes detected in liver, lung and kidney, but no significant amounts were found in skin, bone or muscle (Solvonuk *et al*., 1952). A comparison of the tissue distribution of [^{14}C]menadione and [^{14}C]phylloquinone in rats after intravenous administration of a pharmacological dose (5 mg/kg bw) showed a much higher (24-fold) concentration of radiolabel in the livers of animals given phylloquinone than in those given menadione, and a fivefold greater accumulation of phylloquinone was found in the spleen. As in the studies of Solvonuk *et al*. (1952), no organ-specific accumulation of radiolabel was found in rats given labelled menadione, the highest proportions of radioactivity being found in urine and faeces (Taylor *et al.*, 1957). The rapid, extensive excretion of [^{14}C]menadione in the urine was confirmed by Losito *et al*. (1968) who found that rats excreted about 70% of an intravenous dose in the urine within 24 h compared with only about 10% of a dose of phylloquinone. They also showed that the urinary excretion of menadione (again unlike phylloquinone) was not dependent on an intact liver, as hepatectomized rats excreted the same amount of the dose (70%) as normal rats.

Rats given intracardial injections of a more physiological total dose (10 µg [30 µg/kg bw]) of high-activity 6,7-[^{3}H]menadione showed a pattern of excretion and

tissue distribution similar to that of pharmacological doses, with recovery of 78–83% of the label in the urine after 18 h (Taggart & Matschiner, 1969). A similar pattern was seen in rats given an intraperitoneal injection of about 2 μg of the water-soluble salt menadiol diphosphate; 17 h later, some 43% of the radiolabel had been excreted in urine and about 4% in faeces. The compound was not concentrated in any tissue but was distributed throughout all body organs, and the distribution was the same in vitamin K-replete and -deficient animals. This water-soluble compound underwent rapid conversion to lipid-soluble forms, and the compound and its metabolites were found generally to be associated with the membranous fractions of cells (Thierry & Suttie, 1969).

Three major metabolites of menadione have been isolated from urine. After oral administration of menadione to rabbits, Richert (1951) isolated the sulfated compound 2-methyl-4-hydroxy-1-naphthyl sulfate and noted increased excretion of glucuronic acid. Hoskin *et al*. (1954) resolved three metabolites from rat urine, of which the major product was tentatively identified as 2-methyl-1,4-dihydroxynaphthalene-1,4-diglucuronide and another as the monosulfate conjugate found by Richert (1951). A third, minor metabolite appears to be a phosphate conjugate (Hart, 1958).

Losito *et al.* (1967) showed in an isolated perfused rat liver system that menadione glucuronide or sulfate conjugates are excreted but that the glucuronide is confined to bile and the sulfate to the perfusing blood. In rats *in vivo*, Losito *et al*. (1968) separated three major urinary metabolites, two of which were identified as the same glucuronide and sulfate conjugates as those found in their liver perfusion system (Losito *et al*. 1967). The chromatographic pattern in hepatectomized rats was different, but the major peak was shown to be a glucuronide conjugate, showing that animals have the capacity for extrahepatic conjugation of menadione with glucuronic acid (Losito *et al*., 1968).

(*c*) *Conversion of menadione to menaquinone-4*

The vitamin K activity of menadione and its water-soluble salts depends on its specific metabolic conversion to menaquinone-4 (Suttie, 1985, 1991). The early evidence that both menadione and phylloquinone could be converted in birds and rats has been reviewed (Martius, 1967). The enzymic alkylation of menadione to menaquinone-4 was subsequently confirmed by more sophisticated techniques both *in vivo* in rats (Taggart & Matschiner, 1969) and *in vitro* in chick liver homogenates (Dialameh *et al*., 1970). The greatest alkylating activity was found in the microsomal fraction and was six to seven times higher in chick liver microsomes than in rat liver microsomes (Dialameh *et al*., 1970).

4.2 Toxic effects

4.2.1 *Humans*

(*a*) *Phylloquinone*

Reports of acute toxicity associated with pharmaceutical preparations of vitamin K as phylloquinone are rare and are often attributed to the vehicle of solubilization or other component of the preparation rather than to vitamin K itself. Adverse events associated with two products (Konakion and Konakion MM, currently representing about 50% of the market share worldwide) were monitored in a post-marketing surveillance programme, and the results were analysed and reviewed by Pereira and Williams (1998). During the period 1974 to July 1995, an estimated 635 million adults and 728 million children were prescribed Konakion or Konakion MM, and only 404 adverse events in 286 subjects were reported. Of these, the majority (96%) were associated with the older, Cremophor EL-based Konakion, which accounted for 95% of sales during this period. 'Skin, hair and nail disorders' were the most common adverse effects, accounting for about 25% of those reported. Rare cutaneous reactions to another vitamin K preparation, AquaMephyton, have been reported and are suspected to be immunologically mediated (Sanders & Winkelmann, 1988). This preparation contains a polyoxyethylated fatty acid derivative as the emulsifying agent (Rich & Drage, 1982).

The most serious reaction to vitamin K is anaphylactoid reactions after parenteral administration, but evidence that this effect is due to the polyethoxylated castor oil emulsifier (non-ionic detergent) Cremophor EL (polyethyleneglycolglycerol riconoleate) rather than vitamin K is twofold. Firstly, during the last 12 months of post-marketing surveillance (1994–95), 14 serious adverse events were reported from an estimated 21 million individuals receiving the Cremophor EL-based Konakion but none from the 13 million who received Konakion MM (Pereira & Williams, 1998). Secondly, anaphylactoid reactions in humans have been reported with other drugs solubilized with Cremophor EL, and there is experimental evidence in dogs that Cremophor EL and its components cause histamine release and hypotensive reactions (Lorenz *et al.*, 1982). The mixed-micellar Konakion MM preparation in which the vitamin K is solubilized by the naturally occurring components glycocholic acid and phosphatidylcholine appears to have far fewer anaphylactoid properties, only one probable anaphylactoid reaction having been reported in an estimated 66 million adults and 1–2 million infants and children who received this preparation (Pereira & Williams, 1998). Severe complications resulting in cardiopulmonary arrest were reported after intravenous injection of AquaMephyton (Rich & Drage, 1982).

(*b*) *Menadione*

The potential toxicity of preparations of menadione and its water-soluble derivatives to newborn infants is well established and has been reviewed (Vest, 1966).

The toxic reactions commonly include haemolytic symptoms evidenced by increased reticulocyte counts and Heinz body formation. In severe cases, overt haemolytic anaemia with haemoglobinuria may occur. The increased erythrocyte breakdown may lead to hyperbilirubinaemia and kernicterus. These effects are clearly dose-dependent, as premature infants given 30 mg of menadiol sodium phosphate had higher serum bilirubin concentrations, more Heinz bodies, lower haemoglobin concentrations and lower erythrocyte counts than those given 1 mg. The toxic reactions are more pronounced and may lead to severe haemolysis in premature infants and in infants with a congenital defect of glucose 6-phosphate dehydrogenase.

An explanation for the haemolytic toxicity of menadione is provided by studies showing the high reactivity of the 3-position of menadione with sulfhydryl compounds. Canady and Roe (1956) showed that when menadione is added to blood, it combines directly with blood proteins, probably by forming a thio ether at the 3-position. A later study showed that menadione reacts with both the haem groups and the β-93 thiol groups of haemoglobin and that it oxidizes the haem groups of oxyhaemoglobin, resulting in the formation of methaemoglobin (Winterbourn *et al.*, 1979).

With elucidation of the toxic properties of menadione in newborn infants and, in the 1960s, the industrial synthesis of natural K vitamins, use of menadione for vitamin K prophylaxis in the newborn was discontinued in most countries (Vest, 1966).

4.2.2 *Experimental systems*

(*a*) *Phylloquinone*

Israels *et al.* (1983) suggested that vitamin K compounds may have a regulatory function in the metabolism of benzo[*a*]pyrene and possibly other compounds that are metabolized through the mixed-function oxidase system. This suggestion stemmed from their studies with menadione, which was shown to inhibit the conversion of benzo[*a*]pyrene to its more polar metabolites in rat liver microsomes *in vitro*. The inhibition showed a plateau (25% of control) at a concentration of 100 μmol/L [17 μg/mL]. With phylloquinone, no inhibition to polar metabolites was evident at concentrations up to 50 μmol/L [8.6 μg/mL], but at concentrations of 50–200 μmol/L [34 μg/mL] the inhibition increased rapidly, and at 500 μmol/L [86 μg/mL] the degree of inhibition was similar to that produced by menadione. The authors concluded that menadione acted as an electron acceptor. The weaker effect of phylloquinone at lower concentrations is perhaps due to its much greater lipophilicity and reduced penetration and solubility in microsomal membranes as compared with menadione; this explanation would also be consistent with the absence of a difference in solubility at higher concentrations of phylloquinone. In a later paper, Israels *et al.* (1985) found that microsomal metabolism of benzo[*a*]pyrene to polar metabolites *in vitro* was actually increased when the concentration of phylloquinone was reduced to 25 μmol/L [11.3 μg/mL] but, as in their earlier paper, was decreased at a concentration of 200 μmol/L [90 μg/mL].

In studies of the effects of menadione and phylloquinone on tumorigenesis in mice *in vivo*, the rate of tumour appearance and the death rate of mice given an intraperitoneal injection of benzo[*a*]pyrene were slowed by menadione but increased by phylloquinone. In parallel studies, tumorigenesis was inhibited in mice treated with the vitamin K antagonist warfarin and in mice made vitamin K-deficient by dietary deprivation. In these experiments, the compounds were given either before or both before and after benzo[*a*]pyrene (Israels *et al.*, 1983).

(*b*) *Menadione*

Menadione also causes haemolytic anaemia in animals. The results of studies conducted in the 1940s were confirmed by Munday *et al.* (1991), who gave menadione (in 2% Tween 80) to Sprague-Dawley rats at a single dose of 750 μmol/kg bw per day [equivalent to about 100 mg/kg bw per day] for six consecutive days. This dose resulted in significant increases in splenic weight and decreased blood packed cell volume and haemoglobin concentration. Heinz bodies were observed in stained erythrocytes. There was no evidence that menadione caused haemaglobinaemia, suggesting that the haemolysis is not intravascular but is due to the destruction of damaged erythrocytes by cells of the reticuloendothelial system. Haemolysis was the only toxic change identified in rats dosed with menadione.

Melgar *et al.* (1991) examined the toxicity of menadione by giving Sprague-Dawley rats gradually increasing oral doses of menadione for six weeks, starting at 5 mg/kg bw per day and increasing to 20 mg/kg bw per day in the third week and 40 mg/kg bw per day in the fifth week of treatment. This dose regime was generally well tolerated with no relevant haematological changes, although there was a significant increase in spleen weight.

Many studies have been reported of the cytotoxicity of menadione in isolated and cultured cells of several types, including isolated rat hepatocytes (Mirabelli *et al.*, 1988; Shertzer *et al.*, 1992; Toxopeus *et al.*, 1993), rat renal epithelial cells (Brown *et al.*, 1991), bovine heart microvascular endothelial cells (Kossenjans *et al.*, 1996), Chinese hamster V79 cells (Ochi, 1996) and human hepatoma and leukaemia cell lines (Chiou *et al.*, 1998). The cytotoxicity of menadione has also been studied in isolated rat platelets (Chung *et al.*, 1997).

A characteristic finding in isolated rat hepatocytes treated with menadione is the appearance of numerous protrusions in the plasma membrane, known as blebs. Menadione produced a dose- and time-dependent increase in the frequency of cytoskeletal abnormalities; protein thiol oxidation seems to be intimately related to the appearance of surface blebs (Mirabelli *et al.*, 1988).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No formal investigations of the safety of vitamin K in pregnancy have been found, although it has been proposed that vitamin K deficiency causes congenital malformations (Menger *et al*., 1997). In a study of the efficacy of vitamin K for the prevention of the vitamin K deficiency induced by antiepileptic drugs, 16 women receiving antiepileptic drugs known to induce hepatic microsomal enzymes were treated orally with phylloquinone (Konakion) at 10 mg/day from the 36th week of pregnancy until delivery (mean, 29 days; range, 10–46). A control group of 20 epileptic women on similar antiepileptic drugs did not receive supplemental vitamin K. No adverse effects were observed in the infants of women given vitamin K supplementation. The median maternal plasma concentration of phylloquinone was raised 60-fold and the cord blood concentration was raised 15-fold, for a median maternal:cord blood ratio of 44 (Cornelissen *et al*., 1993b).

4.3.2 *Experimental systems*

The offspring of mice treated with phylloquinone by injection had cleft lip and exencephaly (Schardein, 1993). Six pregnant Sprague-Dawley rats were dosed with 10 mg/kg bw phylloquinone (Konakion) daily on days 9–20 of gestation, and the fetuses were delivered on day 21 and examined for external malformations and the presence of haemorrhages only. No adverse effects were noted when compared with a group of five untreated controls (Howe & Webster, 1990). [The Working Group noted the small numbers of animals and the restricted fetal examination.]

Oral administration of menadione to groups of 10 pregnant Wistar rats throughout gestation at a dose of 0.15, 15 or 150 mg/day [approximately 0.6, 60 or 600 mg/kg bw per day] had no adverse effect on maternal body-weight gain, pregnancy rate or litter size, but the fetuses showed slightly retarded growth and delayed ossification at the high dose. No abnormalities were observed (Kosuge, 1973).

4.4 Genetic and related effects

4.4.1 *Humans*

Cornelissen *et al*. (1991) observed no difference in sister chromatid exchange or chromosomal aberration frequency in peripheral blood lymphocytes from six neonates given intramuscular phylloquinone prophylaxis and in those from six control neonates. The blood was taken 24 h after an intramuscular dose of 1 mg, at which time the plasma concentrations of phylloquinone ranged from 115 to 1150 ng/mL (mean, 536 ng/mL), compared with about 0.15 ng/mL in the control neonates.

Pizer *et al*. (1995) used the glycophorin A mutation assay to assess the risk for somatic mutations of NO and NN variant red cells of 64 infants aged 10 days to six months heterozygous for the MN blood group, who had received either oral, intramuscular or intravenous phylloquinone prophylaxis at birth. All three groups showed a lower variant frequency than a reference group of children aged 1–15 years. For ethical reasons, there was no control group of infants who had not received vitamin K prophylaxis, and the conclusion was therefore limited to a lack of association between the route of vitamin K administration and somatic mutation.

4.4.2 *Experimental systems*

Limited data are available on the genetic and related effects of phylloquinone and menaquinones (Table 9). Phylloquinone did not induce mutation in *Salmonella typhimurium*. It enhanced the frequency of sister chromatid exchange in cultured human maternal lymphocytes at concentrations that are relevant *in vivo*, and a similar increase in sister chromatid exchange frequency was observed in cultured lymphocytes from human placental blood. In fetal sheep that received a catheter in the femoral vein 10–15 days before term, phylloquinone significantly increased the frequency of sister chromatid exchange in peripheral blood lymphocytes sampled 24 h later.

Menaquinone-4 but not phylloquinone inhibited osteoclastic bone resorption by inducing osteoclast apoptosis (Kameda *et al*., 1996). Menaquinone-4 and its derivatives also induced apoptosis in various human leukaemic cell lines (Yaguchi *et al*., 1997).

In preincubation protocols with Ames *Salmonella* tester strains, menadione did not induce reverse mutation in strains TA100, TA102, TA1535, TA1537, TA1538 or TA2638 in the presence or absence of an exogenous metabolic activation system. It was mutagenic in TA98 with metabolic activation and in TA2637 with or without activation. Menadione also induced mutation in strain TA104, but only with metabolic activation by purified NADPH–cytochrome P450 reductase; in another study it was mutagenic in this strain without activation. Menadione did not induce reverse mutation in *Escherichia coli* WP2/pKM101 or WP2*uvr*A/pkM101 in the absence of metabolic activation.

In tests with derivatives of *E. coli* WP2s (*uvr*A *trp*E) that are defective in 7,8-dihydro-8-oxoguanine DNA glycosylase activity (*mut*M) or MutY glycosylase activity on an A:7,8-dihydro-8-oxoguanine mispair (*mut*Y) or give an adaptive response to oxidative stress by superoxide (*sox*RS), to compare the mutability of various reactive oxygen-generating compounds, menadione was not mutagenic; however, it was mutagenic in two strains of *E. coli* WP2 that contain deficiencies in the oxyR function. Menadione induced forward mutation to L-arabinose resistance (Ara^R) in *E. coli* K-12 strains with diminished concentrations of superoxide dismutase and induced a SOS response in PQ37.

This agent induced concentration-dependent single-strand and double-strand DNA breaks in a human breast cancer MCF-7 cell line, in cultured rat hepatocytes, in

Table 9. Genetic and related effects of phylloquinone and menadione

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Phylloquinone				
Salmonella typhimurium TA98, TA100, TA2637, reverse mutation	–	–	100 µg/plate	Tikkanen *et al*. (1983)
Sister chromatid exchange, human peripheral blood lymphocyes *in vitro*	+	NT	0.45	Israels *et al.* (1987)
Sister chromatid exchange, fetal sheep peripheral blood lymphocytes *in vivo*	+		1 mg/animal	Israels *et al*. (1987)
Menadione				
Escherichia coli K12, forward mutation, arabinose resistance	(+)[c]	NT	43 µg/plate	Prieto-Alamo *et al*. (1993)
Escherichia coli WP2s (ZA570, ZA580, ZA590, ZA700, ZA770, ZA780), reverse mutation	–	–	300 µg/plate	Kato *et al*. (1994)
Escherichia coli WP2/pKM101, WPS*uvr*A/pkM101, reverse mutation	–[d]	NT	30 µg/plate	Blanco *et al*. (1998)
Escherichia coli WP2/pKM101, WP2*uvr*A/pKM101, reverse mutation	–	NT	300 µg/plate	Watanabe *et al*. (1998)
Salmonella typhimurium TA102, TA2638, reverse mutation	–	NT	300 µg/plate	Watanabe *et al*. (1998)
Salmonella typhimurium TA102, TA1535, TA1537, TA1538, reverse mutation	–	–	NR	Hakura *et al*. (1994)
Salmonella typhimurium TA2637, reverse mutation	+	+	NR	Hakura *et al*. (1994)
Salmonella typhimurium TA98, reverse mutation	–	+	NR	Hakura *et al*. (1994)
Salmonella typhimurium TA97, TA100, TA104, reverse mutation	+	–	NR	Hakura *et al*. (1994)
Salmonella typhimurium TA104, reverse mutation	NT	+	0.17	Chesis *et al*. (1984)
Salmonella typhimurium TA100, reverse mutation	–	–	140 µg/plate	Tikkanen *et al*. (1983)
Salmonella typhimurium TA98, reverse mutation	–	(+)	140 µg/plate	Tikkanen *et al.* (1983)
Salmonella typhimurium TA2637, reverse mutation	–	+	80	Tikkanen *et al*. (1983)
Drosophila melanogaster, genetic crossing-over or recombination (*white–ivory* assay)	–		10 mmol/L in feed	Ferreiro *et al.* (1997)
*lac*I Mutation, rat embryonic fibroblasts (λ*lac*I-transfected)	+	NT	0.85	Andrew *et al*. (1999)
DNA single-strand breaks, rat primary hepatocytes *in vitro*	+	NT	4.3	Morrison *et al*. (1984)

Table 9 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Menadione (contd)				
DNA single-strand breaks, rat primary hepatocytes *in vitro*	+	NT	1.7	Morrison *et al*. (1985)
DNA single-strand breaks, rat primary hepatocytes *in vitro*	+	NT	8.5	Morgan *et al.* (1992)
DNA fragmentation, rat hepatocytes *in vitro*	+	NT	4.3	Fischer-Nielsen *et al*. (1995)
Cell transformation, BALB/c 3T3 cells (followed by TPA treatment)	+[e]	NT	0.5	Sakai *et al*. (1995)
DNA single-strand breaks, human breast cancer MCF-7 cells *in vitro*	+[f]	NT	0.85	Ngo *et al*. (1991)
DNA single-strand breaks, human primary fibroblasts *in vitro*	+	NT	3.4	Morrison *et al*. (1985)
DNA strand breaks (alkaline single-cell gel electrophoresis assay, comet), human lymphocytes *in vitro*	+	NT	0.17	Woods *et al*. (1997)
DNA single-strand breaks, human chronic myeloid leukaemic K562 cells *in vitro*	+	NT	2.6	Morgan *et al.* (1992); Morgan (1995)
Menaquinone				
Escherichia coli PQ37, SOS response	+	NT	50 µg/plate	Cook *et al*. (1991)

TPA, 12-*O*-tetradecanoylphorbol 13-acetate

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; NR, not reported

[c] LEDs in *E. coli* K12 lacking superoxide dismutase or catalase were ~25 and ~5 times lower, respectively.

[d] Response in cells deficient in OxyR function (WP2 oxyR/pKM101 and WP2*uvr*A oxyR/pKM101) was positive at 10 µg/plate.

[e] Menadione acted as an initiator.

[f] DNA double-strand breaks were induced at 4.3 µg/mL.

human fibroblasts, in human chronic myeloid leukaemic K562 cells and in a single-cell gel electrophoresis assay to measure DNA strand breaks in human lymphocytes at doses as low as 1 μmol/L. At concentrations of 15–100 μmol/L, menadione induced extensive DNA fragmentation in human chronic myeloid leukaemic K562 cells which could be measured in alkaline elution assays. At these doses, no oxidative stress appeared to occur in these cells.

Cantoni *et al*. (1991) reported that hydrogen peroxide produced during the metabolism of menadione does not contribute to the cytotoxic action of the quinone. In isolated rat hepatocytes, menadione induced DNA fragmentation consistent with apoptosis. These effects occurred in the absence of 8-oxo-2′-deoxyguanosine production, and the authors concluded that oxidative modification of DNA bases was unlikely to be involved (Fischer-Nielsen *et al*., 1995). Menadione induced protein-linked DNA breaks in the presence of purified human DNA topoisomerase II but not DNA topoisomerase I (Frydman *et al*., 1997), and it seems likely that DNA topoisomerase II poisoning is involved in DNA breakage by menadione at the lower concentrations, at which oxygen stress does not occur.

Menadione induced morphological transformation of BALB/c 3T3 cells, but only when tested in the presence of the tumour promotor 12-*O*-tetradecanoylphorbol 13-acetate.

Andrew *et al*. (1999) found that menadione enhanced the spontaneous mutation frequency and induced a novel mutation spectrum of *lac*I genes recovered from a rat embryonic fibroblast line transfected with a λ-phage shuttle vector, in both the traditional plaque assay and a positive selection assay.

4.5 Mechanistic considerations

(*a*) *Phylloquinone*

On the basis of studies of microsomal metabolism *in vitro* and studies in rats and mice *in vivo*, Israels *et al.* (1983, 1985) suggested that vitamin K may be mutagenic by affecting the mixed-function oxidase system which metabolizes benzo[*a*]pyrene. Phylloquinone at a high concentration (200 μmol/L) inhibited the conversion of benzo[*a*]pyrene to its more polar metabolites, a property it shares with menadione. Paradoxically, at a lower concentration of phylloquinone (25 μmol/L), but not with menadione, the metabolism of benzo[*a*]pyrene was increased. In this system, therefore, whereas menadione consistently acts as a potential inhibitor of carcinogenesis, phylloquinone could either potentiate or inhibit it, depending on the concentration. The overall weaker inhibitory effect of phylloquinone could be due to the low solubility of this lipophilic compound, but it is difficult to explain the mechanism of the enhanced metabolism of benzo[*a*]pyrene at lower concentrations of phylloquinone.

In studies *in vivo*, Israels and co-workers found that menadione and vitamin K deficiency (nutritional or induced by the vitamin K antagonist, warfarin) both inhibited the

rates of benzo[*a*]pyrene-induced tumour appearance and death, whereas phylloquinone increased the rate of carcinogenesis. They concluded that vitamin K deficiency confers a protective effect against benzo[*a*]pyrene-induced tumour formation. They subsequently tendered the hypothesis that the low vitamin K status of normal newborns confers a biological advantage by reducing the risk of mutagenic events during a period of rapid cell proliferation (Israels *et al.*, 1987; Saxena *et al.*, 1997).

Vervoort *et al*. (1997) reported that metabolic cycling of vitamin K compounds via the vitamin K cycle (Figure 1) confers potent antioxidant activity against lipid peroxidation. They concluded that the antioxidant effect is probably due to radical chain-breaking by vitamin K quinol and that dietary intake of vitamin K may strengthen cellular defences against oxidative stress.

(*b*) *Menadione*

In many of the studies of the cytotoxicity of menadione in cultured cells and blood platelets, menadione was used as a model compound for induction of cellular damage either by arylating protein-bound and soluble thiols or by inducing oxidative stress. The relative importance of these two mechanisms is difficult to determine. The toxicity may result directly from binding of menadione to a critical protein thiol (such as a membrane cation transporter) or indirectly from binding to and decreasing concentrations of reduced glutathione, thereby predisposing the cell to oxidative stress. An alternative mechanism whereby menadione may produce oxidative stress is by redox cycling, which ultimately results in the production of reactive oxygen species. Oxidative stress results when the production of reactive oxygen species exceeds the antioxidant defence mechanisms, which in turn may result in cellular injury and death through a variety of mechanisms. In human cancer cells, menadione-induced cell degeneration was considered to result mainly from lipid peroxidative damage rather than from other mechanisms such as a depleted glutathione content (Chiou *et al.*, 1998).

It has been proposed that menadione causes mutations by generating active oxygen species from semiquinone radicals (e.g. Chesis *et al*., 1984; Smith *et al*., 1987; Hakura *et al*., 1994; Morgan *et al*., 1998). Semiquinones can generate superoxide anion, which itself produces other active species, such as hydrogen peroxide and hydroxyl radical, through enzyme- and metal-catalysed reactions (Chesis *et al*., 1984).

It now seems likely that menadione has an additional mode of action as a mutagen, by acting as a poison of DNA topoisomerase II enzymes. This could well be responsible for the DNA breakage, chromosomal aberrations and apoptosis observed in mammalian cells under conditions that did not lead to oxidative stress (e.g. Sawada *et al*., 1987; Fischer-Nielsen *et al*., 1995; Morgan, 1995). Cells in culture can, however, convert menadione to menaquinone-4, and there is already evidence that this plays a role in apoptosis.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

The term 'vitamin K' refers to a group of 2-methyl-1,4-naphthoquinone derivatives which can fulfil an essential co-factor function in humans in the biosynthesis of a number of calcium-binding proteins, some of which are essential for haemostasis. In nature, vitamin K occurs as phylloquinone in plants and as menaquinones produced by bacteria. The major dietary sources of vitamin K are green leafy vegetables and certain vegetable oils. Clinically, vitamin K is used primarily to prevent or cure deficiency-related bleeding in newborns and patients with malabsorption syndromes and to reverse the anticoagulative effects of vitamin K antagonists.

5.2 Human carcinogenicity data

An association between childhood leukaemia and vitamin K prophylaxis given by the intramuscular route was found in two reports but was not confirmed in a number of studies in various countries. A major limitation of most of the studies is that the fact of intramuscular administration of vitamin K was difficult to establish retrospectively for a substantial proportion of subjects, although the results of the analyses based on individual records and on imputed hospital policies for vitamin K administration are similar. In the studies in which a suggestion of an association was observed, selection bias may have accounted for the result. The possibility cannot be entirely excluded of a small increase in the risk for acute lymphoblastic leukaemia occurring at ages around those of the peak incidence in childhood in children given intramuscular administration of vitamin K.

The few studies that investigated oral administration of vitamin K found no increase in the relative risk for leukaemia.

5.3 Animal carcinogenicity data

No adequate study on the carcinogenicity of vitamin K substances was available to the Working Group.

5.4 Other relevant data

Phylloquinone and menaquinones are absorbed from food into the lymphatic system and carried by triglyceride-rich lipoproteins in the blood. Menaquinones synthesized by the gut microflora may also be absorbed. Phylloquinone is rapidly cleared from the circulation by the liver, metabolized to metabolites with shortened side-chains and excreted in the bile and urine. In animals, menadione is absorbed predominantly by the portal route, does not accumulate in specific organs and is extensively

excreted unchanged in the urine. A fraction of menadione is converted in tissues to menaquinone-4.

Phylloquinone rarely has toxic effects, and the few serious immunological complications observed have been attributed to the vehicle of solubilization. Menadione may cause haemolytic anaemia and induce cellular damage by arylating protein-bound and soluble thiols or by inducing oxidative stress.

No adverse effects have been reported in mothers or infants after administration of vitamin K during pregnancy, whereas vitamin K deficiency is teratogenic. The safety of vitamin K in pregnancy has not been adequately studied experimentally.

Neither phylloquinone nor menaquinones have been adequately studied for mutagenicity. Menadione acts as a bacterial mutagen in several specific strains of *Salmonella typhimurium* and *Escherichia coli*. In mammalian cells, menadione leads to DNA breakage, and there are isolated reports of chromosomal aberrations and sister chromatid exchange.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of vitamin K substances.

There is *inadequate evidence* in experimental animals for the carcinogenicity of vitamin K substances.

Overall evaluation

Vitamin K substances are *not classifiable as to their carcinogenicity to humans (Group 3)*.

6. References

American Hospital Formulary Service (1997) *AHFS Drug Information® 97*, Bethesda, MD, American Society of Health-System Pharmacists, pp. 2834–2836

Andrew, S.E., Hsiao, L., Milhausen, K. & Jirik, F.R. (1999) Comparison of selectable and plaque assay systems to detect menadione- and UV-induced *lacI* mutations in mammalian cells. *Mutat. Res.*, **427**, 89–97

van den Anker, J.N. & Sinaasappel, M. (1993) Bleeding as presenting symptom of cholestasis. *J. Pernatol.*, **13**, 322–324

Ansell, P., Bull, D. & Roman, E. (1996) Childhood leukaemia and intramuscular vitamin K: Findings from a case–control study. *Br. med. J.*, **313**, 204–205

AOAC International (1996) AOAC Official Method 992.27. *trans*-Vitamin K_1 (phylloquinone) in ready-to-feed milk-based infant formula. In: *Official Methods of Analysis of AOAC International*, 16th Ed., 4th rev., Gaithersburg, MD [CD-ROM edition]

Blanco, M., Urios, A. & Martínez, A. (1998) New *Escherichia coli* WP2 tester strains highly sensitive to reversion by oxidative mutagens. *Mutat. Res.*, **413**, 95–101

Booth, S.L & Suttie, J.W. (1998) Dietary intake and adequacy of vitamin K_1. *J. Nutr.*, **128**, 785–788

Booth, S.L., Sadowski, J.A. & Pennington, J.A.T. (1995) Phylloquinone (vitamin K_1) content in the US Food and Drug Administration's Total Diet Study. *J. agric. Food Chem.*, **43**, 1574–1579

Booth, S.L., Pennington, J.A.T. & Sadowski, J.A. (1996) Food sources and dietary intakes of vitamin K-1 (phylloquinone) in the American diet: Data from the FDA Total Diet Study. *J. Am. diet. Assoc.*, **96**, 149–154

Breen, G.A. & St Peter, W.L. (1997) Hypoprothombinemia associated with cefmetazole. *Ann. Pharmacother.*, **31**, 180–184

British Medical Association/Royal Pharmaceutical Society of Great Britain (1998) *British National Formulary*, No. 36, London, pp. 422–423

British Pharmacopoeial Commission (1993) *British Pharmacopoeia 1993*, London, Her Majesty's Stationery Office, Vols I & II, pp. 409–411, 516–517, 999–1000, 1059–1060, S79–S80

Brown, P.C., Dulik, D.M. & Jones, T.W. (1991) The toxicity of menadione (2-methyl-1,4-naphthoquinone) and two thioether conjugates studied with isolated renal epithelial cells. *Arch. Biochem. Biophys.*, **285**, 187–196

Budavari, S., ed. (1996) *The Merck Index*, 12th Ed., Whitehouse Station, NJ, Merck & Co., pp. 994, 1269–1270

Bueno, M.P. & Villalobos, M.C. (1983) Reverse phase high pressure liquid chromatographic determination of vitamin K_1 in infant formulas. *J. Assoc. off. anal. Chem.*, **66**, 1063–1066

Canadian Pharmaceutical Association (1997) *CPS Compendium of Pharmaceuticals and Specialties*, 32nd Ed., Ottawa, Ontario, p. 1730

Canady, W.J. & Roe, J.H. (1956) Studies on the reaction of menadione with blood and denatured proteins. *J. biol. Chem.*, **220**, 571–582

Canfield, L.M., Martin, G.S. & Sugimoto, K. (1988) Vitamin K in human milk. In: Suttie, J.W., ed., *Current Advances in Vitamin K Research*, New York, Elsevier, pp. 499–504

Cantoni, O., Fiorani, M., Cattabeni, F. & Bellomo, G. (1991) DNA breakage caused by hydrogen peroxide produced during the metabolism of 2-methyl-1,4-naphthoquinone (menadione) does not contribute to the cytotoxic action of the quinone. *Biochem. Pharmacol.*, **42**, S220–S222

Carstensen, J. (1992) Intramuscular vitamin K and childhood cancer (Letter to the Editor). *Br. med. J.*, **305**, 709–710

Chesis, P.L., Levin, D.E., Smith, M.T., Ernster, L. & Ames, B.N. (1984) Mutagenicity of quinones: Pathways of metabolic activation and detoxification. *Proc. natl Acad. Sci. USA*, **81**, 1696–1700

Chiou, T.-J., Chou, Y.-T. & Tzeng, W.-F. (1998) Menadione-induced cell degeneration is related to lipid peroxidation in human cancer cells. *Proc. natl Sci. Counc., ROC, Part B: Life Sci.*, **22**, 13–21

Choo, K.E., Tan, K.K., Chuah, S.P., Ariffin, W.A. & Gururaj, A. (1994) Haemorrhagic disease in newborn and older infants: A study in hospitalized children in Kelantan, Malaysia. *Ann. trop. Paediatr.*, **14**, 231–237

Chung, J.-H., Seo, D.-C., Chung, S.-H., Lee, J.-Y. & Seung, S.-A. (1997) Metabolism and cytotoxicity of menadione and its metabolite in rat platelets. *Toxicol. appl. Pharmacol.*, **142**, 378–385

CIS Information Services (1998) *Worldwide Bulk Drug Users Directory 1997/98 Edition*, Dallas, TX [CD-ROM]

Clark, F.I. & James, E.J. (1995) Twenty-seven years of experience with oral vitamin K_1 therapy in neonates. *J. Pediatr*., **127**, 301–304

Conly, J.M. & Stein, K. (1992) Quantitative and qualitative measurements of K vitamins in human intestinal contents. *Am. J. Gastroenterol*., **87**, 311–316

Cook, A., Stovicek, R., D'Odorico, A., Tkac, A. & Bilton, R.F. (1991) Menaquinone mediated free radical generation: A possible mutagenic mechanism. *Biochem. Soc. Trans*., **19**, 426S

Cornelissen, M., Smeets, D., Merkx, G., De Abreu, R., Kollée, L. & Monnens, L. (1991) Analysis of chromosome aberrations and sister chromatid exchanges in peripheral blood lymphocytes of newborns after vitamin K prophylaxis at birth. *Pediatr. Res*., **30**, 550–553

Cornelissen, E.A.M., Kollée, L.A.A., De Abreu, R.A., van Baal, J.M., Motohara, K., Verbruggen, B. & Monnens, L.A.H. (1992) Effects of oral and intramuscular vitamin K prophylaxis on vitamin K_1, PIVKA-II, and clotting factors in breast fed infants. *Arch. Dis. Child*., **67**, 1250–1254

Cornelissen, E.A.M., van Lieburg, A.F., Motohara, K. & van Oostrom, C.G. (1992) Vitamin K status in cystic fibrosis. *Acta paediatr*., **81**, 658–661

Cornelissen, M., Steegers-Theunissen, R., Kollée, L., Eskes, T., Vogels-Mentink, G., Motohara, K., De Abreu, R. & Monnens, L. (1993a) Increased incidence of neonatal vitamin K deficience resulting from maternal anticonvulsant therapy. *Am. J. Obstet. Gynecol*., **168**, 923–928

Cornelissen, M., Steegers-Theunissen, R., Kollée, L., Eskes, T., Motohara, K. & Monnens, L. (1993b) Supplementation of vitamin K in pregnant women receiving anticonvulsant therapy prevents neonatal vitamin K deficiency. *Am. J. Obstet. Gynecol*., **168**, 884–888

Cornelissen, M., von Kries, R., Loughnan, P. & Schubiger, G. (1997) Prevention of vitamin K deficiency bleeding: Efficacy of different multiple oral dose schedules of vitamin K. *Eur. J. Pediatr*., **156**, 126–130

Council of Europe (1997) *European Pharmacopoeia*, 3rd Ed., Strasbourg, pp. 1154–1155, 1332–1333

Crowther, M.A., Donovan, D., Harrison, L., McGinnis, J. & Ginsberg, J. (1998) Low-dose oral vitamin K reliably reverses over-anticoagulation due to warfarin. *Thromb. Haemostasis*, **79**, 1116–1118

Davidson, R.T., Foley, A.L., Engelke, J.A. & Suttie, J.W. (1998) Conversion of dietary phylloquinone to tissue menaquinone-4 in rats is not dependent on gut bacteria. *J. Nutr*., **128**, 220–223

Denda, A., Sai, K.M., Tang, Q., Tsujiuchi, T., Tsutsumi, M., Amanuma, T., Murata, Y., Nakae, D., Maruyama, H., Kurokawa, Y. & Konishi, Y. (1991) Induction of 8-hydroxydeoxyguanosine but not initiation of carcinogenesis by redox enzyme modulations with or without menadione in rat liver. *Carcinogenesis*, **12**, 719–726

Dialameh, G.H., Yekundi, K.G. & Olson, R.E. (1970) Enzymatic alkylation of menaquinone-0 to menaquinones by microsomes from chick liver. *Biochim. biophys. Acta*, **223**, 332–338

Doran, O., Austic, N.C. & Taylor, B.J. (1995) Vitamin K administration in neonates: Survey of compliance with recommended practices in the Dunedin area. *N.Z. J. Med.*, **108**, 337–339

Draper, G. & McNinch, A. (1994) Vitamin K for neonates: The controversy. *Br. med. J.*, **308**, 867–868

Draper, G.J. & Stiller, C.A. (1992) Intramuscular vitamin K and childhood cancer (Letter to the Editor). *Br. med. J.*, **305**, 709

Durie, P.R. (1994) Vitamin K and the management of patients with cystic fibrosis. *Can. med. Assoc. J.*, **151**, 933–936

Editions du Vidal (1998) *Dictionnaire Vidal 1998*, 74th Ed., Paris, OVP, pp. 1989–1990

Ekelund, H. (1991) Late haemorrhagic disease in Sweden 1987–89. *Acta paediatr. scand.*, **80**, 966–968

Ekelund, H., Finnström, O., Gunnarskog, J., Källén, B. & Larsson, Y. (1993) Administration of vitamin K to newborn infants and childhood cancer. *Br. med. J.*, **307**, 89–91

Ferland, G. & Sadowski, J.A. (1992) Vitamin K_1 (phylloquinone) content of edible oils: Effects of heating and light exposure. *J. agric. Food Chem.*, **40**, 1869–1873

Fetrow, C.W., Overlock, T. & Leff, L. (1997) Antagonism of warfarin-induced hypoprothrombinemia with use of low-dose subcutaneous vitamin K1. *J. clin. Pharmacol.*, **37**, 751–757

Fischer-Nielsen, A., Corcoran, G.B., Poulsen, H.E., Kamendulis, L.M. & Loft, S. (1995) Menadione-induced DNA fragmentation without 8-oxo-2′-deoxyguanosine formation in isolated rat hepatocytes. *Biochem. Pharmacol.*, **49**, 1469–1474

Food & Drug Administration (1999) Food and drugs. *US Code Fed. Regul.*, **Title 21**, Part 107.100, Subpart D, pp. 186–187

Fournier, B., Sann, L., Guillaumont, M. & Leclercq, M. (1987) Variations of phylloquinone concentration in human milk at various stages of lactation and in cow's milk at various seasons. *Am. J. clin. Nutr.*, **45**, 551–558

Frydman, B., Marton, L.J., Sun, J.S., Neder, K., Witiak, D.T., Liu, A.A., Wang, H.-M., Mao, Y., Wu, H.-Y., Sanders, M.M. & Liu, L.F. (1997) Induction of DNA topoisomerase II-mediated DNA cleavage by beta-lapachone and related naphthoquinones. *Cancer Res.*, **57**, 620–627

Gennaro, A.R. (1985) *Remington's Pharmaceutical Sciences*, 17th Ed., Easton, PA, Mack Publishing Co., pp. 1010–1011

Gennaro, A.R. (1995) *Remington: The Science and Practice of Pharmacy*, 19th Ed., Easton, PA, Mack Publishing Co., Vol. II, pp. 1114–1115

Gijsbers, B.L.M.G., Jie, K.-S.G. & Vermeer, C. (1996) Effect of food composition on vitamin K absorption in human volunteers. *Br. J. Nutr.*, **76**, 223–229

Golding, J., Paterson, M. & Kinlen, L.J. (1990) Factors associated with childhood cancer in a national cohort study. *Br. J. Cancer*, **62**, 304–308

Golding, J., Greenwood, R., Birmingham, K. & Mott, M. (1992) Childhood cancer, intramuscular vitamin K, and pethidine given during labour. *Br. med. J.*, **305**, 341–346

Greer, F.R., Marshall, S., Cherry, J. & Suttie, J.W. (1991) Vitamin K status of lactating mothers, human milk, and breast-feeding infants. *Pediatrics*, **88**, 751–756

Greer, F.R., Marshall, S.P., Foley, A.L. & Suttie, J.W. (1997) Improving the vitamin K status of breastfeeding infants with maternal vitamin K supplements. *Pediatrics*, **99**, 88–92

Guillaumont, M., Sann, L., Leclercq, M., Dostalova, L., Vignal, B. & Frederich, A. (1993) Changes in hepatic vitamin K1 levels after prophylactic administration to the newborn. *J. pediatr. Gastroenterol. Nutr.*, **16**, 10–14

Hagstrom, J.N., Bovill, E.G., Soll, R.F., Davidson, K.W. & Sadowski, J.A. (1995) The pharmacokinetics and lipoprotein fraction distribution of intramuscular vs. oral vitamin K_1 supplementation in women of childbearing age: Effects on hemostasis. *Thromb. Haemostasis*, **74**, 1486–1490

Hakura, A., Mochida, H., Tsutsui, Y. & Yamatsu, K. (1994) Mutagenicity and cytotoxicity of naphthoquinones for Ames *Salmonella* tester strains. *Chem. Res. Toxicol.*, **7**, 559–567

Hanawa, Y. (1992) Vitamin K deficiency in infancy: The Japanese experience. *Acta paediatr. jpn.*, **34**, 107–116

Hansen, K.B. & Ebbesen, F. (1996) Neonatal vitamin K prophylaxis in Denmark: Three years' experience with oral administration during the first three months of life compared with one oral administration at birth. *Acta pediatr.*, **85**, 1137–1139

Haroon, Y., Shearer, M.J., Rahin, S., Gunn, W.G., McEnery, G. & Barkhan, P. (1982) The content of phylloquinone (vitamin K_1) in human milk, cows' milk and infant formula foods determined by high-performance liquid chromatography. *J. Nutr.*, **112**, 1105–1117

Hart, K.T. (1958) Study of hydrolysis of urinary metabolites of 2-methyl-1,4-naphthoquinone. *Proc. Soc. exp. Biol. Med.*, **97**, 848–851

Hassan, M.M.A., Mossa, J.S. & Taragan, A.H.U.K. (1988) Analytical profile of phytonadione. In: Florey, K., ed., *Analytical Profiles of Drug Substances*, New York, Academic Press, Vol. 17, pp. 449–531

Hewson, D. & Bennett, A. (1987) Childbirth research data: Medical records or women's reports? *Am. J. Epidemiol.*, **125**, 484–491

Hill, R.J. (1994) The uptake of the third oral vitamin K dose in general practice. *N.Z. Med. J.*, **107**, 177–178

Hodges, S.J., Bejui, J., Leclercq, M. & Delmas, P.D. (1993) Detection and measurement of vitamins K_1 and K_2 in human cortical and trabecular bone. *J. Bone Min. Res.*, **8**, 1005–1008

Hoskin, F.C.G., Spinks, J.W.T. & Jaques, L.B. (1954) Urinary excretion products of menadione (vitamin K_3). *Can. J. Biochem. Physiol.*, **32**, 240–250

Houwen, R.H.J., Bouquet, J. & Bijleveld, C.M.A. (1987) Bleeding as the first symptom of extrahepatic biliary atresia. *Eur. J. Pediatr.*, **146**, 425–426

Howe, A.M. & Webster, W.S. (1990) Exposure of the pregnant rat to warfarin and vitamin K1: An animal model of intraventricular hemorrhage in the fetus. *Teratology*, **42**, 413–420

Hwang, S.-M. (1985) Liquid chromatographic determination of vitamin K_1 *trans*- and *cis*-isomers in infant formula. *J. Assoc. off. anal. Chem.*, **68**, 684–689

Indyk, H.E. & Woollard, D.C. (1997) Vitamin K in milk and infant formulas: Determination and distribution of phylloquinone and menaquinone-4. *Analyst*, **122**, 465–469

Israels, L.G., Walls, G.A., Ollmann, D.J., Friesen, E. & Israels, E.D. (1983) Vitamin K as a regulator of benzo(a)pyrene metabolism, mutagenesis, and carcinogenesis. Studies with rat microsomes and tumorigenesis in mice. *J. clin. Invest.*, **71**, 1130–1140

Israels, L.G., Ollmann, D.J. & Israels, E.D. (1985) Vitamin K_1 as a modulator of benzo(a)-pyrene metabolism as measured by *in vitro* metabolite formation and *in vivo* DNA-adduct formation. *Int. J. Biochem.*, **17**, 1263–1266

Israels, L.G., Friesen, E., Jansen, A.H. & Israels, E.D. (1987) Vitamin K1 increases sister chromatid exchange *in vitro* in human leukocytes and *in vivo* in fetal sheep cells: A possible role for 'vitamin K deficiency' in the fetus. *Pediatr. Res.*, **22**, 405–408

Japan Medical Products Trade Association (1996) *Japan Pharmaceutical Reference (JPR). Products and Administration in Japan*, 4th Ed., Tokyo, pp. 161–164, 181–186

Jaques, L.B., Millar, G.J. & Spinks, J.W.T. (1954) The metabolism of the K vitamins. *Schweiz. med. Wochenschr.*, **84**, 792–796

Kameda, T., Miyazawa, K., Mori, Y., Yuasa, T., Shiokawa, M., Nakamaru, Y., Mano, H., Hakeda, Y., Kameda, A. & Kumegawa, M. (1996) Vitamin K2 inhibits osteoclastic bone resorption by inducing osteoclast apoptosis. *Biochem. biophys. Res. Commun.*, **220**, 515–519

Kato, T., Watanabe, M. & Ohta, T. (1994) Induction of the SOS response and mutations by reactive oxygen-generating compounds in various *Escherichia coli* mutants defective in the *mutM*, *mutY* or *soxRS* loci. *Mutagenesis*, **9**, 245–251

Keller, C., Matzdorff, C. & Kemkes-Matthes, B. (1999) Pharmacology of warfarin and clinical implication. *Semin. Thromb. Hemostasis*, **25**, 13–16

Klebanoff, M.A., Read, J.S., Mills, J.L. & Shiono, P.H. (1993) The risk of childhood cancer after neonatal exposure to vitamin K. *New Engl. J. Med.*, **329**, 905–908

Kohlmeier, M., Salomon, A., Saupe, J. & Shearer, M.J. (1996) Transport of vitamin K to bone in humans. *J. Nutr.*, **126**, 1192S–1196S

Kossenjans, W., Rymaszewski, Z., Barankiewicz, J., Bobst, A. & Ashraf, M. (1996) Menadione-induced oxidative stress in bovine heart microvascular endothelial cells. *Microcirculation*, **3**, 39–47

Kosuge, Y. (1973) [Study of developmental pharmacology on vitamin K3. Part 1. Effect of vitamin K3 on the rat fetus.] *Folia pharmacol. jpn.*, **69**, 285–291 (in Japanese)

Kowdley, K.V., Emond, M.J., Sadowski, J.A. & Kaplan, M.M. (1997) Plasma vitamin K_1 level is decreased in primary biliary cirrhosis. *Am. J. Gastroenterol.*, **92**, 2059–2061

von Kries, R. (1991) Neonatal vitamin K—Prophylaxis for all. *Br. med. J.*, **303**, 1083–1084

von Kries, R. (1999) Oral versus intramuscular phytomenadione: Safety and efficacy compared. *Drug Saf.*, **21**, 1–6

von Kries, R. & Hanawa, Y. (1993) Neonatal vitamin K prophylaxis. Report of Scientific and Standardization Subcommittee on Perinatal Haemostasis. *Thromb. Haemostasis*, **69**, 293–295

von Kries, R., Becker, A. & Göbel, U. (1987a) Vitamin K in the newborn: Influence of nutritional factors on acarboxy-prothrombin detectability and factor II and VII clotting activity. *Eur. J. Pediatr.*, **146**, 123–127

von Kries, R., Shearer, M., McCarthy, P.T., Haug, M., Harzer, G. & Göbel, U. (1987b) Vitamin K_1 content of maternal milk: Influence of the stage of lactation, lipid composition, and vitamin K_1 supplements given to the mother. *Pediatr. Res.*, **22**, 513–517

von Kries, R., Shearer, M.J. & Gobel, U. (1988) Vitamin K in infancy. *Eur. J. Pediatr.*, **147**, 106–112

von Kries, R., Göbel, U., Hachmeister, A., Kaletsch, U. & Michaelis, J. (1996) Vitamin K and childhood cancer: A population based case–control study in Lower Saxony, Germany. *Br. med. J.*, **313**, 199–203

Lamon-Fava, S., Sadowski, J.A., Davidson, K.W., O'Brien, M.E., McNamara, J.R. & Schaefer, E.J. (1998) Plasma lipoproteins as carriers of phylloquinone (vitamin K_1) in humans. *Am. J. clin. Nutr.*, **67**, 1226–1231

LINFO Läkemedelsinformation AB (1998) *FASS 1998 Läkemedel i Sverige*, Stockholm, pp. 694–695

Lorenz, W., Schmal, A., Schult, H., Lang, S. Ohmann, C., Weber, D., Kapp, B., Lüben, L. & Doenicke, A. (1982) Histamine release and hypotensive reactions in dogs by solubilizing agents and fatty acids: Analysis of various components in Cremophor EL and development of a compound with reduced toxicity. *Agents Actions*, **12**, 64–80

Losito, R., Owen, C.A., Jr & Flock, E.V. (1967) Metabolism of [^{14}C]menadione. *Biochemistry*, **6**, 62–68

Losito, R., Owen, C.A., Jr & Flock, E.V. (1968) Metabolic studies of vitamin K_1-^{14}C and menadione-^{14}C in the normal and hepatectomized rats. *Thromb. Diath. Haemorrh.*, **19**, 383–388

Loughnan, P.M. & McDougall, P.N. (1996) Does intramuscular vitamin K_1 act as an unintended depot preparation? *J. paediatr. Child Health*, **32**, 251–254

Mandelbrot, L., Guillaumont, M., Leclercq, M., Lefrère, J.J., Gozin, D., Daffos, F. & Forestier, F. (1988) Placental transfer of vitamin K_1 and its implications in fetal hemostasis. *Thromb. Haemostasis*, **60**, 39–43

Martius, C. (1967) Chemistry and function of vitamin K. In: Seegers W.H., ed., *Blood Clotting Enzymology*, New York, Academic Press, pp. 551–575

McBurney, A., Shearer, M.J. & Barkhan, P. (1980) Preparative isolation and characterization of the urinary aglycones of vitamin K_1 (phylloquinone) in man. *Biochem. Med.*, **24**, 250–267

McKinney, P.A., Juszczak, E., Findlay, E. & Smith, K. (1998) Case–control study of childhood leukaemia and cancer in Scotland: Findings for neonatal intramuscular vitamin K. *Br. med. J.*, **316**, 173–177

McNinch, A.W., Upton, C., Samuels, M., Shearer, M.J., McCarthy, P., Tripp, J.H. & Orme, R.L.'E. (1985) Plasma concentrations after oral or intramuscular vitamin K_1 in neonates. *Arch. Dis. Child.*, **60**, 814–818

Melgar, M.J., Anadon, A. & Bello, J. (1991) Effects of menadione on the cardiovascular system. *Vet. hum. Toxicol.*, **33**, 110–114

Menger, H., Lin, A.E., Toriello, H.V., Bernert, G. & Spranger, J.W. (1997) Vitamin K deficiency embryopathy: A phenocopy of the warfarin embryopathy due to a disorder of embryonic vitamin K metabolism. *Am. J. med. Genet.*, **72**, 129–134

Meyer, T.C. & Angus, J. (1956) The effect of large doses of 'Synkavit' in the newborn. *Arch. Dis. Child.*, **31**, 212–215

Mezick, J. A., Tomkins, R.K. & Cornwell, D.G. (1968) Absorption and intestinal lymphatic transport of ^{14}C-menadione. *Life Sci.*, **7**, 153–158

Mirabelli, F., Salis, A., Marinoni, V., Finardi, G., Bellomo, G., Thor, H. & Orrenius, S. (1988) Menadione-induced bleb formation in hepatocytes is associated with the oxidation of thiol groups in actin. *Arch. Biochem. Biophys.*, **264**, 261–269

Morgan, W.A. (1995) DNA single-strand breakage in mammalian cells induced by redox cycling quinones in the absence of oxidative stress. *J. biochem. Toxicol.*, **10**, 227–232

Morgan, W.A., Hartley, J.A. & Cohen, G.M. (1992) Quinone-induced DNA single strand breaks in rat hepatocytes and human chronic myelogenous leukaemic K562 cells. *Biochem. Pharmacol.*, **44**, 215–221

Morgan, W.A., Kaler, B. & Bach, P.H. (1998) The role of reactive oxygen species in adriamycin and menadione-induced glomerular toxicity. *Toxicol. Lett.*, **94**, 209–215

Morrison, H., Jernström, B., Nordenskjöld, M., Thor, H. & Orrenius, S. (1984) Induction of DNA damage by menadione (2-methyl-1,4-naphthoquinone) in primary cultures of rat hepatocytes. *Biochem. Pharmacol.*, **33**, 1763–1769

Morrison, H., Di Monte, D., Nordenskjöld, M. & Jernström, B. (1985) Induction of cell damage by menadione and benzo(a)pyrene-3,6-quinone in cultures of adult rat hepatocytes and human fibroblasts. *Toxicol. Lett.*, **28**, 37–47

Motohara, K., Matsukura, M., Matsuda, I., Iribe, K., Ikeda, T., Kondo, Y., Yonekubo, A., Yamamoto, Y. & Tsuchiya, F. (1984) Severe vitamin K deficiency in breast-fed infants. *J. Pediatr.*, **105**, 943–945

Munday, R., Smith, B.L. & Fowke, E.A. (1991) Haemolytic activity and nephrotoxicity of 2-hydroxy-1,4-naphthoquinone in rats. *J. appl. Toxicol.*, **11**, 85–90

National Research Council (1989) *Recommended Dietary Allowances*, 10th Ed., Washington DC, National Academy Press, pp. 107–114

Ngo, E.O., Sun, T.-P., Chang, J.-Y., Wang, C.-C., Chi, K.-H., Cheng, A.-L. & Nutter, L.M. (1991) Menadione-induced DNA damage in a human tumor cell line. *Biochem. Pharmacol.*, **42**, 1961–1968

Nowak, D., Chudzik, J., Pietras, T. & Bialasiewicz, P. (1997) Severe haemorrhagic diathesis in an adult patient with cystic fibrosis after long-term antibiotic treatment of pulmonary infection. *Monaldi Arch. Chest Dis.*, **52**, 343–345

Oakley, A., Rajan, L. & Robertson, P. (1990) A comparison of different sources of information about pregnancy and childbirth. *J. biosoc. Sci.*, **22**, 477–487

O'Brien, D.P., Shearer, M.J., Waldron, R.P., Horgan, P.G. & Given, H.F. (1994) The extent of vitamin K deficiency in patients with cholestatic jaundice: A preliminary communication. *J. R. Soc. Med.*, **87**, 320–322

Ochi, T. (1996) Menadione causes increases in the level of glutathione and in the activity of γ-glutamylcysteine synthetase in cultured Chinese hamster V79 cells. *Toxicology*, **112**, 45–55

Øie, S., Trenk, D., Guentert, T.W., Mosberg, H. & Jähnchen, E. (1988) Disposition of vitamin K_1 after intravenous and oral administration to subjects on phenprocoumon therapy. *Int. J. Pharm.*, **48**, 223–230

Olsen, J.H., Hertz, H., Blinkenberg, K. & Verder, H. (1994) Vitamin K regimens and incidence of childhood cancer in Denmark. *Br. med. J.*, **308**, 895–896

Park, B.K., Scott, A.K., Wilson, A.C., Haynes, B.P. & Breckenridge, A.M. (1984) Plasma disposition of vitamin K_1 in relation to anticoagulant poisoning. *Br. J. clin. Pharmacol.*, **18**, 655–662

Parker, L., Cole, M., Craft, A.W. & Hey, E.N. (1998) Neonatal vitamin K administration and childhood cancer in the north of England: Retrospective case–control study. *Br. med. J.*, **316**, 189–193

Passmore, S.J., Draper, G., Brownbill, P. & Kroll, M. (1998a) Case–control studies of relation between childhood cancer and neonatal vitamin K administration. *Br. med. J.*, **316**, 178–184

Passmore, S.J., Draper, G., Brownbill, P. & Kroll, M. (1998b) Ecological studies of relation between hospital policies on neonatal vitamin K administration and subsequent occurrence of childhood cancer. *Br. med. J.*, **316**, 184–189

Pereira, S.P. & Williams, R. (1998) Adverse events associated with vitamin K_1: Results of a worldwide postmarketing surveillance programme. *Pharmacoepidemiol. Drug Saf.*, **7**, 173–182

Pietersma-de Bruyn, A.L.J.M., van Haard, P.M.M., Beunis, M.H., Hamulyák, K. & Kuijpers, J.C. (1990) Vitamin K_1 levels and coagulation factors in healthy newborns till 4 weeks after birth. *Haemostasis*, **20**, 8–14

Pindur, G., Mörsdorf, S., Schenk, J.F., Krischek, B., Heinrich, W. & Wenzel, E. (1999) The overdosed patient and bleeding with oral anticoagulation. *Semin. Thromb. Hemostasis*, **25**, 85–88

Pizer, B., Boyse, J., Hunt, L. & Mott, M. (1995) Neonatal vitamin K administration and in vivo somatic mutation. *Mutat. Res.*, **347**, 135–139

Prieto-Alamo, M.J., Abril, N. & Pueyo, C. (1993) Mutagenesis in *Escherichia coli* K-12 mutants defective in superoxide dismutase or catalase. *Carcinogenesis*, **14**, 237–244

Rennie, J.M. & Kelsall, A.W.R. (1994) Vitamin K prophylaxis in the newborn—again. *Arch. Dis. Child.*, **70**, 248–251

Renzulli, P., Tuchschmid, P., Eich, G., Fanconi, S. & Schwobel, M.G. (1998) Early vitamin K deficiency bleeding after maternal phenobarbital intake: Management of massive intracranial haemorrhage by minimal surgical intervention. *Eur. J. Pediatr.*, **157**, 663–665

Rich, E.C. & Drage, C.W. (1982) Severe complications of intravenous phytonadione therapy. *Postgrad. Med.*, **72**, 303–306

Richert, D.A. (1951) Studies on the detoxification of 2-methyl-1,4-naphthoquinone in rabbits. *J. biol. Chem.*, **189**, 763–768

Roman, E., Ansell, P. & Bull, D. (1997) Leukaemia and non-Hodgkin's lymphoma in children and young adults: Are prenatal and neonatal factors important determinants of disease? *Br. J. Cancer*, **76**, 406–415

Rote Liste Sekretariat (1998) *Rote Liste 1998*, Frankfurt, Rote Liste Service GmbH, pp. 84-171, 84-177, 84-180

Royal Pharmaceutical Society of Great Britain (1999) *Martindale, The Extra Pharmacopoeia*, 13th Ed., London, The Pharmaceutical Press [MicroMedex Online: Health Care Series]

Sadtler Research Laboratories (1980) *Sadtler Standard Spectra, 1980 Cumulative Index*, Philadelphia, PA, p. 881

Sakai, A., Miyata, N. & Takahashi, A. (1995) Initiating activity of quinones in the two-stage transformation of BALB/3T3 cells. *Carcinogenesis*, **16**, 477–481

Sanders, M.N. & Winkelmann, R.K. (1988) Cutaneous reactions to vitamin K. *J. Am. Acad. Dermatol.*, **19**, 699–704

Sann, L., Leclercq, M., Frederich, A., Bourgeois, J., Bethenod, M. & Bourgeay-Causse, M. (1985) Pharmacokinetics of vitamin K_1 in low-birth-weight neonates. *Dev. Pharmacol. Ther.*, **8**, 269–279

Sawada, M., Sofuni, T., Hatanaka, M. & Ishidate, M., Jr (1987) Induction of chromosome aberrations in active oxygen-generating systems. 4. Studies with hydrogen peroxide-resistant cells in culture (Abstract No. 52). *Mutat. Res.*, **182**, 376

Saxena, S.P., Fan, T., Li, M., Israels, E.D. & Israels, L.G. (1997) A novel role for vitamin K_1 in a tyrosine phosphorylation cascade during chick embryogenesis. *J. clin. Invest.*, **99**, 602–607

Schardein, J.L. (1993) *Chemically Induced Birth Defects*, 2nd Ed., New York, Marcel Dekker, p. 552

Schneider, D.L., Fluckiger, H.B. & Manes, J.D. (1974) Vitamin K_1 content of infant formula products. *Pediatrics*, **53**, 273–275

Schneiderman, M.A., Sharma, A.K., Mahanama, K.R.R. & Locke, D.C. (1988) Determination of vitamin K1 in powdered infant formulas, using supercritical fluid extraction and liquid chromatography with electrochemical detection. *J. Assoc. off. anal. Chem.*, **71**, 815–817

Schubiger, G., Tönz, O., Grüter, J. & Shearer, M.J. (1993) Vitamin K_1 concentration in breast-fed neonates after oral or intramuscular administration of a single dose of a new mixed-micellar preparation of phylloquinone. *J. pediatr. Gastroenterol. Nutr*., **16**, 435–439

Schubiger, G., Grüter, J. & Shearer, M.J. (1997) Plasma vitamin K_1 and PIVKA-II after oral administration of mixed-micellar or Cremophor EL-solubilized preparations of vitamin K_1 to normal breast-fed newborns. *J. pediatr. Gastroenterol. Nutr*., **24**, 280–284

Sharma, R.K., Marwaha, N., Kumar, P. & Narang, A. (1995) Effect of oral water soluble vitamin K on PIVKA-II levels in newborns. *Indian Pediatr*., **32**, 863–867

Shearer, M.J. (1992) Vitamin K metabolism and nutriture. *Blood Rev*., **6**, 92–104

Shearer, M.J. (1995) Fat-soluble vitamins. Vitamin K. *Lancet*, **345**, 229–234

Shearer, M.J. (1997) The roles of vitamins D and K in bone health and osteoporosis prevention. *Proc. Nutr. Soc*., **56**, 915–937

Shearer, M.J., Barkhan, P. & Webster, G.R. (1970) Absorption and excretion of an oral dose of tritiated vitamin K_1 in man. *Br. J. Haematol*., **18,** 297–308

Shearer, M.J., Mallinson, C.N., Webster, G.R. & Barkhan, P. (1972) Clearance from plasma and excretion in urine, faeces and bile of an intravenous dose of tritiated vitamin K_1 in man. *Br. J. Haematol*., **22**, 579–588

Shearer, M.J., McBurney, A. & Barkhan, P. (1974) Studies on the absorption and metabolism of phylloquinone (vitamin K_1) in man. In: Harris, R.S., Munson, P.L., Diczfalusy, E. & Glover, J., *Vitamins and Hormones, Advances in Research and Applications*, New York, Academic Press, Vol. 32, pp. 513–542

Shearer, M.J., Rahm, S., Barkhan, P. & Stimmler, L. (1982) Plasma vitamin K_1 in mothers and their newborn babies. *Lancet*, **ii**, 460–463

Shearer, M.J., McCarthy, P.T., Crampton, O.E. & Mattock, M.B. (1988) The assessment of human vitamin K status from tissue measurements. In: Suttie J.W., ed., *Current Advances in Vitamin K Research*, New York, Elsevier, pp. 437–452

Shearer, M.J., Bach, A. & Kohlmeier, M. (1996) Chemistry, nutritional sources, tissue distribution and metabolism of vitamin K with special reference to bone health. *J. Nutr*., **126** (Suppl. 4), 1181S–1186S

Shertzer, H.G., Låstbom, L., Sainsbury, M. & Moldéus, P. (1992) Menadione-mediated membrane fluidity alterations and oxidative damage in rat hepatocytes. *Biochem. Pharmacol.*, **43**, 2135–2142

Shinzawa, T., Mura, T., Tsunei, M. & Shiraki, K. (1989) Vitamin K absorption capacity and its association with vitamin K deficiency. *Am. J. Dis. Child*., **143**, 686–689

Smith, P.F., Alberts, D.W. & Rush, G.F. (1987) Menadione-induced oxidative stress in hepatocytes isolated from fed and fasted rats: The role of NADPH-regenerating pathways. *Toxicol. appl. Pharmacol*., **89**, 190–201

Society of Japanese Pharmacopoeia (1996) *The Japanese Pharmacopoeia JP XIII*, 13th Ed., Tokyo, p. 569

Soedirman, J.R., De Bruijn, E.A., Maes, R.A.A., Hanck, A. & Grüter, J. (1996) Pharmacokinetics and tolerance of intravenous and intramuscular phylloquinone (vitamin K_1) mixed micelles formulation. *Br. J. clin. Pharmacol*., **41**, 517–523

Solvonuk, P.F., Jaques, L.B., Leddy, J.E., Trevoy, L.W. & Spinks, J.W.T. (1952) Experiments with C^{14}-menadione (vitamin K_3). *Proc. Soc. exp. Biol. Med*., **79**, 597–604

Stoeckel, K., Joubert, P.H. & Grüter, J. (1996) Elimination half-life of vitamin K_1 in neonates is longer than is generally assumed: Implications for the prophylaxis of haemorrhagic disease of the newborn. *Eur. J. clin. Pharmacol*., **49**, 421–423

Sutor, A.H., von Kries, R., Cornelissen, M., McNinch, A.W. & Andrew, M. (1999) Vitamin K deficiency bleeding (VKDB) in infancy. *Thromb. Haemostasis*, **81**, 456–461

Suttie, J.W. (1985) Vitamin K. In: Diplock, A.T., ed., *Fat-soluble Vitamins. Their Biochemistry and Applications*, Lancaster, PA, Technomic Publishing Co., pp. 225–233, 295–311

Suttie, J.W. (1987) Recent advances in hepatic vitamin K metabolism and function. *Hepatology*, **7**, 367–376

Suttie, J.W. (1991) Vitamin K. In: Machlin, L.J., ed., *Handbook of Vitamins*, 2nd Ed., New York, Marcel Dekker, pp. 145–194

Suttie, J.W. (1995) The importance of menaquinones in human nutrition. *Ann. Rev. Nutr*., **15**, 399–417

Swiss Pharmaceutical Society, ed. (1999) *Index Nominum, International Drug Directory*, 16th Ed., Stuttgart, Medpharm Scientific Publishers [MicroMedex Online: Health Care Series]

Taggart, W.V. & Matschiner, J.T. (1969) Metabolism of menadione-6,7-^{3}H in the rat. *Biochemistry*, **8**, 1141–1146

Taylor, J.D., Millar, G.J. & Wood, R.J. (1957) A comparison of the concentration of C^{14} in the tissues of pregnant and nonpregnant female rats following the intravenous administration of vitamin K_1-C^{14} and vitamin K_3-C^{14}. *Can. J. Biochem. Physiol*., **35**, 691–697

Thierry, M.J. & Suttie, J.W. (1969) Distribution and metabolism of menadiol diphosphate in the rat. *J. Nutr*., **97**, 512–516

Thijssen, H.H.W. & Drittij-Reijnders, M.J. (1996) Vitamin K status in human tissues: Tissue-specific accumulation of phylloquinone and menaquinone-4. *Br. J. Nutr*., **75**, 121–127

Thomas, J., ed. (1998) *Australian Prescription Products Guide*, 27th Ed., Victoria, Australian Pharmaceutical Publishing Co., Vol. 1, pp. 687, 1562–1563, 1566, 1570, 1908–1909

Thorp, J.A., Gaston, L., Caspers, D.R. & Pal, M.L. (1995) Current concepts and controversies in the use of vitamin K. *Drugs*, **49**, 376–387

Tikkanen, L., Matsushima, T., Natori, S. & Yoshihira, K. (1983) Mutagenicity of natural naphthoquinones and benzoquinones in the *Salmonella*/microsome test. *Mutat. Res*., **124**, 25–34

Tönz, O. & Schubiger, G. (1988) [Neonatal vitamin K prophylaxis and vitamin K deficiency hemorrhage in Switzerland 1986–1988.] *Schweiz. med. Wochenschr*., **118**, 1747–1752 (in German)

Toxopeus, C., van Holsteijn, I., Thuring, J.W.F., Blaauboer, B.J. & Noordhoek, J. (1993) Cytotoxicity of menadione and related quinones in freshly isolated rat hepatocytes: Effects on thiol homeostasis and energy charge. *Arch. Toxicol.*, **67**, 674–679

Uchida, K. & Komeno, T. (1988) Relationships between dietary and intestinal vitamin K, clotting factor levels, plasma vitamin K, and urinary Gla. In: Suttie J.W., ed., *Current Advances in Vitamin K Research*, New York, Elsevier, pp. 477–492

US Pharmacopeial Convention (1994) *The 1995 US Pharmacopeia*, 23rd Rev./*The National Formulary*, 18th Rev., Rockville, MD, pp. 946–948, 1224–1226

US Pharmacopeial Convention (1998) *USP Dispensing Information*, Vol. I, *Drug Information for the Health Care Professional*, 18th Ed., Rockville, MD, pp. 2984–2986

Usui, Y., Tanimura, H., Nishimura, N., Kobayashi, N., Okanoue, T. & Ozawa, K. (1990) Vitamin K concentrations in the plasma and liver of surgical patients. *Am. J. clin. Nutr*., **51**, 846–852

Van Arnum, S.D. (1998) Vitamin K. In: Kroschwitz, J.I. & Howe-Grant, M., eds, *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th Ed., New York, John Wiley & Sons, Vol. 25, pp. 269–283

Vervoort, L.M.T., Ronden, J.E. & Thijssen, H.H.W. (1997) The potent antioxidant activity of the vitamin K cycle in microsomal lipid peroxidation. *Biochem. Pharmacol*., **54**, 871–876

Vest, M. (1966) Vitamin K in medical practice: Pediatrics. *Vitam. Horm.*, **24**, 644–663

Watanabe, K., Sakamoto, K. & Sasaki, T. (1998) Comparisons on chemically-induced mutation among four bacterial strains, *Salmonella typhimurium* TA102 and TA2638, and *Escherichia coli* WP2/pKM101 and WP2 *uvrA*/pKM101: Collaborative study II. *Mutat. Res*., **412**, 17–31

Weber, F. & Rüttimann, A. (1996) Vitamins. 5. Vitamin K. In: Elvers, B. & Hawkins, S., eds, *Ullmann's Encyclopedia of Chemical Technology*, 5th Ed., Weinheim, VCH Verlagsgesellschaft, Vol. A27, pp. 488–506

Winterbourn, C.C., French, J.K. & Claridge, R.F.C. (1979) The reaction of menadione with haemoglobin. Mechanism and effect of superoxide dismutase. *Biochem. J.*, **179**, 665–673

Woods, J.A., Young, A.J., Gilmore, I.T., Morris, A. & Bilton, R.F. (1997) Measurement of menadione-mediated DNA damage in human lymphocytes using the comet assay. *Free Radic. Res*., **26**, 113–124

Yaguchi, M., Miyazawa, K., Katagiri, T., Nishimaki, J., Kizaki, M., Tohyama, K. & Toyama, K. (1997) Vitamin K2 and its derivatives induce apoptosis in leukemia cells and enhance the effect of all-*trans* retinoic acid. *Leukemia*, **11**, 779–787

Yang, Y.-M., Simon, N., Maertens, P., Brigham, S. & Liu, P. (1989) Maternal–fetal transport of vitamin K_1 and its effect on coagulation in premature infants. *J. Pediatr*., **115**, 1009–1013