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Dietary Reference Values for vitamin K

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Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) derives Dietary Reference Values (DRVs) for vitamin K. In this Opinion, the Panel considers vitamin K to comprise both phylloquinone and menaquinones. The Panel considers that none of the biomarkers of vitamin K intake or status is suitable by itself to derive DRVs for vitamin K. Several health outcomes possibly associated with vitamin K intake were also considered but data could not be used to establish DRVs. The Panel considers that Average Requirements and Population Reference Intakes for vitamin K cannot be derived for adults, infants and children, and therefore sets Adequate Intakes (AIs). The Panel considers that available evidence on occurrence, absorption, function and content in the body or organs of menaquinones is insufficient, and, therefore, sets AIs for phylloquinone only. Having assessed additional evidence available since 1993 in particular related to biomarkers, intake data and the factorial approach, which all are associated with considerable uncertainties, the Panel maintains the reference value proposed by the Scientific Committee for Food (SCF) in 1993. An AI of 1 µg phylloquinone/kg body weight per day is set for all age and sex population groups. Considering the respective reference body weights, AIs for phylloquinone are set at 70 µg/day for all adults including pregnant and lactating women, at 10 µg/day for infants aged 7–11 months, and between 12 µg/day for children aged 1-3 years and 65 µg/day for children aged 15–17 years.

Keywords: vitamin K, phylloquinone, menaquinones, factorial approach, Adequate Intake, Dietary Reference Value

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Summary

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a Scientific Opinion on Dietary Reference Values for the European population, including vitamin K.

Vitamin K represents a family of fat-soluble compounds with the common chemical structure of 3-substituted 2-methyl-1,4-napthoquinone. It naturally occurs in food as phylloquinone (vitamin K1) and menaquinones (vitamin K2). Phylloquinone has a phytyl side chain and is the primary dietary form of vitamin K in Europe: it is mainly found in dark green leafy vegetables (e.g. spinach, lettuce and other salad plants) and Brassica. Menaquinones are a group of compounds with an unsaturated side chain from 4 to 13 isoprenyl units (vitamin K2 or MK-n) and are found mainly in animal products such as meat, cheese and eggs. Apart from MK-4 that is formed via metabolic conversion of phylloquinone during its absorption in the intestinal mucosa and in other organs, menaquinones are produced by bacteria capable of food fermentation and specific anaerobic bacteria of the colon microbiota. In this Opinion, the Panel considers vitamin K to comprise both phylloquinone and menaquinones.

Vitamin K acts as a cofactor of the γ-glutamyl carboxylase (GGCX) that catalyses the carboxylation of glutamic acid (Glu) residues into γ-carboxyglutamic acid (Gla) residues in vitamin K-dependent proteins (Gla-proteins), which convert them into their active forms. These Gla-proteins are involved in different physiological processes, including blood coagulation or bone mineralisation. MK-7 may have a greater bioactivity compared to phylloquinone in stimulating γ-carboxylation, but the available data are insufficient to set different activity coefficients for phylloquinone and menaquinones.

In adults, vitamin K deficiency is clinically characterised by a bleeding tendency in relation to a low activity of blood coagulation factors, resulting in an increase in prothrombin time (PT) or partial thromboplastin time (or activated partial thromboplastin time). Symptomatic vitamin K deficiency and impairment of normal haemostatic control in healthy adults may take more than two to three weeks to develop at a 'low' phylloquinone intake (i.e. < 10 µg/day). Exclusively breastfed infants are susceptible to bleeding, due to the low vitamin K content of human milk and their small body pool of vitamin K. Administration of phylloquinone at a pharmacological dose, either orally or by intramuscular injection, is usual practice for prevention of haemorrhagic disease in newborn infants.

Phylloquinone is absorbed in the intestine in the presence of dietary fat. Studies on absorption of phylloquinone in healthy adults show widely variable results. The data for absorption of some dietary menaquinones (MK-4, MK-7 or MK-9) in comparison with phylloquinone are also limited. Absorption of menaquinones produced by gut bacteria in the distal intestine remains uncertain, and therefore their contribution to vitamin K status is unclear. The Panel considers that it is not possible to estimate precisely an average absorption of phylloquinone, menaquinones, and thus vitamin K from the diet.

After intestinal absorption, phylloquinone and individual menaquinones are transported into the blood by lipoproteins. The clearance of MK-7 and MK-9 from serum/plasma is slower than for phylloquinone. Vitamin K accumulates primarily in the liver, but is also present in bones and other tissues. The liver contains widely variable concentrations of phylloquinone and menaquinones. Vitamin K has a fast turnover in the body. In the liver, phylloquinone and menaquinones are catabolised to the same metabolites, excreted in bile and urine. Phylloquinone crosses the placenta in small quantities, whilst for menaquinones, this is unclear.

PT is the only vitamin K biomarker for which a change (increase) has been associated with vitamin K deficiency. Possible changes in the other biomarkers (concentration/activity of blood coagulation factors, blood concentration of the undercarboxylated forms of vitamin-K dependent proteins, blood concentration of vitamin K, urinary concentration of Gla residues or of the 5C and 7C metabolites) according to phylloquinone intake are difficult to interpret, as no cut-off value to define adequate

vitamin K status is available. There is no biomarker for which a dose-response relationship with phylloquinone intake has been established. Studies investigating the relationship between biomarkers and menaquinone intake often used doses much higher than the limited observed intake data available in Europe. Thus, the Panel concludes that none of these biomarkers is suitable by itself to assess vitamin K adequacy. The Panel also concludes that data are insufficient for deriving the requirement for vitamin K according to sex or for 'younger' and 'older' adults.

The Panel notes the uncertainties in the food composition data and available consumption data related to phylloquinone, individual menaquinones or vitamin K.

After having reviewed the available evidence, the Panel also concludes that available data on intake of phylloquinone or menaquinones and health outcomes cannot be used to derive DRVs for vitamin K.

The Panel considers a total body pool of phylloquinone of about 0.55 µg/kg body weight in healthy adults at steady state not to be associated with signs of vitamin K deficiency and to be a desirable body pool size for phylloquinone. The Panel notes that available data do not allow to estimate the daily dietary intake of phylloquinone required to balance total phylloquinone losses through urine and bile and to maintain an adequate body pool of phylloquinone. There is no data on the total body pool of menaquinones.

The Panel considers that Average Requirements and Population Reference Intakes for vitamin K cannot be derived for adults, infants and children, and therefore sets Adequate Intakes (AIs). The Panel considers that available evidence on intake, absorption, function and content in the body or organs of menaquinones is insufficient, thus sets AIs for phylloquinone only. Having assessed additional evidence available since 1993 related on biomarkers, intake data and the factorial approach, the Panel concludes that all possible approaches investigated to set DRVs for vitamin K are associated with considerable uncertainties and that the available scientific evidence is insufficient to update the previous reference value. Therefore, the Panel maintains the reference value proposed by the Scientific Committee for Food (SCF) in 1993. Thus, an AI of 1 µg phylloquinone/kg body weight per day is set for all age and sex population groups.

For adults, the Panel considers the respective reference body weights of men and women and after rounding up, sets the same AI of 70 µg phylloquinone/day. The Panel notes that the proposed AI in adults is close to the median phylloquinone intake of 76 µg/day in the 2012 German national survey that used updated phylloquinone composition data. The Panel considers that there is no evidence of different vitamin K absorption and different losses according to age in adults, thus sets the same AI for 'younger' and 'older' adults.

For infants and children, the Panel considers that the requirement for growth would be covered by an intake of 1 µg phylloquinone/kg body weight per day. Considering the respective reference body weights, and after rounding up, AIs for phylloquinone are set at 10 µg/day for infants aged 7-11 months, and between 12 µg/day for children aged 1–3 years and 65 µg/day for children aged 15-17 years.

For pregnant women, taking into account the mean gestational increase in body weight and the reference body weight of non-pregnant women, the AI set for pregnant women is the same as that for non-pregnant women obtained after rounding. For lactating women, the Panel considers that the AI of 1 µg/kg body weight per day of phylloquinone set for non-lactating women covers the small excretion of vitamin K in breast milk. Thus, the AI for pregnant or lactating women is set at 70 µg phylloquinone/day.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and if necessary to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on the nutrient and energy intakes for the European Community.[1] The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No. 178/2002,[2] the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on population reference intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, *trans* fatty acids;

[1] Scientific Committee for Food, Nutrient and energy intakes for the European Community, Reports of the Scientific Committee for Food 31st series, Office for Official Publication of the European Communities, Luxembourg, 1993.

[2] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1-24.

- Protein;
- Dietary fibre.

Following on from the first part of the task, EFSA is asked to advise on population reference intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

306 ASSESSMENT

307 ## 1. Introduction

308 In 1993, the Scientific Committee for Food (SCF) adopted an opinion on the nutrient and energy
309 intakes for the European Community (1993). For vitamin K, SCF (1993) did not set any average
310 requirement (AR) or population reference intake (PRI). The SCF considered that an intake of 1 µg/kg
311 body weight per day, provided by a usual mixed diet, is adequate.

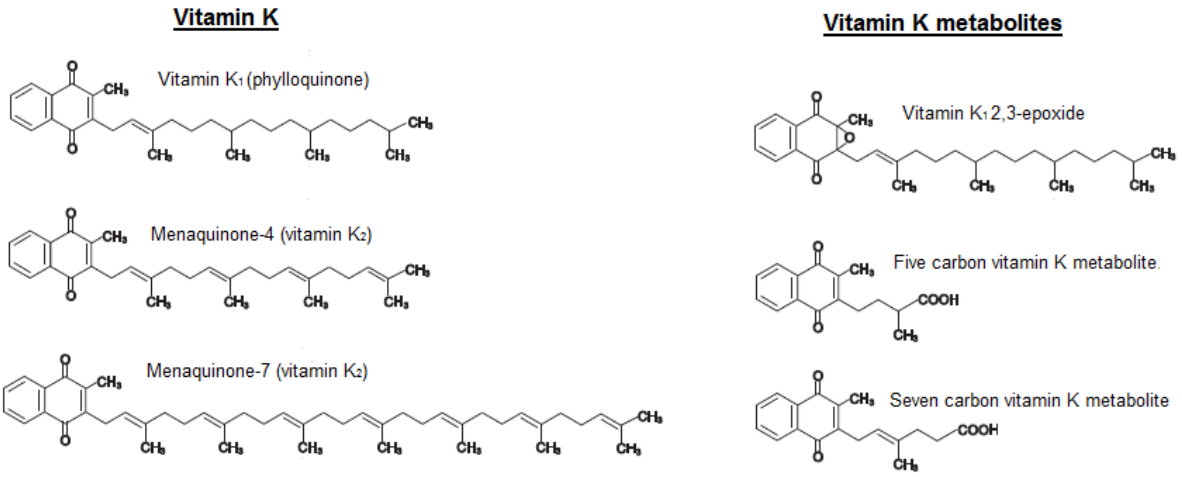
312 The purpose of this Opinion is to review dietary reference values (DRVs) for vitamin K. Vitamin K
313 naturally occurs in food as phylloquinone (vitamin K1) and menaquinones (vitamin K2, MK-n). The
314 Panel notes that dietary reference values set by other authorities and bodies (Section 4) are mainly
315 related to data on phylloquinone and that the role of MK-n in meeting vitamin K requirement is often
316 not considered. However, some new data are available on both types of components. Therefore, the
317 Panel considers that MK-n should be included, in addition to phylloquinone, in this assessment. In this
318 Scientific Opinion, the Panel considers that vitamin K comprises both phylloquinone and
319 menaquinones.

320 ## 2. Definition/category

321 The data discussed in this Opinion include data on vitamin K administered orally, but also parenterally
322 when the data provide additional information on the role of vitamin K in the body.

323 ### 2.1. Chemistry

324 Vitamin K represents a family of fat-soluble compounds with the common chemical structure
325 3-substituted 2-methyl-1,4-napthoquinone (Figure 1).



326

327 **Figure 1:** Chemical structures of vitamin K and metabolites
328 Molecular masses – Phylloquinone: 450.7 g/mol; MK-4: 444.7 g/mol; MK-7: 648.9 g/mol; 5C-metabolite: 272.3 g/mol;
329 7C-metabolite: 298.3 g/mol (see above).

330 **Phylloquinone** (also called *phytonadione* or *phytomenadione*) is from plant origin. It contains a phytyl
331 group and is the primary dietary form of vitamin K, mainly found in green leafy vegetable plants and
332 Brassica (Section 3.1).

333 **Menaquinones** are a group of compounds with unsaturated side chains of varying length (MK-n)[3]
334 from 4 to 13 isoprenyl units at the 3-position of the 2-methyl-1,4-napthoquinone group and found in

[3] MK-5 = 512.8 g/mol; MK-6 = 580.9 g/mol; MK-8 = 717.1 g/mol; MK-9: 785.2 g/mol; MK-10 = 853.4 g/mol; MK-11 = 921.5 g/mol; MK-12 = 989.6 g/mol; MK-13 = 1,057.7 g/mol

animal products such as meat, cheese and egg (Section 3.1).

Most menaquinones, i.e. the medium-chain and long-chain MK-n (MK-6 or higher) but not the short-chain MK-4 (also called *menatetrenone*), are produced by bacteria, including bacteria capable of food fermentation, gut bacteria in animals, and anaerobic bacteria of the human colon microbiota (Conly and Stein, 1992). In breast-fed infants, the production of menaquinones by gut microbiota is probably low, as most bacteria of their microbiota, including *Bifidobacterium*, *Lactobacillus* and *Clostridium* species, do not produce menaquinones; and with weaning, there is a progressive colonisation of the gut by MK-producing bacteria such as *Bacteroides fragilis* and *Escherichia coli* (Greer, 2010; Shearer et al., 2012). In humans, MK-4 is produced via metabolic conversion of phylloquinone during its absorption in the intestinal mucosa and in other organs (Section 2.3.5.).

Menadione (unsubstituted 2-methyl-1,4-napthoquinone, a chemical analogue of 1,4-naphthoquinone with a methyl group in the 2-position, and that is also called *vitamin K3*) is a water soluble synthetic form of vitamin K that plays a role as an intermediate in the metabolic conversion of phylloquinone to MK-4 (Section 2.3.5.). *Menadiol sodium phosphate* (also called *vitamin K4*) is a synthetic water-soluble form derived from menadione by reduction. *Dihydrophylloquinone* is present in foods made with partially hydrogenated fat like hydrogenated soybean oil (Section 3.1.).

2.2. Function of vitamin K

2.2.1. Biochemical functions

Vitamin K (i.e. either phylloquinone or menaquinones) acts as a cofactor of the enzyme γ-glutamyl carboxylase (GGCX) that catalyses the post-translational carboxylation of glutamic acid (Glu) residues into γ-carboxyglutamic acid (Gla) residues in the amino-terminal domain of different vitamin K-dependent proteins. This reaction converts these proteins, also called Gla-proteins, into their active form (Stafford, 2005). These proteins all display calcium-mediated actions, with the Gla residues located at their specific calcium binding sites (Ferland, 1998; Litwack, 2008).

One group of vitamin K-dependent proteins comprises blood coagulation factors, including factors II (prothrombin), VII, IX and X and the anticoagulant proteins C and S. These proteins are synthesised and secreted by the liver in inactive forms (with Glu residues), and are converted in the blood to their active forms (with Gla residues) by GGCX, in the presence of vitamin K. The protein induced by vitamin K absence or antagonism-II (PIVKA-II), the precursor of the active coagulation protein prothrombin, has ten Glu residues that are carboxylated to Gla residues, leading to the formation of prothrombin. After the formation of Gla residues and in the presence of calcium ions, the clotting factors bind to phospholipids at the surface of the membrane of platelets and endothelial cells, where they form membrane-bound complexes with other clotting cofactors, and these complexes are cleaved after coagulation is initiated in the plasma.

Another group of vitamin K-dependent proteins include e.g. osteocalcin (OC), matrix γ-carboxyglutamic acid protein (MGP), and growth arrest-specific protein 6 (GAS 6), synthesised by osteoblasts or other tissues (e.g. vascular smooth muscle cells for GAS 6 and MGP, chondrocytes for MGP). Osteocalcin, one of the most abundant non-collagenous proteins in bone, is involved in bone mineralisation, and some authors suggest that osteocalcin, MGP and GAS 6 may be involved in the control of soft tissue calcification, but this remains questionable (Ferland, 1998; Bellido-Martin and de Frutos, 2008; Danziger, 2008; Booth, 2009; Shiozawa et al., 2010; Walther et al., 2013).

During the γ-glutamyl carboxylation of vitamin K-dependent proteins, the active (reduced) form of vitamin K (hydroquinone) is converted to vitamin K epoxide, the oxidized form of vitamin K, that is subsequently reduced back to hydroquinone (Furie et al., 1999; Tie et al., 2005). This redox cycle, called vitamin K cycle, takes place in different tissues, particularly in the liver and bone. It involves the integral membrane enzymes GGCX and vitamin K epoxide reductase (VKOR), acting on membrane-bound vitamin K (Stafford, 2005; Tie et al., 2005; Oldenburg et al., 2008; Tie and Stafford,

2008; Wu et al., 2011). VKOR controls a critical step of the vitamin K cycle that is blocked by warfarin and is at the bottom of warfarin's anticoagulant activity (Garcia and Reitsma, 2008). Unlike in adults, vitamin K epoxide is detectable in newborn cord plasma, and may reflect 'low' concentrations of VKOR (Bovill et al., 1993). Infants born with a rare genetic deficiency of VKOR may present with severe coagulopathy and/or skeletal defects (Oldenburg et al., 2000).

Data on *in vitro* and *in vivo* animal experiments suggest that vitamin K is involved in the down-regulation of expression of genes involved in acute inflammatory response (Ohsaki et al., 2006). The mechanisms (Hanck and Weiser, 1983; Reddi et al., 1995; Li et al., 2003) and relevance in humans (Juanola-Falgarona et al., 2013) are unclear.

MK-n have the same function as phylloquinone (γ-carboxylation), but MK-7 may have a greater bioactivity compared to phylloquinone in stimulating γ-carboxylation. A cross-over study (n = 18), using equimolar doses of either phylloquinone or MK-7 (0.22 µmol/day[4]) as supplements consumed with a meal for 6 weeks (with a wash-out period of 12 weeks), showed that MK-7 induced a higher ratio of serum γ-carboxylated OC/undercarboxylated OC (cOC/ucOC) compared to phylloquinone (Schurgers et al., 2007). Another cross-over study in the same paper (n = 12), which used the vitamin K γ-carboxylation antagonist acenocoumarol with weekly-increasing oral doses of either phylloquinone or MK-7 as supplements (0–500 and 0–285 µg/day, respectively, with a wash-out period of two weeks), showed that MK-7 was about 2.5 times more potent than phylloquinone to counter-act the effect of acenocoumarol (i.e. 130 versus 315 µg/day, respectively, to obtain a comparable effect).

The Panel notes that dietary vitamin K (i.e. either phylloquinone or menaquinones) acts as cofactor of the enzymatic conversion of vitamin K-dependent proteins (Gla-proteins) into their active form, by carboxylation of Glu residues to Gla residues in the amino-terminal domain. These proteins are involved in different physiological processes, including blood coagulation, bone mineralisation and possibly control of soft tissue calcification. The Panel also notes that MK-7 may have a greater bioactivity compared to phylloquinone in stimulating γ-carboxylation, but that the available data are insufficient to set different activity coefficients for phylloquinone and menaquinones.

2.2.2. Health consequences of deficiency and excess

2.2.2.1. Deficiency

In adults, vitamin K deficiency is clinically characterised by a bleeding tendency in relation to a low activity of the blood coagulation factors. This can be demonstrated by a vitamin K-responsive increase in prothrombin time (PT) or partial thromboplastin time (PTT also called activated partial thromboplastin time, APTT). PT and PTT are indicators of the activity of the extrinsic and intrinsic coagulation pathways, respectively, assessed by the time it takes for a fibrin clot to form. More information on the sensitivity of the PT test compared to other biomarkers is provided in Section 2.4.

In ten healthy subjects fed for three weeks a diet considered as free of vitamin K by the authors (and that probably contained less than 10 µg/day vitamin K), there was an increase in average weekly PT (from 14.8 to 16 s, $p < 0.05$) (Udall, 1965). Other depletion/repletion studies however showed that healthy adults fed diets containing 5–10 µg phylloquinone/day for two weeks showed no change in coagulation time, either measured by PT or PTT (Allison et al., 1987; Ferland et al., 1993) (n = 33 and 32, respectively). A study in ten adult patients with apoplexy unable to eat and with parenteral administration of vitamins without vitamin K, showed after 21 to 28 days prolonged PTs (assessed by % Quick test) in seven patients treated with antibiotics ('affecting the intestinal flora') but not in the three subjects not treated with antibiotics (Frick et al., 1967). This induced deficiency responded to increasing phylloquinone doses administered intravenously, from which the authors concluded that the amount of phylloquinone needed to restore a normal Quick value is between 0.03 and 1.5 µg/kg body

[4] 99 and 143 µg/day, respectively.

weight per day phylloquinone. The Panel notes that these studies suggest that symptomatic vitamin K deficiency and impairment of normal haemostatic control in healthy adults may take more than two to three weeks to develop at 'low' phylloquinone intake (i.e. < 10 µg/day).

Exclusively breastfed infants are more susceptible to bleeding than formula-fed infants (Shearer, 2009), due to the low phylloquinone content of human milk (Section 2.3.6.3.) compared to infant formulas, which usually provide average daily intakes of about 50 µg of phylloquinone (50-fold higher than human milk) (Greer et al., 1991). Phylloquinone concentrations were undetectable in cord blood of infants of unsupplemented mothers unless the pregnant women received phylloquinone intravenously before delivery (Shearer et al., 1982). Liver tissue contents of phylloquinone and of menaquinones in neonates are low (MK-n were undetectable until 14 days post partum), although these low vitamin K stores seem to be sufficient to maintain normal haemostasis during fetal life (von Kries et al., 1988) (Section 2.3.4.3). Incidence rates of vitamin K deficiency bleeding (VKDB) in infants not given vitamin K prophylaxis have been reviewed (Sutor et al., 1999; Zipursky, 1999; Shearer, 2009). Studies cited in these reviews reported that the incidence of early VKDB (< 24 h of life) ranged from less than 6 to 12% of births and that the incidence of classical VKBD (first week of life) ranged from $5.4/10^5$ births to 1.7% of births in Western European countries, and between $25/10^5$ births and 0.9% in Africa and South-East Asia. The incidence of late VKDB (after the first week of life, up to 6 months, with a peak at 3–8 weeks of life) was reported to range from 4.4 to $7.2/10^5$births in Western European countries, and from 10.5 to $72/10^5$ births in South East Asia (Japan and Thailand). The relative risk (RR) for developing late VKDB is estimated to be 81 times greater for infants not given vitamin K prophylaxis (McNinch and Tripp, 1991). The incidence of VKDB declines at 12 weeks of age, and spontaneous bleeding beyond that age is rare and as a rule limited to lipid malabsorption syndromes.

Administration of phylloquinone at a pharmacological dose, either orally or by intramuscular injection, is usual practice for prevention of haemorrhagic disease in newborn infants (Clarke et al., 2006; Busfield et al., 2007; Strehle et al., 2010; Mihatsch et al., 2016). Oral pharmacological doses of MK-4 (2 mg at birth, and 4 mg at one week of age, n = 72,000) have been successfully used in newborns for prophylaxis of haemorrhagic diseases in Japan (Matsuzaka et al., 1987).

More recently, studies have investigated possible relationships between 'low' vitamin K intake and abnormal calcification including osteoporosis or arterial calcification (as reviewed in Kaneki et al. (2006) and Vermeer and Braam (2001)) and possible associations between plasma phylloquinone and the risk of osteoarthritis (Neogi et al., 2006). This is discussed further in Sections 2.4. and 5.2.

2.2.2.2. Excess

The SCF (2003a) reviewed data on phylloquinone and identified two studies in humans (Craciun et al., 1998; Booth et al., 1999b), which showed no evidence of adverse effects associated with supplementation up to 10 mg/day for one month. The SCF considered that these limited human data are supported by animal studies, which showed no adverse effect after daily administration of 2,000 mg/kg body weight for 30 days. The SCF concluded that there was no appropriate evidence to derive a tolerable upper intake level (UL) for vitamin K. The Panel notes that revising the UL for vitamin K is not within the scope of the present Opinion.

A review showed that prophylactic vitamin K administration to newborns of supraphysiological parenteral doses (ranging from 0.2 mg/kg to a 1 mg bolus dose) can induce mean/median serum phylloquinone concentrations in the first week of life up to 1,000-fold higher than non-fasting adult 'normal' values (Clarke, 2010). However, in studies in term or preterm infants investigating different doses of parenteral vitamin K prophylaxis, the increase in production of vitamin K metabolites, of vitamin K recycling and of vitamin K catabolic pathways (Sections 2.2.1. and 2.3.5.), showed that infants are capable of metabolising large vitamin K doses (Clarke et al., 2006; Harrington et al., 2010). No adverse effect has been reported with these high prophylactic doses.

2.3. Physiology and metabolism

The way dietary vitamin K is absorbed and transported in the body is complex (Figure 2).

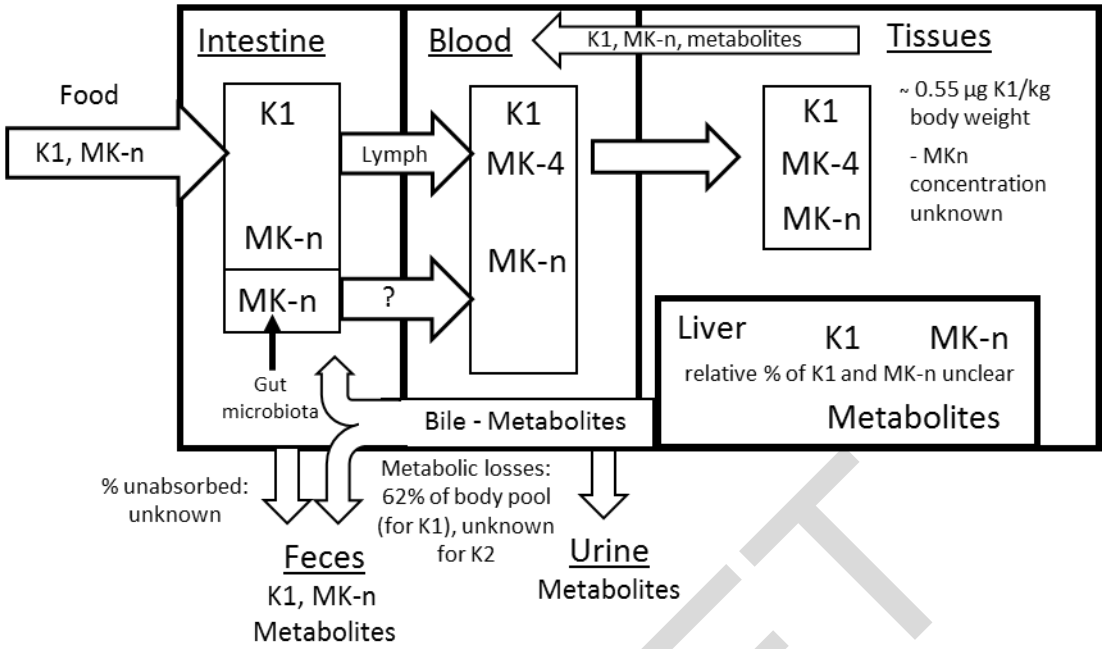


Figure 2: Metabolism of vitamin K in adults.
Legend: K1: phylloquinone, K2: MK-n: menaquinones. Absorption of menaquinones synthesised from gut microbiota in the large intestine remains uncertain (hence the '?' in the figure) (Section 2.3.1.).

2.3.1. Intestinal absorption

2.3.1.1. Intestinal absorption of phylloquinone

Phylloquinone is absorbed in the intestine, together with lipophilic compounds, and in the presence of dietary fat in a process that includes bile salts and requires proper pancreatic function for uptake of mixed micelles into the enterocytes and packaging with dietary lipids into nascent chylomicron particles (Blomstrand and Forsgren, 1968; Shearer et al., 1974; Shearer et al., 2012). Absorption of phylloquinone depends on the food/meal matrix, as shown by differences in absorption of ^{13}C-labelled phylloquinone from a supplement consumed with different types of meals (Jones et al., 2009).

Studies investigating phylloquinone absorption in (usually small) samples of healthy adults, generally based on measurements of phylloquinone concentration in blood, differ in design. They used a variety of forms of phylloquinone (free or naturally present in various plant foods), of modes of preparation and administration (foods either cooked or fresh, with or without fat, supplements consumed with or without a meal), of phylloquinone intakes, or of experimental methods (isotope-labelled or unlabelled phylloquinone, kinetic model, area-under-the-curve AUC).

Absorption of **free phylloquinone from a supplement** ranges from **13 ± 9%** (mean ± standard deviation (SD), range 2-26%) to about **80%** of the ingested dose in two studies. The lower value was calculated from a kinetic study using labelled phylloquinone in oil and given as gelatine capsules without a meal, and measuring plasma phylloquinone concentration (Jones et al., 2008) (Section 2.3.4.). The higher value was obtained from the measurement of unchanged phylloquinone out of the total amount of radioactivity (unchanged form and metabolites) recovered from the faeces, after ingestion of labelled phylloquinone mixed with detergent solubilised phylloquinone and given as a supplement consumed with a meal containing fat, as discussed in the review by Shearer et al. (1974).

Mean relative absorption of unlabelled **phylloquinone naturally present** in plant foods (broccoli, spinach or lettuce; fresh or cooked, with or without fat), assessed as plasma AUC, ranged from approximately **4%** to about **60–64%** of the absorption of free phylloquinone in three studies. These studies used a variety of comparators (exogenous free phylloquinone added to the oil consumed with a baseline diet that also contained phylloquinone from foods, detergent-solubilised free phylloquinone supplement or free phylloquinone from a tablet) that were all efficiently absorbed as indicated by their respective AUCs. The lower mean relative absorption of 4.1% referred to the absorption of 1 mg phylloquinone from cooked spinach without butter (Gijsbers et al., 1996), while the higher mean relative absorption of about 60–64% referred to the absorption of 377 µg phylloquinone/day from cooked broccoli (consumed daily for five days) in a baseline diet, in different age groups (Booth et al., 2002). A third study provided intermediate mean relative absorption values (Garber et al., 1999). Compared to a tablet providing 500 µg phylloquinone consumed with fat (27% energy), mean relative absorptions were about **17%** for 150 g fresh spinach (450 µg phylloquinone) but about **9%** for 50 g fresh spinach (165 µg phylloquinone) both consumed with fat (about 25% of energy) (significant difference between their respective AUC, $p < 0.05$). Mean relative absorptions were about **14%** for fresh broccoli (214 µg phylloquinone) and about **23%** for the same amount of cooked broccoli (184 µg phylloquinone) both consumed with fat in a meal (about 30% energy) (no significant difference in their respective AUC). Mean relative absorptions were about **11%** for fresh romaine lettuce (179 µg phylloquinone) consumed with fat in a meal (30% of energy) and about **16%** for the same amount of fresh lettuce (179 µg phylloquinone) consumed with more fat (45% of energy) (no significant difference in their respective AUC).

Absorption of **phylloquinone** (70 µg) **present in intrinsically labelled cooked kale** consumed with 30 g oil was calculated to be **4.7 ± 4.8%** (mean ± SD, range 1–14%) or **7%**. The first value was obtained from a kinetic study in subjects who consumed a diet providing daily 119 µg phylloquinone per 8.4 MJ during one week prior to kale ingestion and during the blood collection of about four weeks (Novotny et al., 2010) (Sections 2.3.4. and 5.1.1.5.), while the second value was obtained from a study in one man who consumed a controlled diet of unknown phylloquinone content (Kurilich et al., 2003).

Relative absorption of phylloquinone (1 mg) from cooked spinach was enhanced up to about three times (i.e. to 13.3%) by dietary fat (butter) (Gijsbers et al., 1996), but this was not observed with fresh lettuce consumed with different fat intakes (Garber et al., 1999).

No significant sex differences (Jones et al., 2009) or age differences in adults (Booth et al., 2002) in phylloquinone absorption were observed (no data on phylloquinone absorption in infants or children are available).

2.3.1.2. Intestinal absorption of menaquinones

The contribution of medium and long-chain **menaquinones produced by gut microbiota** to vitamin K status is unclear, as they are probably not easily absorbed from the distal bowel (Conly and Stein, 1992; Shearer, 1992). Menaquinones produced by the gut microbiota are not utilised in sufficient amounts to compensate for experimental dietary phylloquinone depletion in subjects not using antibiotics, as demonstrated by observed changes in vitamin K biomarkers during phylloquinone depletion (Paiva et al., 1998; Booth et al., 2001; Booth et al., 2003a) (Section 2.4.).

In healthy adults, absorption of **MK-4, MK-7 or MK-9** has been studied in comparison with phylloquinone (either free or in plant food), based on measurements of peak serum concentration and/or AUC. As phylloquinone in plant foods is tightly bound to chloroplasts in plant cells (Manzotti et al., 2008; Reumann, 2013), thus not easily available for absorption when plant foods are ingested, the description below focusses on the results of the comparison with free phylloquinone.

MK-4 and MK-9 are less well absorbed than free phylloquinone (Gijsbers et al., 1996; Schurgers and Vermeer, 2002). The designs of these studies differed, as e.g. MK-4 and MK-9 were provided as free forms (consumed with fat and with or without a meal) and free phylloquinone was either consumed

with fat within a meal or from a supplement containing detergent-solubilised phylloquinone consumed without a meal.

MK-7 is more absorbed than free phylloquinone (Schurgers and Vermeer, 2000; Schurgers et al., 2007). The designs of these studies differed, as e.g. MK-7 was consumed either in a food (natto) or as a supplement, free phylloquinone was consumed either in a detergent-solubilised form within a meal with fat, or as a supplement in a meal of unspecified fat content, and vitamin K was given as a single dose or over several weeks.

MK-7 is more absorbed than MK-4, each provided as a single supplement dose (gelatine capsules) consumed with a meal containing fat (Sato et al., 2012).

2.3.1.3. Conclusions on intestinal absorption

The Panel notes that data on **phylloquinone** absorption in healthy adults, measured from different food sources and matrices, are variable, that absorption of phylloquinone from cooked plant foods may be enhanced by dietary fat by up to three-fold, and that limited data suggest no significant sex or age differences in phylloquinone absorption in adults.

The Panel notes that all the studies that used the **AUC** approach to assess relative absorption of **phylloquinone naturally present in cooked or fresh plant foods (with or without fat)** had a sufficient duration of serum/plasma phylloquinone measurements to calculate the AUC (9 to 24 h) (Gijsbers et al., 1996; Garber et al., 1999; Booth et al., 2002). Assuming, as reference, 80% absorption for free phylloquinone (as a supplement consumed with fat (Shearer et al., 1974)), the Panel estimated from these three studies an absolute value of mean absorption of **about 3% to 50%**. The Panel also notes that absorption assessed by AUC of plasma concentration or assessed by the peak concentration can be underestimated, as the peak concentration value is influenced not only by absorption, but also by disposal and elimination rate. The Panel also notes that the results do not allow a direct measurement of an absolute value of phylloquinone absorption as no fractional absorption rate can be calculated from these studies. Other data on **intrinsically labelled cooked kale consumed with fat** showed that absorption of **phylloquinone** from plant food was about **5–7%** (Kurilich et al., 2003; Novotny et al., 2010)**.** Mean absorption of **free phylloquinone from a supplement** ranges from **13%** (provided in oil in a hydrophilic matrix, i.e. gelatin, without a meal (Jones et al., 2008)) to about **80%** (mixed with detergent solubilised phylloquinone and given as a supplement consumed with a meal containing fat (Shearer et al., 1974)).

The Panel notes that absorption of **menaquinones produced by gut bacteria** in the distal intestine remains uncertain, and therefore the contribution of medium and long-chain menaquinones produced by gut microbiota to vitamin K status is unclear. For **dietary menaquinones**, the Panel considers that available results indicate that MK-4 and MK-9 are less efficiently absorbed, and MK-7 is more efficiently absorbed, than synthetic free phylloquinone; however, MK-7 does not contribute much to MK-n intake in Europe (Section 3.2.2.). The Panel notes that these results are based on studies using serum concentrations (peak concentration or AUC) of menaquinones and phylloquinone that are known to have different kinetics in plasma (Section 2.3.2.), and that these results do not allow to directly quantify MK-4, MK-7 or MK-9 absorption as, again, no fractional absorption rate can be calculated.

The Panel considers that it is not possible to estimate precisely an average absorption of **phylloquinone**, **menaquinones**, and thus **vitamin K** from the diet.

2.3.2. Transport in blood

The predominant circulating form of vitamin K in blood is phylloquinone (Hodges et al., 1993a; Thijssen et al., 2002; Gentili et al., 2014), except in populations with high intakes of MK-7 as in Japan (Tsugawa et al., 2006).

After intestinal absorption, radiolabeled **phylloquinone** first appears in the lymph (Blomstrand and Forsgren, 1968) and then enters the blood stream incorporated in chylomicrons (Shearer et al., 1970a). No specific carrier protein for phylloquinone in blood has been identified. Its main transporters during the postprandial phase of absorption are triglyceride (TG)-rich lipoproteins (TRL) (about 75–90% of plasma phylloquinone), primarily chylomicron remnants and very low-density lipoproteins (VLDL) (Kohlmeier et al., 1996; Lamon-Fava et al., 1998; Schurgers and Vermeer, 2000, 2002; Erkkila et al., 2004). The remainder is approximately equally distributed between low- and high-density lipoproteins (LDL and HDL), with lesser amounts in the intermediate-density lipoprotein (IDL) fraction.

Studies on ingestion of labelled or unlabelled phylloquinone show that it peaks in plasma/serum about 4–10 h after ingestion and it peaks in the TRL fraction 3 h later than the TG present in the test meal (Shearer et al., 1970a; Lamon-Fava et al., 1998; Schurgers and Vermeer, 2000; Dolnikowski et al., 2002; Schurgers and Vermeer, 2002; Kurilich et al., 2003; Erkkila et al., 2004; Fu et al., 2009; Novotny et al., 2010). Phylloquinone half-life ($t_{1/2}$) in plasma has been determined to range between 0.22–8.80 h, depending on studies, study durations and methodologies (Shearer et al., 1972; Shearer et al., 1974; Bjornsson et al., 1979; Schurgers and Vermeer, 2000; Olson et al., 2002; Jones et al., 2008; Novotny et al., 2010) (Section 2.3.5.).

After ingestion of equimolar doses (2 µmol[5]) of phylloquinone, MK-4 and MK-9, all dissolved in a meal containing fat, serum **MK-4** peaked at 2 h at the same time as the peak of TGs from the test meal, then was transferred to LDL and then to HDL (Schurgers and Vermeer, 2002). Serum phylloquinone and **MK-9** peaked at 4 h and 5 h, respectively. MK-9 was found only with LDL but not in HDL. Phylloquinone or MK-4 disappeared from the circulation overnight, while MK-9 serum concentration after 24 h was still about 25% of the peak value and remained detectable until the last measurement at 48h (Schurgers and Vermeer, 2002). After ingestion of 3.1 µM of **MK-7** in the form of natto compared to 3.5 µM phylloquinone in the form of spinach and consumed with fat,[6] serum phylloquinone and MK-7 peaked at 6 h following consumption and a quick disappearance of phylloquinone from serum was observed within 24 h while MK-7 showed complex (biphasic) pharmacokinetics in serum and remained detectable for at least 72 h (Schurgers and Vermeer, 2000). After ingestion of equal quantities of phylloquinone and MK-7 (1 mg of each) in oil within a meal containing fat, the peak values were seen at about 4 h after the meal, and serum phylloquinone declined by 86% in the following 4 h, while MK-7 showed a biphasic decline and was still present at 96 h (Schurgers et al., 2007).

The Panel notes that the main transporters of phylloquinone are TRL, and that menaquinones are also transported by lipoproteins. The Panel also notes that phylloquinone and individual menaquinones have different kinetics in serum/plasma, and that the clearance of MK-7 and MK-9 from serum/plasma is slower (48-96 h) than for phylloquinone.

2.3.3. Distribution to tissues

The **liver** is the primary organ that efficiently accumulates absorbed **phylloquinone** transported in chylomicrons (Section 2.3.4.). The uptake of chylomicron remnants by the liver involves different apolipoproteins and high-affinity lipoprotein receptors that mediate internalization of the lipoprotein particles (Cooper, 1997). There is no conclusive information on the mechanism of uptake of **menaquinones** by the liver.

Bone matrix contains several vitamin K-dependent proteins synthesised by the osteoblasts (Section 2.2.1.), and vitamin K (phylloquinone and menaquinones) needs to be transported to osteoblasts for the γ-glutamyl carboxylation of these proteins. Osteoblasts and osteoblast-like cells are able to internalise **phylloquinone** from various lipoprotein fractions, as shown with human cell lines (Newman et al., 2002; Niemeier et al., 2005) and reviewed by Kohlmeier et al. (1996). The

[5] i.e. 0.90 mg phylloquinone, 0.89 mg MK-4 and 1.57 mg MK-9.
[6] i.e. about 1.6 mg phylloquinone and 2 mg MK-7.

mechanism of cellular uptake of phylloquinone associated with TRL in the bone is dependent on both heparan sulfate proteoglycans (HSPG) and ApoE (Newman et al., 2002) and human osteoblasts express several receptors: the LDL receptor, the LDL receptor-related protein 1, and to a lesser degree the VLDL receptor (Niemeier et al., 2005). There is no information on the mechanism of uptake of **menaquinones** by bones.

During pregnancy, only small quantities of **phylloquinone** cross the **placenta** from mother to fetus (Greer, 1995). Blood concentrations of phylloquinone in the full-term newborn are about half of that of the mothers and the phylloquinone concentration in cord blood is low (< 0.1 nmol/L) (Shearer et al., 1982; Pietersma-de Bruyn and van Haard, 1985; Greer et al., 1988; Mandelbrot et al., 1988). No information is available on the amount of **menaquinones** crossing the placenta.

2.3.4. Storage

2.3.4.1. Kinetic studies on the total body pool of phylloquinone

A kinetic study involved seven healthy US adults (3 women and 4 men; mean ± SD: 46 ± 14 years, 71 ± 8 kg mean body weight), who received a controlled diet providing daily 119 µg phylloquinone per 8.4 MJ (Novotny et al., 2010) (Section 2.3.1.). Blood samples were taken on the intervention day and then for about four weeks. Intervention consisted of a single serving of labelled kale (equivalent to 70 µg unlabelled phylloquinone). A modelling of phylloquinone kinetics was developed, considering three compartments (for the gastrointestinal tract, the plasma and a body tissue pool). The authors used this compartmental modelling to determine the vitamin K utilisation rate and tissue storage pool, considering US mean body weights of 86 and 74 kg, and plasma phylloquinone concentrations of 1.43 and 1.47 nmol/L for men and women respectively (as reported in Booth et al. (1997); McDowell et al. (2005)). The model indicated 'tissue storage pools' of 41 and 46 µg phylloquinone for women and men, respectively (or 0.55 and 0.53 µg/kg body weight, respectively).

In another kinetic study (Olson et al., 2002), seven healthy subjects (six men including five followed as in-patients in a metabolic unit, and one woman, aged 22–49 years) consumed a diet (control period) providing a mean phylloquinone intake of 75 µg/day for one to two weeks (n = 7). Then they consumed a 'low-vitamin K' diet providing a mean of 8 µg phylloquinone/day (n = 5 out of 7 subjects[7]) for three weeks (n = 2) to eight weeks (n = 3, whose average body weight was about 72 kg (read on figure)). Both diets provided a mean energy intake of about 8–12.8 MJ/day. Subjects received 0.3 µg isotopic-labelled phylloquinone administered intravenously at the end of each period, and provided blood, urine and faeces samples for six days after each injection (Section 2.3.6). Based on a two-compartment model, dilution of labelled phylloquinone indicated that the mean (± SD) total body pool of phylloquinone in the control or 'low-vitamin K' periods were 87.6 (± 55.6) µg and 44.7 (± 25.1) µg, respectively. However, according to the authors, plasma phylloquinone (used in the calculation of the body pool) was overestimated[8] due to the presence of an interference inherent to the analytical method used (method of Ueno and Suttie (1983)). Taking into account the 'lower' values for plasma phylloquinone, considered by the authors as more accurate, and the body weights of the participants (not reported for all), the authors calculated that the mean 'exchangeable body pool size' in subjects on the control diet would drop from 1.14 (SD 0.64) µg/kg to 0.57 (SD 0.32) µg/kg body weight. The Panel notes that the results were similar to the results by Novotny et al. (2010) and that the study has several limitations.

Ten healthy men and women (aged 22–31 years, mean body weight of 61 ± 10.7 kg), consumed ^{13}C-labelled phylloquinone (3 times 3 µg per day) with food (phylloquinone intake from food not provided) for six days and then received a single intravenous dose of either 6 µg (n = 6) or 30 µg (n = 4) phylloquinone plus an oral dose of 4 µg ^{2}H-labelled phylloquinone (Jones et al., 2008) (Section 2.3.1.). Blood samples were collected the day before and on the day of the intravenous

[7] Two subjects dropped-out before the end of the phylloquinone restriction.
[8] Plasma phylloquinone concentration in the range of 0.82–3.33 nmol/L on the control diet.

phylloquinone injection over 6 hours post-dose. Phylloquinone in plasma was measured by high performance liquid chromatography (HPLC) and isotope ratios by gas chromatography/mass spectrometry (GC/MS). The use of a two-compartment model to calculate the total body pool size of phylloquinone resulted in a mean of 2.3 µg (or 0.04 µg/kg body weight). The Panel notes the shorter length of measurements (6 h post-dose) compared to the other studies, the different design, the absence of information on phylloquinone intake from food, and that this 'total body pool size' of phylloquinone appears to be underestimated.

Another study aimed to investigate, in four men receiving intravenous doses of radiolabelled phylloquinone, the potential interaction between clofibrate and warfarin on vitamin K disposition (Bjornsson et al., 1979). The authors indicate that the pool size of vitamin K in the body is 'small' but could not be calculated for these subjects.

The Panel notes the uncertainties and methodological limitations of the studies by Jones et al. (2008) and Bjornsson et al. (1979), and considers that no conclusion can be drawn from these two studies to assess the total body pool of phylloquinone.

2.3.4.2. Measurements of phylloquinone and menaquinones in the liver of adults

In livers obtained by autopsy (Rietz et al., 1970; Duello and Matschiner, 1972), MK-7, MK-8, MK-10 and MK-11 were identified (as well as MK-4 and MK-9 in Duello and Matschiner (1972)). The authors approximated phylloquinone content to be about 50% of the total amount of vitamin K in the liver on a weight basis, visually from relative intensity of thin-layer chromatographic detection (Rietz et al., 1970) or 'nearly one-half' of vitamin K in the liver i.e. about 60 ng/g of wet liver weight, as assessed by thin-layer chromatography and mass spectrometry (Duello and Matschiner, 1972). The Panel notes that the method in these two studies does not allow a quantitative estimation of phylloquinone and menaquinone concentrations in the liver.

In livers obtained by autopsy or donated for transplantation (thus with no information on previous intake), vitamin K concentration was assessed by HPLC in three studies. Concentration in ng/g, and the ratio between phylloquinone and MK-n on a molar basis, were either reported or recalculated:

- The phylloquinone concentration in livers of 32 adults showed a wide range between 1.1 and 21.3 ng/g wet liver weight, whilst the medians of 5.5 ng/g for men and 5.4 ng/g for women were quite similar (Shearer et al., 1988). The same authors also describe a semi-quantitative analysis of menaquinones (i.e. by HPLC and comparison of peak area with that of phylloquinone) of 10 liver samples of adults. Menaquinones accounted for (median, range) 92% (75-97%) of the total amount of vitamin K in the liver on a molar basis. Chromatographic profiles of 17 livers of adults showed MK-6, MK-7, and MK-8 to MK-11 to be present.
- The mean concentration of phylloquinone in livers of three adults was 34 ng/g liver (range: about 8-83 ng/g) and that of menaquinones (MK-4 and MK-7 to MK-11 in most samples) was 21 ng/g liver (range: about 12–36 ng/g) (Kayata et al., 1989). Phylloquinone accounted for (mean, range) 74% (33–90%) of the total amount of vitamin K in the liver on a molar basis.
- The mean concentration of phylloquinone in liver samples of three men and three women was about 7 ng/g (range: about 2–23 ng/g wet liver) (Thijssen and Drittij-Reijnders, 1996). The mean concentration of menaquinones (MK-4 and MK-6 to MK-11) was about 50 ng/g (range: about 21-87 ng/g wet liver). Phylloquinone accounted for (mean, range) about 21% (about 4 to 48%) of the total amount of vitamin K in the liver on a molar basis.

Fresh liver specimens (n = 15) were obtained by biopsy in patients who underwent gastrointestinal surgery, with known phylloquinone and menaquinone intake (Usui et al., 1990). Seven patients had been put on a standard diet (150–450 µg phylloquinone/day, < 2 µg/day each of MK-4 to MK-8), and eight on a low phylloquinone diet (per day 5 µg phylloquinone, 16 µg of MK-9, and MK-4, 5, 7, 8 and 10 each about 1–3 µg), for three days before operation. Concentrations of phylloquinone and menaquinones (MK-4 to 13) were measured by HPLC. The mean liver concentration of phylloquinone was about 13 ng/g and 3 ng/g of wet liver weight with the standard and low phylloquinone diets,

respectively (significantly different, $p < 0.01$). Phylloquinone accounted for (mean, range) about 10% (about 9–12%) of the total amount of vitamin K in the liver on a molar basis with the standard diet, while the mean percentange was 2.4% (about 2–4%) on the low phylloquinone diet. Total MK-n concentrations in the liver were not significantly different between the two groups, and were (mean, range) about 205 ng/g (137–409 ng/g liver) on the standard diet and about 239 ng/g (166-321 ng/g) on the low phylloquinone diet. Mean total concentrations of vitamin K in the liver were about 217 ng/g with the standard diet and 242 ng/g with the low phylloquinone diet, which are higher than the values reported by Thijssen and Drittij-Reijnders (1996) and Kayata et al. (1989). The Panel notes that, whilst plasma phylloquinone was decreased by a low phylloquinone diet (and by pre-operative fasting) and liver phylloquinone was decreased by three days of a low phylloquinone diet, the total concentration of vitamin K in the liver was not. The Panel notes that this study conducted in patients suggests that phylloquinone in the liver may be more rapidly depleted and catabolized than MK-n.

The Panel notes that the mean/median phylloquinone concentration ranged between about 3 and 34 ng/g of liver, that the mean concentration of menaquinones (MK-4 up to MK-13 according to the studies considered) ranged from about 21 to 239 ng/g of liver, and that the mean/median percentage of phylloquinone in the total content of vitamin K of the liver ranged, on a molar basis, from 2.4 to 74%. The Panel also notes that the range of the content of phylloquinone in the human liver is large, due to possible variability in phylloquinone intake and status, but also to possible conversion of phylloquinone to MK-4 (Sections 2.1. and 2.3.5.) and degradation of phylloquinone during tissue handling and storage. The Panel notes that the reason for the high concentration of menaquinones in the liver in the study by Usui et al. (1990) in view of their dietary intake remains unclear.

2.3.4.3. Measurements of phylloquinone and menaquinones in the liver of fetuses and newborns

Phylloquinone concentration was in the range 0.4–3.7 ng/g in 21 fetal livers at 10 to 27 weeks of gestation (median of 1.3 ng/g in n = 18 at 19–27 weeks of gestation), and in the range 0.1–8.8 ng/g liver for 10 term newborns (median 1.0 ng/g) (Shearer et al., 1988) (Section 2.3.4.2.). Median phylloquinone concentrations in the liver of fetuses and neonates did not significantly differ, but were significantly lower than those observed in adults in this study ($p < 0.01$). The authors could not identify any menaquinones in livers of fetuses or neonates.

Liver samples from autopsies of full-term infants who died from sudden infant death syndrome, who were formula-fed and received a phylloquinone intramuscular injection at birth were also analysed (Kayata et al., 1989) (Section 2.3.4.2.). Mean concentrations were 36 ng/g liver for phylloquinone and 5.5 ng/g liver for menaquinones in infants aged less than two weeks (n = 2), and were 45 ng/g liver for phylloquinone and 36 ng/g liver for menaquinones (MK-4 and MK-7 to MK-10 in most samples) in infants aged 2 to 4 months (n = 5). The statistical difference with adult values (mean of 34 ng phylloquinone per g liver, Section 2.3.4.2.) was not tested.

The Panel notes that data are limited on phylloquinone concentration in the liver of fetuses, neonates and infants, and that these studies suggest that, at birth, the concentration of menaquinones is low in the liver (compared to adults) and increases during the first year of life. This increase could be related to the addition of complementary foods to the diet of infants and/or to the progressive colonisation of the gut by MK-producing bacteria (Section 2.1.).

2.3.4.4. Measurements of phylloquinone and menaquinones in extra-hepatic tissues

Phylloquinone and MK-n occur not only in liver and plasma, but data on tissue content in humans are limited. In tissue samples from autopsies (Thijssen and Drittij-Reijnders, 1996) (Section 2.3.4.2.), apart from the liver, the concentrations of phylloquinone were highest in the heart and pancreas, and lowest in the lung, kidney and brain. In this study, MK-4 concentrations were highest in pancreas, kidney and brain and lowest in heart and lung. Molar ratios of MK4:phylloquinone showed that there was more MK-4 than phylloquinone in the kidney and brain, similar amounts of both forms in pancreas and more phylloquinone than MK-4 in the heart. In a study on six men and women who had a hip replacement (mean age: 69.7 ± 8.8 years) (Hodges et al., 1993b), concentrations in cortical and

trabecular bone taken from the femoral neck ranged between 0.06 and 8.37 ng/g dry weight for phylloquinone and between 0.25 and 7.24 ng/g dry weight for MK-6 to MK-8.

2.3.4.5. Conclusions on storage

The total body pool of phylloquinone depends on phylloquinone intake, and is small, according to kinetic analyses. The Panel notes the limitations of available data from studies on total body pool of phylloquinone in adults (Bjornsson et al., 1979; Olson et al., 2002; Jones et al., 2008) (Section 2.3.4.1.). The Panel considers that the most accurate values of the body pool of phylloquinone come from a compartmental analysis of phylloquinone kinetics in women and men (Novotny et al., 2010), as it takes into account the fast kinetics of phylloquinone. This study found 'tissue storage pools' of 46 and 41 µg for men and women respectively, or 0.55 and 0.53 µg/kg body weight. The Panel also notes that the study by Olson et al. (2002), when taking into account the value for plasma phylloquinone considered as more accurate by the authors, provides a mean body pool of phylloquinone of 0.57 µg/kg body weight, a value which is close to the values of 0.53–0.55 µg/kg body weight obtained by Novotny et al. (2010). The Panel considers that **a total body pool of phylloquinone of about 0.55 µg/kg body weight in healthy adults** at steady state is associated with no signs of vitamin K deficiency.

The Panel notes that there is no data on the total body pool of **menaquinones**. Various organs contain phylloquinone and different menaquinones. The Panel notes that the liver is the organ that contains the highest concentration of vitamin K, as a mixture of phylloquinone and menaquinones (MK-4 up to MK-13 according to the studies considered), which contents are widely variable. The Panel also notes that relatively small amounts of vitamin K are reported in the liver of the newborn, in which phylloquinone predominates over menaquinones.

2.3.5. Metabolism

The turnover of **phylloquinone** in the body proceeds through two phases. The first phase of fast turnover of phylloquinone has been associated with a *plasma/serum half-life* ($t_{1/2}$) in the range of 0.22-8.80 h (Section 2.3.2.), and the second phase of slower turnover has been associated with *a tissue* $t_{1/2}$ in the range of 1.8–215 h, depending on studies and methodologies (Shearer et al., 1972; Shearer et al., 1974; Bjornsson et al., 1979; Schurgers and Vermeer, 2000; Olson et al., 2002; Erkkila et al., 2004; Jones et al., 2008; Novotny et al., 2010). The value of 215 hours was obtained in the study of longest duration (three weeks) (Novotny et al., 2010), but studies of shorter duration provided smaller values (of 22.8-27.6 h (Olson et al., 2002; Erkkila et al., 2004) or a few hours in the remaining studies). In the kinetic study by Olson et al. (2002) (Sections 2.3.4. and 2.3.6.), the mean turnover times were 39.7 and 36.1 h on the control and low phylloquinone diets, repectively.

Phylloquinone is converted to menadione (Section 2.1.) that is converted by cellular alkylation to **MK-4**, which is not commonly produced by bacteria in contrast to other MK-n (Section 2.1.). This tissue-specific conversion from phylloquinone has been observed in *animals* (e.g. rats, chicken), independently of gut bacteria since it occurs in germ-free rats (Will et al., 1992; Thijssen and Drittij-Reijnders, 1994; Davidson et al., 1998; Ronden et al., 1998; Al Rajabi et al., 2012). Data in human cells/humans are more limited and often refer to high doses of vitamin K. MK-4 epoxide accumulated in *human kidney cells* incubated in the presence of 2.2 and 22 µmol/L of phylloquinone (Davidson et al., 1998) and menadione was converted into MK-4 in *cultures of several human cell lines* (Thijssen et al., 2006). Authors believe the conversion of phylloquinone to menadione and MK-4 occurs also in humans, during absorption in the intestinal mucosa and/or in other organs (Thijssen and Drittij-Reijnders, 1996; Thijssen et al., 2002; Thijssen et al., 2006). Urinary excretion of menadione increased following single *oral* phylloquinone supplementation (10 mg) in healthy men, but not after a subcutaneous *injection* (Thijssen et al., 2006). Urinary excretion of menadione was also stimulated by the intake of single doses of MK-4 (15 mg), MK-7 (1 mg) or menadione (10 mg). The authors calculated that daily urinary excretion of menadione corresponded on a molar basis to 1.6–5.6% of the phylloquinone oral dose and 1–2.5% of the MK-4 oral dose. In lactating women, the site of the conversion from phylloquinone to MK-4 was suggested to be the *mammary tissue*, as MK-4 concentration in breast milk was significantly correlated with phylloquinone concentration and

increased with phylloquinone supplementation of the mothers (0.8, 2 or 4 mg/day compared with an unsupplemented group) (Thijssen et al., 2002). The enzyme UbiA prenyltransferase domain-containing protein 1 (UBIAD1) has been identified in *humans* and catalyses the initial side chain cleavage of phylloquinone to release menadione and the prenylation of menadione to form MK-4 (Nakagawa et al., 2010).

The hepatic and extra-hepatic metabolism of **menadione** has been assessed in isolated rat livers perfused with menadione (Losito et al., 1967) or in rats administered menadione orally (Hoskin et al., 1954; Losito et al., 1967; Thompson et al., 1972), but no data on menadione metabolism in humans are available.

Phylloquinone in the **liver** has a fast turnover and is catabolised to metabolites that are rapidly transferred to plasma, urine and mainly bile, according to studies using radiolabelled tracer and unlabelled pharmacological doses of phylloquinone in humans (Shearer and Barkhan, 1973; Shearer et al., 1974; McBurney et al., 1980) (Section 2.3.6.).

The catabolism of phylloquinone and menaquinones in the liver proceeds through a common degradative pathway. The side chain is metabolised by an initial ω-hydroxylation, followed by a progressive side-chain shortening via the β-oxidation pathway (Shearer and Newman, 2014), until the side chain is shortened to two major metabolites with 7- and 5-carbon side chains. The **5C-metabolite** has the structure 2-methyl-3-(3'-3'-carboxymethylpropyl)-1,4-naphthoquinone and the **7C-metabolite** has the structure 2-methyl-3-(5'-carboxy-3'-methyl-2'-pentenyl)-1,4-naphthoquinone (Figure 1 in Section 2.1., and Section 2.4). These two metabolites are conjugated with glucuronic acid and excreted in the **bile** (Shearer et al., 1972; Shearer et al., 1974) and the **urine** (Shearer et al., 1970b; Shearer and Barkhan, 1973; Shearer et al., 1974; McBurney et al., 1980) (Section 2.3.6.). The ingestion of a large single pharmacological dose of phylloquinone (400 mg) by subjects treated with warfarin (Section 2.2.1.) resulted in the isolation of a third aglycone metabolite in **urine**, identified as 2-methyl-3-(7'-carboxy-3',7'-dimethyl-2'-heptenyl)-1,4-naphthoquinone (**10C-metabolite**) (McBurney et al., 1980).

The Panel notes that vitamin K has a fast turnover in the body. Phylloquinone can be converted in humans to menadione and MK-4, independently of the gut microflora. In the liver, phylloquinone and menaquinones are efficiently catabolised. The metabolism of phylloquinone and menaquinones produces the same metabolites, excreted in urine (5C, 7C or 10C) and bile (5C, 7C).

2.3.6. Elimination

2.3.6.1. Faeces

In the review by Shearer et al. (1974) (Section 2.3.1.), in healthy subjects (n = 3) who ingested 1 mg of radioactive phylloquinone with a meal, the radioactivity recovered from the faeces over a period of three days was **54–60%** of the dose. From this, 15–23% was identified by the authors as unmodified, presumably unabsorbed phylloquinone and the remaining lipid-soluble radioactivity consisted of more polar metabolites that were separated by thin-layer chromatography.

The radioactivity in faeces after five days after an intravenous dose of 1 mg radioactive phylloquinone represented **34 and 38%** of the dose in two subjects, respectively (Shearer et al., 1972; Shearer et al., 1974). No detectable faecal levels of radioactivity were present in a patient who also received this intravenous dose and whose total bile was collected for a period of three days, which indicates that the biliary route is the major route by which vitamin K metabolites pass into the intestinal lumen and are excreted in the faeces (Shearer et al., 1972). Shearer et al. (1974) also reported that, in one study in a subject injected with 45 µg radioactive phylloquinone, **51%** of the dose was excreted in the faeces.

In the study by Olson et al. (2002) (Sections 2.3.4.1. and 2.3.5.), in seven adults on the control diet providing a mean intake of 75 µg phylloquinone/day and receiving 0.3 µg isotope-labelled phylloquinone administered intravenously, the total losses, measured by the excretion of radioactive products of phylloquinone during six days following the injection, accounted for (mean ± standard

error of the mean (SEM)) 61.8 ± 2% of the isotopic dose, with **31.8** ± 0.81% excreted in faeces through the bile. This decreased to a mean (± SEM) of 13.3 ± 0.51% ($p < 0.001$) excreted in faeces when on the low phylloquinone diet (providing 8 µg/day).

Both phylloquinone and menaquinones are more prevalent in the stools of formula-fed infants compared to breast-fed infants (Greer et al., 1988; Fujita et al., 1993).

2.3.6.2. Urine

After a 1 mg intravenous dose of tritiated phylloquinone, in three adults, the cumulative excretion within three days was **19–26%** of the dose via the urine (Shearer et al., 1972). In healthy adults who received an injection of 1 mg labelled phylloquinone with a meal, the urinary excretion of the 'polar metabolites' was found to be virtually complete after three days, accounting for 8–26% of the administered dose (mean of **19 %**) (Shearer et al., 1974). Shearer et al. (1974) also reported that, in one study in a subject injected with 45 µg radioactive phylloquinone, **18%** of the dose was excreted in the urine. The major urinary metabolites are glucuronide conjugates.

In the study by **Olson et al. (2002)** (Sections 2.3.4.1. 2.3.5 and 2.3.6.1.), in seven adults consuming the control diet providing 75 µg/day and receiving 0.3 µg isotope-labelled phylloquinone administered intravenously, losses of phylloquinone metabolites in urine, measured by the excretion of radioactive products of phylloquinone (24-h urinary samples) during six days following the injection, were (mean ± SEM) **30** ± 1.8 % of the isotopic dose. This value was 38.8 ± 9.8% on the low phylloquinone diet providing 8 µg/day. As plasma showed no detectable radioactivity after six days, the authors hypothesised that the radioactivity unaccounted for in faeces (Section 2.3.6.1.) and urine remained in the adipose tissue.

The **5C- and 7C-metabolites** are common products of the metabolism of phylloquinone and menaquinones (Figure 1 and Section 2.3.5.). The 5C-metabolite was shown as the main urinary vitamin K metabolite in adults either unsupplemented or consuming various doses/intakes of phylloquinone, MK-4 or MK-7 (Harrington et al., 2005; Harrington et al., 2007) (Section 2.4.) and in term infants before or after vitamin K prophylaxis (Harrington et al., 2010). Urinary excretion of the 5C- and 7C-metabolites increases in adults also in response to supplementation with menadione (20 mg) and reflects the process of inter-conversion of menadione to MK-4 (Harrington et al., 2005).

In term infants, only 0.03% of a parenterally administered phylloquinone dose was excreted as urinary metabolites within the first 24 hours post-prophylaxis (Harrington et al., 2010), which suggests that the rate of phylloquinone clearance to the urine in neonates is slower than in adults. This is supported by the prolonged presence of phylloquinone in term neonate blood after its oral administration up to four days (Schubiger et al., 1993; Schubiger et al., 1997).

2.3.6.3. Breast milk

SCF (2003c) noted that breast milk contains 'low' concentrations of vitamin K (mostly phylloquinone), between about 0.6 and 10 µg/L (von Kries et al., 1987b; Fomon, 2001). SCF (2003c) also noted that the supply of vitamin K in breast milk is not sufficient to meet the requirements of all young infants. The SCF concluded that vitamin K supplementation is generally recommended in young infants in addition to the supply with breast milk. Based on data reported by IOM (2001), mean phylloquinone concentrations in breast milk around 2.5 µg/L, but varying from 0.85 to 9.2 µg/L, were noted (EFSA NDA Panel, 2013a).

Phylloquinone concentration in (mostly mature) breast milk of lactating women either not supplemented or supplemented with phylloquinone, and menaquinone concentration in mature breast milk, in countries of the European Union (EU), US and Japan, are described in Appendix A, with details on stage of lactation.

In the EU, **mean/median** concentration of **phylloquinone** in breast milk of **unsupplemented** mothers of full term infants was 1.2 µg/L in Germany (von Kries et al., 1987a), about 1.7 µg/L in Austria (Pietschnig et al., 1993), 2.1 µg/L in the UK (Haroon et al., 1982), about 2.2 µg/L in The Netherlands (Thijssen et al., 2002), and 9.18 µg/L in France (Fournier et al., 1987). The concentration of phylloquinone in breast milk is affected by maternal oral supplementation (about 0.1-5 mg phylloquinone/day or up to 20 mg as one dose) in EU and US studies, with mean concentration reaching up to about 130 µg/L. When available, Appendix A reports on maternal vitamin K intake (Pietschnig et al., 1993) or status (Thijssen et al., 2002).

Limited data are available on **menaquinone** concentration in breast milk. In unsupplemented women in the Netherlands (Thijssen et al., 2002), mean MK-4 concentration in breast milk was about 0.8-0.9 µg/L at 16-19 days post partum, and increased with phylloquinone supplementation (2 or 4 mg/day, $p < 0.05$ compared with the unsupplemented group). Mean concentration in breast milk in two Japanese studies (Kojima et al., 2004; Kamao et al., 2007b) were in the range of about 1.2-1.9 µg/L for MK-4 and about 0.8–1.7 µg/L for MK-7.

2.3.6.4. Conclusions on elimination

The Panel notes that, with high oral doses of phylloquinone (e.g. 1 mg), non-absorbed phylloquinone plus phylloquinone metabolites excreted via the bile are eliminated via faeces in large amounts, up to 60%. The Panel notes that the study by Olson et al. (2002), which measured losses both through collection of urine and faeces over six days, considered a lower intake (mean of 75 µg phylloquinone/day) that is closer to observed intake estimates (Section 3.2.). Based on this study, the Panel considers that a mean of about **62%** of injected phylloquinone is excreted as radioactive metabolites in urine (mean of **30%**) and faeces (mean of about **32**%). No similar experiment was available to assess losses of metabolites in urine and faeces after menaquinone ingestion. The Panel also notes that the 5C-metabolite was the main urinary vitamin K metabolite in studies in adults and term infants.

The Panel notes that breast milk of unsupplemented women in the EU contains 'low' mean/median concentration of phylloquinone, varying from about 1.2 to 9.2 µg/L. The concentration of phylloquinone in breast milk is increased by maternal oral supplementation. Data on menaquinone concentration in breast milk in the EU are limited, and mean concentration is in the range of 1.8-2.2 µg/L for MK-4 in unsupplemented women.

2.3.7. Interaction with other nutrients

Vitamin K intake is associated with changes in calcium balance that can positively influence bone calcium content (EFSA NDA Panel, 2015b). The vitamin D metabolite $1,25(OH)_2D$ is needed for the synthesis of osteocalcin in the osteoblasts together with vitamin K, and it regulates the expression of osteocalcin (EFSA NDA Panel, 2016).

Vitamin K and α-tocopherol (vitamin E) share common metabolic pathways, including blood transport via lipoproteins, catabolism and biliary excretion (Schmolz et al., 2016). Up-regulation of these pathways in response to increased α-tocopherol intake can increase the rate of vitamin K catabolism and/or urinary and faecal excretion (Traber, 2008). α-Tocopherol can also interfere with the vitamin K-activation of the pregnane X receptor, leading to modulation of the expression of oxidative and conjugation enzymes (Landes et al., 2003). A cross-sectional study suggested that about 10% of the variation in plasma phylloquinone concentrations could be explained by plasma concentrations of other fat-soluble vitamins, particularly α-tocopherol (Thane et al., 2006). A competitive inhibition was described between tocopherol quinone and the phylloquinone hydroquinone for the vitamin K-dependent gamma-carboxylase (EFSA NDA Panel, 2015a). In its assessment of the UL for vitamin E, SCF (2003b) concluded that 'high' intakes of 'vitamin E' in subjects with 'low' vitamin K status (caused by malabsorption, impairment of the gut microbiota, or therapy with anticoagulants) can cause impairment of blood coagulation. The SCF indicated that this would be a result of a reduction of the cyclooxygenase pathway, therefore of the thromboxane synthesis, thus impairing the thromboxane-

dependent blood coagulation and decreasing the coagulation factor II and VII. In healthy adults, 'high' intake of α-tocopherol or α-tocopherol given intravenously can result in bleeding, prolonged PT, lowered vitamin K-dependent coagulation factors and appearance of undercarboxylated prothrombin in the blood (Booth et al., 2004b). α-Tocopherol supplementation during 10 years had a mild anti-thrombotic effect (Glynn et al., 2007). Doses of RRR-α-tocopherol above the UL can result in an increase in PIVKA-II in adults in blood with normal coagulation status (Booth et al., 2004b).

The Panel notes that 'high' intakes of α-tocopherol in subjects with 'low' vitamin K status can cause impairment of blood coagulation, and considers that data on interactions of vitamin K with other nutrients are limited.

2.4. Biomarkers

2.4.1. Prothrombin time (PT) test and partial thromboplastin time (PTT) test

The PT and PTT tests can reflect vitamin K deficiency (Section 2.2.2.1.). PT has a usual range of 10–16 s for infants and 11–14 s for adults; and PTT is 25.4–59.8 s in healthy full-term infants aged 5 days and 26.6–40.3 s in adults, according to reviews (Andrew, 1997; Greer and Zachman, 1998).

The review by Suttie (1992) reports on an experiment in which 'normal' human plasma was mixed with plasma from a warfarin-treated patient (25% of the 'normal' concentration of prothrombin) in varying amounts. The curve of PT according to the percentage of 'normal' prothrombin shows that PT was still 'normal'[9] in samples with only 50% of the 'normal' prothrombin, and that it increases only at lower percentages (Suttie, 1992; IOM, 2001), suggesting a low sensitivity of the PT test.

From patients with apoplexy fed parenterally without receiving vitamin K, some of them also treated with antibiotics (Frick et al., 1967) (Section 2.2.2.1.), the authors estimated that the amount of phylloquinone needed to recover a normal PT is between 0.03 and 1.5 µg/kg body weight per day in adults (body weight not given in the paper). The Panel notes that the results of this study showed a large range of values (that may be explained by methodological limitations in measuring small differences in phylloquinone concentrations).

Depletion/repletion studies in healthy individuals who consumed diets 'low' in phylloquinone, i.e. < 10 µg/day for two to three weeks, showed an increased coagulation time measured as PT in some subjects (Udall, 1965), but not in others (Allison et al., 1987; Ferland et al., 1993; Paiva et al., 1998), measured either as PT or PTT (Section 2.2.2.1.). Dietary restriction of phylloquinone to 18 µg/day for 28 days (Booth et al., 2003a) or to about 35 µg/day for 40 days (Suttie et al., 1988)[10] did not affect PT (Suttie et al., 1988) or PT and PTT (Booth et al., 2003a). Increasing phylloquinone intake from 100 µg/day to around 400 µg/day did not induce any change in PT or PTT (Booth et al., 1999b).

PT and PTT cannot be considered as biomarkers of all the functions controlled by vitamin K (Section 2.2.1.). A disturbed coagulation time (increase of PT or PTT) may also indicate hepatic dysfunction or haematological disease not related to vitamin K deficiency and several other acute or chronic conditions, as reviewed by Booth and Al Rajabi (2008). Thus, PT and the PTT are markers of vitamin K status, but they are not specific.

The Panel considers that the PT and the PTT are not sensitive markers of vitamin K intake and status and non-specific indicators of vitamin K deficiency. PT and PTT cannot be considered as markers of all the functions controlled by vitamin K. The Panel also notes that depletion/repletion studies show that vitamin K intakes sufficient for an adequate PT (e.g. equal or above 10 µg phylloquinone/day) may not be enough for the other functions controlled by vitamin K (as suggested by results on e.g. plasma phylloquinone, urinary Gla excretion, serum PIVKA II, %ucOC) (Sections 2.4.2. to 2.4.7).

[9] i.e. 10–11s according to Suttie (1992).
[10] Used by the SCF to set DRVs for vitamin K, see Section 4.

2.4.2. Plasma concentration and activity of blood coagulation factors

Among the vitamin K-dependent blood coagulation factors, i.e. factor II (prothrombin), VII, IX, and X, synthetised by the liver as inactive forms (Section 2.2.1), factor VII (FVII) is the most frequently used, on the basis of its relatively short half-life (approximately six hours) (Ferland et al., 1993; Kamali et al., 2001). Normal laboratory ranges of FVII reported in studies in adults were about 70% to 130% of 'normal' values, with 100% corresponding to the FVII value observed in normal pooled plasma i.e. 0.011 µM, or as Unit/mL (Allison et al., 1987; Andrew et al., 1988; Ferland et al., 1993). Authors considered values of FVII less than 60% as abnormal (Allison et al., 1987).

The depletion study of Allison et al. (1987) (Sections 2.2.2.1. and 2.4.1.) was undertaken in 11 groups of three men each (aged 21–49 years, as inpatients in a ward) fed a diet containing less than 5 µg phylloquinone/day) for two weeks, with different antibiotics given orally or intravenously during the last 10 days in ten of these groups. FVII concentration decreased after antibiotics treatment and was < 60% of 'normal" value on at least one day in 2/3 or 1/3 of treated subjects depending on the type of antibiotic, but not in individuals without antibiotics. The Panel notes that, in the subjects without antibiotics, a phylloquinone intake of 5 µg/day for two weeks did not lead to a decrease in FVII concentration. **The Panel** also notes that it is unknown if the antibiotics tested, some being well absorbable or given intravenously, decreased menaquinone production by the gut microbiota.

In the depletion/repletion study of Ferland et al. (1993) (Sections 2.2.2.1., 2.4.1. and 4.), 32 healthy adults aged 20–40 and 60–80 years, in a metabolic unit, not receiving antibiotics, were subjected to a 4-day baseline diet, a 13-day depletion diet (about 10 µg phylloquinone/day) and a 16-day repletion period (additional phylloquinone of 5, 15, 25 and 45 µg/day). No statistically different changes in the production of FVII were observed during the study as mean FVII 'functional activity' remained between 103 and 105%, while in both age-groups, PIVKA-II antigen concentration (Section 2.4.3.) was increased significantly ($p < 0.05$) at the end of depletion compared to baseline.

Another depletion/repletion study was undertaken on 9 younger (20–28 years) and 9 older (55-75 years) men on their normal diet restricted in phylloquinone-rich foods and providing 83 µg phylloquinone/day (younger adults, about 1 µg/kg body weight per day) and 164 µg/day (older adults, 'about twice' the amount consumed by younger adults) (Bach et al., 1996) (Section 4.). Subjects received after 3 days, and for 14 days daily, 1 mg warfarin ('acquired vitamin K-deficiency'), and thereafter for 5 days 1 mg/day phylloquinone. Mean FVII activity was not affected by warfarin treatment whilst PIVKA-II concentrations (Section 2.4.3.) increased by > 30% by day 10 of warfarin treatment (exact increase depending on analytical method used to assess PIVKA-II and age group), and while %ucOC (Section 2.4.3.) increased continuously with time during depletion.

These studies in adults suggest that the depletion phase of about two weeks was too short for a change in FVII concentration/activity to occur. Both plasma concentration and functional activity of blood coagulation factors (in particular FVII) have a low sensitivity as biomarkers of vitamin K intake. FVII activity can be modified by other causes than vitamin K deficiency e.g. genetic or liver diseases (Green et al., 1976; Mariani et al., 2003), thus is not a specific marker of vitamin K status.

Prophylactic administration of phylloquinone to pregnant women (20 mg/day orally for at least three days, during the second trimester or at birth) led to total prothrombin (factor II) activity in the fetuses (n = 41) or full-term neonates (n = 33) that were comparable to that of fetuses or neonates from unsupplemented mothers. The values were lower than 'normal' adult values (pool of 30 healthy donors) (difference not tested) (Mandelbrot et al., 1988). Thus, phylloquinone administered to the mother does not change factor II activity in newborns. At day 1 in full-term infants who all received 1 mg intramuscular 'vitamin K' at birth (n = 59 to 61 depending on the clotting factor), factor II, VII, IX, and X average activities were about 40–60% of adult values (n = 29) (Andrew et al., 1987). The authors report that the activity of these four factors at six months were in the adult range.

The Panel notes that FVII concentration/activity is not a sensitive biomarker of phylloquinone intake: for a change in FVII concentration/activity, the depletion phase of about two weeks in available

studies may have been too short. The Panel also notes that FVII concentration/activity is not a specific marker of vitamin K status. FVII concentration/activity does not represent all functions that are controlled by vitamin K (as shown in studies indicating no change in FVII activity during depletion while PIVKA-II concentration increased).

2.4.3. Circulating concentration of the undercarboxylated form of vitamin K-dependent proteins

Insufficient availability of vitamin K results in the secretion into plasma of biologically inactive undercarboxylated vitamin K-dependent proteins (Ferland et al., 1993; Booth et al., 2000a; Booth et al., 2003a) (Section 2.2.1.). Their concentrations have been proposed as biomarkers of vitamin K status/stores for certain tissues (liver, bone, vessels (vascular calcification) (Liska and Suttie, 1988; Szulc et al., 1993; Rennenberg et al., 2010; Schurgers et al., 2010).

2.4.3.1. Protein induced by vitamin K absence or antagonism-II (PIVKA-II) and S:E ratio

Normal blood concentration of PIVKA-II (Section 2.2.1.) has been defined as ≤ 2 µg/L (Booth et al., 2000a; Booth et al., 2001; Booth et al., 2003a). A review by Shea and Booth (2016) indicates that commercially available PIVKA-II assays have low sensitivity for detecting variation in usual vitamin K intakes in healthy populations. The result of the assay for plasma concentration of functionally active prothrombin is also expressed as the S:E ratio, which compares the amount of prothrombin generated in the test sample by action of a commercial thromboplastin preparation (Simplastin) with that generated with a snake venom protease from *E. carinatus*.

PIVKA-II blood concentration changes according to vitamin K intake. In metabolic[11] depletion/repletion studies in adults (Section 2.4.1.), it increases significantly in response to dietary restriction of phylloquinone (restriction to 10–18 µg/day) (Ferland et al., 1993; Booth et al., 2001; Booth et al., 2003a) and decreases significantly in response to dietary repletion with phylloquinone (Booth et al., 2000a; Booth et al., 2001; Booth et al., 2003a).

In particular, PIVKA-II significantly dropped between end of depletion (at 10–11 µg phylloquinone/day) and end of repletion (at 200 µg/day for ten days), and was restored to a value of ≤ 2 µg/L (Booth et al., 2000a; Booth et al., 2001). In the study by Booth et al. (2003a) in post-menopausal women, that comprised a baseline diet (90 µg phylloquinone/day for 14 days) followed by a dietary depletion phase (18 µg/day for 28 days), mean PIVKA-II decreased during the three consecutive phases of repletion (86, 200 and 450 µg phylloquinone/day for 14 days each) compared to the end of depletion. The decrease was not statistically significant with 86 µg phylloquinone/day (concentration above 2 µg/L) but became significant with 200 µg/day (concentration below 2 µg/L), until it attained the baseline value with 450 µg/day (concentration of about 1.4 µg/L, read on figure). **The Panel notes** the discrepancy in the results of this study, in that PIVKA-II concentration did not return to normal with an intake of 86 µg phylloquinone/day for 14 days, while it was normal with the baseline diet corresponding to a similar intake of 90 µg/day for 14 days that is a finding indicating vitamin K sufficiency.

In the depletion/repletion study by Suttie et al. (1988) (Section 2.4.1.), used by the SCF to set DRVs for vitamin K (Section 4), ten young men (mean ± SD: 72 ± 9 kg body weight) followed a 'normal' diet with an intake of 82 µg phylloquinone/day for seven days and continued with a restricted diet for 21 days. Median phylloquinone intake at day 9 and 27 was 40 and 32 µg/day, respectively, analytically measured in duplicate portions of all foods and beverages consumed. Subjects were then supplemented with either 50 or 500 µg phylloquinone/day from day 29 to 40 in addition to the same restricted diet, then with 1 mg/day from day 41 to 47. The mean S:E ratio was significantly lower ($p < 0.01$) at the end of the restriction period compared with the 'normal' diet (0.9111 versus 1.024, respectively), and was restored to normal with either 50 or 500 µg/day supplementation.

[11] Well-controlled studies in which participants were housed in a metabolic unit are termed metabolic studies.

Most infants with vitamin K deficiency have 'high' PIVKA-II concentrations, although it is not necessarily a predictor of haemorrhagic disease. Detection rates of PIVKA-II in cord blood ranged from about 10% to 30% of full-term infants (Motohara et al., 1985; Motohara et al., 1990; von Kries et al., 1992; Bovill et al., 1993). In full-term newborns (n = 156 enrolled), 47% of cord blood samples had PIVKA-II blood concentrations ≥ 0.1 AU/mL (Greer et al., 1998).

2.4.3.2. Undercarboxylated osteocalcin (OC) and matrix γ-carboxyglutamic acid protein (MGP)

The serum concentrations or proportions of ucOC or desphospho-uncarboxylated MGP (dp-ucMGP) (Sections 2.2.1. and 2.3.3) expressed as percentage of the total form (e.g. %ucOC), have been proposed as biomarkers for the extra-hepatic vitamin K status. **The Panel notes** that this expression as % is more precise, because of the variability in the concentration of the total form. The relationship between vitamin K supplementation (phylloquinone or MK-4 or MK-7) and absolute concentration of dp-ucMGP has been investigated (Cranenburg et al., 2010; Shea et al., 2011; Dalmeijer et al., 2012), showing a decrease in its concentration in the supplemented subjects compared to placebo. **The Panel notes** that interpretation of change in dp-ucMGP with regard to vitamin K status is unclear. Concentration or % ucOC in serum have been proposed as a biomarker of bone vitamin K status, as described below.

A randomised cross-over metabolic depletion/repletion study compared the effects of phylloquinone or dihydrophylloquinone (dK) on a number of markers in 15 healthy adults (20–40 years) (Booth et al., 2001) (Section 2.4.3.1.). The two residency periods of 30 days each, separated by at least four weeks, consisted of: (1) a five-days control diet (mean: 93.1 µg phylloquinone/day, no dK), (2) a 15-days depletion diet (mean: 11.0 µg phylloquinone/day, no dK) and (3) a 10-days repletion diet (mean: 206 µg phylloquinone/day with no dK, or 240 µg dK/day with 11.0 µg phylloquinone/day). Mean %uOC was about 28–29% during the control diet, significantly increased ($p < 0.01$) after the depletion period (to about 42–47%), then significantly decreased ($p < 0.01$) during the phylloquinone repletion (to about 20%, not significantly different from the control diet), but not during the dihydrophylloquinone repletion. **The Panel notes** that this study showed not significantly different mean %ucOC with the daily intakes of about 90 µg and about 200 µg phylloquinone.

In the randomised cross-over metabolic study by Booth et al. (1999b) (Section 2.4.1.) with three residency periods of 15 days each, 36 healthy younger and older adults (20–40 and 60–80 years) consumed a mixed diet containing 100 µg phylloquinone/day or the same diet supplemented for days 6–10 with broccoli or fortified oil, thus providing 377 or 417 µg phylloquinone/day, respectively. Younger adults had significantly higher %ucOC than older adults on a mixed diet ($p = 0.001$, about 23% versus about 18% (read on figures), respectively), but there was no difference between sexes. In both age-groups, mean %ucOC significantly decreased five days after the start of the supplemented diets (no difference between supplemented diets), while it did not significantly change on the mixed diet (i.e. about 20% (older adults) or 25% (younger adults) over the 15 days (read on figure)).

In a cross-sectional study in 396 healthy Japanese women (30–88 years) with high natto consumption (phylloquinone or menaquinone intake not reported), women older than 70 years (n = 136) had significantly higher ($p < 0.003$) %ucOC in blood than women < 70 years (Tsugawa et al., 2006). This is in contrast to the previous study by Booth et al. (1999b).

In RCTs (Binkley et al., 2002; Bolton-Smith et al., 2007; Kanellakis et al., 2012), in adult populations with mean baseline phylloquinone intake in the range of about 80–120 µg/day, different high doses of phylloquinone (100–1,000 µg/day, from supplements or fortified foods) compared to placebo/control significantly decreased mean % ucOC.

In a prospective cohort study of 245 healthy girls aged 3–16 years (Kalkwarf et al., 2004) (Section 5.2.), baseline median phylloquinone intake (assessed by three-day food records, from food and supplements) was 45 µg/day (range: 6–275 µg/day). There was no association between phylloquinone intake and %uOC after adjustment for energy intake or energy intake and age.

Based on data that used the same assay for %ucOC (Booth et al., 1999b; Booth et al., 2001), a cut-off of 20% has been proposed by McKeown et al. (2002) as the %ucOC above which the risk for dietary vitamin K insufficiency (defined in relation to US DRVs for phylloquinone, see Section 4) increases. In this observational study (Section 2.4.4.), the lowest quintile of phylloquinone intake (i.e: median of 64 µg/day in women, 54 µg/day in men) was associated with a significantly higher risk of having a %ucOC above or equal to 20% (odds ratio (OR) (95% confidence interval (CI)): 2.51 (1.23–5.11), p = 0.01 in women; 2.75 (1.29–5.87), p = 0.009 in men), compared to the highest quintile (median of 307 µg/day in women and of 254 µg/day in men) (McKeown et al., 2002). However, **the Panel notes** that there is no clear reference level of γ-carboxylation that can be considered as optimal related to functions controlled by vitamin K status.

Cross-sectional analyses on 766 men and 925 women, either premenopausal or postmenopausal with or without current oestrogen use (all groups having similar vitamin K intake), showed that postmenopausal women not using hormonal replacement therapy had higher mean %ucOC in blood (23.5%) compared to the other groups (14–16%, difference not tested) (Booth et al., 2004a). Untreated early postmenopausal women (n = 19, 40–52 years), also had significantly higher mean %ucOC than premenopausal women (n = 40 women aged 20–30 or 40-52 years) (21.9 versus 17.4%, p = 0.02) (Lukacs et al., 2006). These authors note that whether estrogen directly modulates carboxylation of OC remains unclear. In addition to vitamin K intake, %ucOC is influenced by non-genetic factors such as TG and smoking status (Shea et al., 2009).

A number of RCTs designed to investigate bone health (Section 5.2.) have been done in Japanese or European adult populations using **MK-4 or MK-7** supplementation (Schurgers et al., 2007; Koitaya et al., 2009; Emaus et al., 2010; Bruge et al., 2011; Kanellakis et al., 2012; Nakamura et al., 2014; Inaba et al., 2015), and using MK-7 in children (van Summeren et al., 2009). **The Panel notes** the observed changes in the ratio between carboxylated and undercarboxylated osteocalcin according to menaquinone intake. However, doses used were much higher (45–360 µg/day for MK-7, 300-1,500 µg/day for MK-4) than the limited habitual intake data in European populations (Section 3), and baseline vitamin K intake was not always reported (Schurgers et al., 2007; Emaus et al., 2010; Bruge et al., 2011; Nakamura et al., 2014). **The Panel considers** that these data are not relevant to conclude on relationship of this biomarker with usual dietary menaquinone intake in European populations, thus, that no conclusion can be drawn from these studies for setting DRVs for vitamin K.

2.4.3.3. Conclusions on circulating concentration of the undercarboxylated form of vitamin K-dependent proteins

The Panel notes that concentrations of circulating undercarboxylated forms of vitamin K-dependent proteins (in particular PIVKAII and ucOC) have been proposed as biomarkers of vitamin K status/stores for certain tissues (in particular liver and bone). They are sensitive to phylloquinone over a certain range of intake. Data on concentrations of circulating ucOC and menaquinone intake (MK-4 or MK-7) have been obtained with doses much higher than the limited observed intake data in Europe.

Normal blood concentration of PIVKA-II has been defined as ≤ 2 µg/L but commercially available PIVKA-II assays have low sensitivity for detecting variation in usual vitamin K intakes in healthy populations. A cut-off value of ≤ 20% for ucOC has been proposed above which the risk for dietary vitamin K insufficiency, defined in relation to US DRVs for phylloquinone, increases. The Panel notes that the relationship between %ucOC and bone mineral density (BMD) or risk of hip fracture has been investigated (Szulc et al., 1993; Szulc et al., 1994; Schaafsma et al., 2000; Booth et al., 2004a), but the relevance of the 20% cut-off for %ucOC with regard to these outcomes remains to be established. The Panel notes that oestrogen status may be one determinant of vitamin K status assessed as %ucOC independent of diet in women, while the limited data on the influence of age on %ucOC in adults are contradictory. The Panel notes that dietary intakes of phylloquinone or menaquinones required for full γ-carboxylation of PIVKA-II or OC or MGP have not been determined and that the optimal extent of carboxylation is not known.

2.4.4. Circulating concentration of vitamin K

Most of the data on plasma vitamin K concentration are related to phylloquinone, and data on circulating menaquinone concentration (MK-4, MK-5 and MK-7) are limited, as reviewed by Shea and Booth (2016).

Because of the fast turnover of vitamin K (Section 2.3.4), plasma phylloquinone concentration reflects recent intake of phylloquinone, and responds to an increase in phylloquinone intake within 24 hours (Sokoll et al., 1997) or to phylloquinone depletion within three days (Allison et al., 1987). In adults, circadian variation in the circulating mean vitamin K concentration (mainly phylloquinone) shows minimal and maximal levels at 10:00 and 22:00 hours respectively (Kamali et al., 2001), and plasma TG mirrored changes in plasma vitamin K concentration. In healthy adults, fasting plasma phylloquinone concentrations (not adjusted for TG) have a higher intra-individual than interindividual variability (Booth et al., 1997).

Circulating phylloquinone concentration decreased with phylloquinone restriction and increased with phylloquinone supplementation (doses up to 1,000 µg/day) (Ferland et al., 1993; Booth et al., 2000a; Binkley et al., 2002; Booth et al., 2003a; Bolton-Smith et al., 2007). Considering phylloquinone absorption and transport, and the correlation observed between plasma phylloquinone and TG concentration (Booth et al., 2004a; Tsugawa et al., 2006; Azharuddin et al., 2007), plasma phylloquinone concentration should be adjusted for TGs (nmol phylloquinone/mmol TG) (Shea and Booth, 2016). This is often not the case in available studies (Ferland et al., 1993; Booth et al., 1999b; Booth et al., 2000a; Binkley et al., 2002; Booth et al., 2003a; Bolton-Smith et al., 2007).

- After phylloquinone restriction (18 µg/day for 28 days or 10 µg/day for about two weeks) (Sections 2.4.1, 2.4.2. and 2.4.3.) (Ferland et al., 1993; Booth et al., 2003a), plasma phylloquinone concentration significantly increased after repletion with 450 µg phylloquinone/day for two weeks (but not with 86 or 200 µg/day), although it did not return to initial levels (Booth et al., 2003a). However, in the other study (Ferland et al., 1993), it started to increase slightly only within the last repletion phase (additional 45 µg phylloquinone/day for four days) without reaching baseline values.
- Mean plasma phylloquinone not adjusted for TGs was significantly higher in older than in younger adults (Ferland et al., 1993; Booth et al., 1999b) (Sections 2.4.2. and 2.4.3.2.). However, in an observational study, plasma phylloquinone concentrations adjusted for TGs were significantly lower in older adults (65–92 years, n = 195) compared to younger adults (20–49 years, n = 131) (Sadowski et al., 1989). In younger and older adults (Booth et al., 2002) (Section 2.3.1.), whose plasma phylloquinone was measured for 24 hours, and who consumed diets providing on average 100, 377 or 417 µg phylloquinone/day, there was a significant overall age effect when comparing plasma phylloquinone concentration, either unadjusted or adjusted for TG, at 0 and 24 h, although there were no age differences in the 24 h-AUC for plasma phylloquinone adjusted for TGs.

A significant positive relationship between phylloquinone intake (from food or food and supplements) and phylloquinone plasma concentration was also observed in large observational studies in adults, over a large range of intake (5–1,000 µg/day measured by seven-day food record (Thane et al., 2006); 50–200 µg/day measured by a food frequency questionnaire (FFQ) (McKeown et al., 2002)).

In full-term infants (Greer et al., 1991), mean plasma phylloquinone concentrations were lower in exclusively breast-fed compared to formula-fed infants (range: 0.29–0.53 nmol/L in 23 breast-fed infants between 6 and 26 weeks, versus 9.8–13.3 nmol/L in 11 formula-fed infants), in relation to their different phylloquinone intake.[12]

[12] Mean at 6, 12 and 26 weeks: 0.07–0.12 µg/kg body weight per day in breast-fed infants, and 7–9.3 µg/kg body weight per day in formula-fed infants.

The Panel notes that the circulating concentration of phylloquinone in blood is a biomarker of short-term phylloquinone intake in adults. Circulating phylloquinone decreases during phylloquinone dietary depletion and increases with phylloquinone supplementation. Plasma phylloquinone concentration needs to be adjusted for TG concentration, which is often not done in available data. The exact dose-response relationship is unclear. Data on circulating menaquinone concentrations are limited. The Panel also notes that no cut-off value of plasma phylloquinone or menaquinone concentration has been set to define vitamin K adequacy (Shea and Booth, 2016).

2.4.5. Urinary concentration of γ-carboxyglutamic acid (Gla) residues

In protein catabolism, Gla residues contained in the vitamin K-dependent proteins are not further metabolised and are excreted in the urine (Shea and Booth, 2016). As a result, urinary Gla excretion has been used as an indicator of vitamin K status in adults. Urinary Gla excretion is a measure of the overall body content of vitamin K-dependent proteins, including proteins whose functions are not related to hemostasis but have not been clearly established, as reviewed by Ferland (1998).

In the randomised cross-over metabolic depletion/repletion study in young men and women by Booth et al. (2001) (Section 2.4.3.), mean urinary Gla concentration (measured in 24-h urine samples) significantly decreased during phylloquinone depletion (about 10 µg/day for 15 days) compared with the control diet (about 100 µg phylloquinone/day for 5 days), then significantly increased with phylloquinone repletion (about 200 µg/day for 10 days) without returning to baseline values within the time frame of repletion (and it did not react to dK supplementation). In the depletion-repletion study in young adults by Suttie et al. (1988) (Sections 2.4.1. and 2.4.3.), urinary Gla concentration was measured in 3-day composite urine samples and expressed as a percentage of the 'normal' diet period (i.e. a diet with a median intake of 82 µg phylloquinone/day). Mean urinary Gla excretion at the end of the phylloquinone depletion period was significantly decreased (i.e. about 78 % of the value of the normal diet period, $p < 0.01$), then significantly increased with phylloquinone supplementation (50 or 500 µg/day) compared to the depletion phase ($p < 0.01$, to reach about 97% of the value of the 'normal' diet, the two supplemented groups were combined as not significantly different).

In the depletion-repletion metabolic study in younger and older adults (Ferland et al., 1993) (Sections 2.2.2.1., 2.4.1., 2.4.2. and 4.), mean urinary Gla concentration (measured in 24-h urine samples) significantly decreased in response to dietary phylloquinone depletion (~ 10 µg/day for 13 days) in young adults compared to baseline (100 µg phylloquinone/day for 4 days). This was not observed in the older adults (significant difference between age group, $p < 0.03$). Urinary Gla concentration increased after phylloquinone supplementation (with additional 15, 25, and 45 µg/day, days 22–33, but not with additional 5 µg/day during days 18–21) in adults, respectively, with urinary Gla excretion reaching 96% of baseline values in young adults even with the supplementation at 45 µg phylloquinone/day (i.e. about 55 µg/day in total for four days). 24h-urine concentrations (µM, mean ± SEM) at baseline, at end of depletion and at end of repletion were 38.5 ± 1.5, 35.2 ± 1.4, and 36.7 ± 1.1 for the young adults and 38.2 ± 2.6, 38.0 ± 2.4, and 39.4 ± 2.7 for the older adults, respectively.

In the randomised cross-over study by Booth et al. (1999b) (Sections 2.4.1. and 2.4.3.), urinary Gla concentration (measured in 24-h urine samples) did not change significantly during the 15-day mixed-diet period (100 µg/day) in younger and older adults. Urinary Gla concentration was expressed as percentage of baseline and the mixed diet was compared with the supplemented diets (377 or 417 µg phylloquinone/day from days 6 to 10): there was no significant difference in urinary Gla concentration between the three diets on day 10 (i.e. mean of about 101% of baseline values for each diet). As well, in the metabolic depletion/repletion study in postmenopausal women by Booth et al. (2003a) (Sections 2.4.1. and 2.4.3.), mean urinary Gla concentration (measured in 24-h urine samples) was significantly lower ($p < 0.05$) at the end of the dietary depletion phase (18 µg/day for 28 days) compared to the start of the baseline diet (90 µg/day for 14 days), but did not significantly change during the three consecutive phases of dietary repletion (86, 200 and 450 µg phylloquinone/day for 14 days each).

The Panel notes that urinary concentration of Gla residues, that is a measure of the overall body content of vitamin K-dependent proteins, is sensitive to phylloquinone dietary depletion and may be sensitive to phylloquinone supplementation over several days in studies in adults, but data on a possible relationship between urinary Gla concentration and phylloquinone supplementation are conflicting. Thus, a dose-response relationship between urinary concentrations of Gla residues with phylloquinone intake cannot be precisely established. The Panel is not aware of any data on the relationship between urinary Gla concentration and menaquinone intake in the range of observed intake in Europe (Section 3.). The Panel notes that dietary intakes of phylloquinone or menaquinones required for maximal or optimal urinary Gla excretion have not been determined. The Panel also notes that there are no agreed cut-offs values for urinary Gla concentration that would indicate vitamin K adequacy. The available data suggest that the response of urinary Gla excretion to these dietary changes is age-specific.

2.4.6. Urinary concentration of vitamin K metabolites 5C and 7C

The measurement of the urinary concentrations of the 5C- and 7C-metabolites, common to both phylloquinone and menaquinones metabolism (Sections 2.3.5 and 2.3.6.), has also been proposed as a marker of the total body pool of vitamin K in adults, as reviewed by Card et al. (2014). The 5C and 7C metabolites have been measured in 24-h or spot urine samples in unsupplemented healthy adults on two consecutive days, and these concentrations respond to high-dose supplementation with phylloquinone (2 or 50 mg), MK-4 (45 mg), MK-7 (1 mg) or menadione (20 mg) in adults or in neonates (intramuscular phylloquinone, 1 mg) (Harrington et al., 2005).

In a randomised cross-over study in 9 adults residing in a metabolic unit for two 30 day-periods (separated by at least four weeks), subjects consumed a control diet (93 µg phylloquinone/day for five days), then a phylloquinone-restricted diet (11 µg/day for 15 days), then a repletion diet with either 206 µg phylloquinone/day or 240 µg dK/day for 10 days in separate residency periods (Harrington et al., 2007). Urinary 5-C and 7-C metabolites concentrations, measured in 24-h urine samples,[13] reacted differently to phylloquinone restriction. The urinary 5-C metabolite concentration significantly decreased ($p = 0.001$) after phylloquinone restriction while the urinary 7-C metabolite concentration did not. Both significantly increased after phylloquinone repletion to reach a plateau after four days.[14] **The Panel** notes that only one level of intake of phylloquinone was investigated during repletion.

The Panel notes that urinary concentrations of the 5C- and 7C-metabolites, which have been proposed as a biomarker of total vitamin K status, are sensitive to phylloquinone or menaquinone supplementation, but limited data showed that only the urinary 5C- metabolite concentration decreased during phylloquinone dietary depletion. The usefulness of the measurement of urinary concentrations of the 5C and 7C-metabolites to assess vitamin K status is limited by the proportion of these metabolites also excreted in the bile (Sections 2.3.5. and 2.3.6.). The Panel considers that the dose-response relationship with vitamin K intake (phylloquinone or menaquinones) is not established, and notes that no agreed cut-off for vitamin K adequacy has been identified.

2.4.7. Conclusions on biomarkers

Vitamin K deficiency leads to an increased PT, which is the only vitamin K biomarker that has been associated with adverse clinical symptoms. Symptomatic vitamin K deficiency and impairment of normal haemostatic control in healthy adults may take more than two to three weeks to develop at 'low' phylloquinone intake (i.e. < 10 µg/day) (Section 2.2.1.).

The other biomarkers (concentration/activity of blood coagulation factors, blood concentrations of undercarboxylated forms of vitamin-K dependent proteins or of vitamin K, urinary concentrations of

[13] Mean of 3.55 and 1.33 µg/day after the control period, respectively.
[14] 5-C: mean of 2.89 µg/day at the end of depletion and of 8.48 µg/day at the end of repletion; 7-C: mean of 1.10 µg/day at the end of depletion, and of 2.71 µg/day at the end of repletion.

Gla residues or of vitamin K metabolites 5C and 7C) may change according to vitamin K dietary intake (biomarker of intake). In the available studies, dietary vitamin K restriction results in lower phylloquinone plasma concentration, higher plasma concentration of undercarboxylated vitamin K dependent proteins, lower urinary Gla excretion, and mostly not in PT increase (possibly in relation to the short study duration). The Panel concludes that there are no biomarkers for which a dose-response relationship with phylloquinone intake has been established. The available studies generally assessed whether the biomarkers returned to baseline values with phylloquinone supplementation/dietary repletion after phylloquinone depletion. However, for these biomarkers, no cut-off value to define adequate vitamin K status is available, so these changes in biomarkers are difficult to interpret. Studies investigating the relationship between biomarkers and menaquinone intake often used doses much higher than the limited observed intake data in Europe (Section 3.). Thus, the Panel considers that none of these biomarkers is suitable by itself to assess vitamin K adequacy. The Panel also considers that data on the effect of age and sex on vitamin K status in adults are insufficient for deriving the requirement for vitamin K according to sex or for 'younger' and 'older' adults.

2.5. Effects of genotypes

The response of biomarkers to vitamin K intake varies among healthy individuals (Shea and Booth, 2016). Meta-analysis of genome-wide association studies for single nucleotide polymorphisms (SNPs) associated with circulating phylloquinone concentrations identified multiple candidate genes related to lipoprotein and phylloquinone metabolism (Dashti et al., 2014).

A common polymorphism of the gene for the enzyme **GGCX** (Section 2.2.1.) in human populations has been associated with transcriptional activity and sensitivity to warfarin (Shikata et al., 2004; Wadelius et al., 2005; Vecsler et al., 2006). The *GGCX rs699664* SNP induces an increased carboxylase activity (Kinoshita et al., 2007). In community-dwelling older adults, significant cross-sectional association was observed between plasma phylloquinone concentration and/or plasma %ucOC and polymorphisms of *GGCX* (Crosier et al., 2009).

In the VKOR protein structure (Section 2.2.1.), the VKOR complex subunit 1 (**VKORC1**) is involved in enzymatic activity (Goodstadt and Ponting, 2004) and common polymorphisms of the *VKORC1* gene are associated with variability in the effect of warfarin (Li et al., 2006; Montes et al., 2006; Obayashi et al., 2006; Rettie and Tai, 2006; Garcia and Reitsma, 2008; Owen et al., 2010). In community-dwelling older adults, significant cross-sectional association was observed between plasma phylloquinone concentration and/or plasma %ucOC and polymorphisms of VKORC1 (Crosier et al., 2009). In a Chinese cohort, SNPs and haplotypes within the *VKORC1* locus were significantly associated with ucOC and PIVKA-II concentrations (Wang et al., 2006). Genetic polymorphisms in the coagulation factor FVII (F7 -323Ins10) and VKORC1 were found to have an impact on the coagulation profile and the risk to develop intraventricular haemorrhage in a cohort (n = 90) of preterm infants (Schreiner et al., 2014).

Among the three common alleles of the gene encoding **ApoE** (i.e. E2, E3, and E4), the ability to clear intestinal lipoproteins rich in vitamin K from the blood is greatest with E4 and lowest with E2 (Kohlmeier et al., 1995; Newman et al., 2002). However, the magnitude of the effect of *ApoE* genotype on vitamin K status remains unclear, because in some studies, the highest frequency of E4 allele was associated with lower %ucOC in blood but also with higher or no different plasma phylloquinone concentration (Beavan et al., 2005; Yan et al., 2005).

Cytochrome P450 4F2 (**CYP4F2**) is involved in the hydroxylation of tocopherols and acts as a phylloquinone oxidase to produce the phylloquinone metabolite ω-hydroxyvitamin K1 (McDonald et al., 2009). A *CYP4F2* DNA variant (*rs2108622; V433M*) is present with a minor allele frequency of 5.8–26.7% in different ethnic groups (American, Chinese, Japanese and Africans) (Caldwell et al., 2008). Carriers of this polymorphism need an increased warfarin dose for the anticoagulation activity (Caldwell et al., 2008), have lower CYP4F2 protein concentrations in liver and a reduced capacity to metabolise phylloquinone and may require lower dietary intakes of vitamin K compared to non-carriers to maintain an equivalent vitamin K status (McDonald et al., 2009).

The Panel notes that potential genetic determinants of vitamin K status include polymorphisms in the genes involved in the activity, transport, uptake, metabolism, tissue-specific availability and recycling of vitamin K, but **considers** that data on the effect of genotypes are insufficient to be used for deriving the requirement for vitamin K according to genotype variants.

3. Dietary sources and intake data

3.1. Dietary sources

Phylloquinone, present in all photosynthetic plants, is the predominant dietary form of vitamin K in the human diet (Gross et al., 2006). The primary sources of phylloquinone include dark green leafy vegetables (e.g. spinach, lettuce and other salad plants) and Brassica (flowering, head or leafy), with contents of about 60–365 µg and about 80–585 µg per 100 g, respectively, according to European food composition database (Section 3.2.1.) of the European Food Safety Authority (EFSA). Other sources of phylloquinone include some seed oils, spreadable vegetable fats and blended fats/oils (Piironen et al., 1997; Peterson et al., 2002), with content of about 25–60 µg/100 g, based on this EFSA database.

For (**total or individual) menaquinones**, food composition data are limited, in the EU (Schurgers et al., 1999; Koivu-Tikkanen et al., 2000; Schurgers and Vermeer, 2000; Anses/CIQUAL, 2013; Manoury et al., 2013), in the US (Elder et al., 2006; Ferreira et al., 2006; USDA, 2015; Fu et al., 2016) and in Japan (Hirauchi et al., 1989; Kamao et al., 2007a).

Menaquinones are found in **animal-based foods**, in particular in *liver products*: mostly MK-4 in the range 0.3–369 µg/100 g in the EU, MK-9 to MK-11 in the range 0.4–492 µg/100 g in the USA, and MK-6 to MK-14 in the range 0.03–44 µg/100 g in Japan. Menaquinones are also found in *meat and meat products* (mostly MK-4, in the range 0.1–42 µg/100 g in the available data), and in *poultry* products that are particularly rich in MK-4, as poultry feed is a rich source of menadione, subsequently converted to MK–4 in certain tissues of the poultry (in the range 5.8-60 µg/100 g in the EU, and 9-39 µg/100 g in the USA and Japan). Menaquinones are also present in some *cheese and other dairy* products: EU data on MK-4 to MK-10 (in particular MK-9) are in the range 0.1–94 µg/100 g, while US and Japanese data, mainly on MK-4, are in the range 1–21 µg/100 g. In **natto**, the most abundant menaquinone is MK-7, in the range of about 850 µg–1,000 µg/100 g (EU and Japanese data). Limited data on menaquinones are also available in a number of other products: in **eggs** (in particular in egg yolk) the most abundant menaquinone is MK-4, in the range 10–30 µg/100 g in the EU, or 9-64 µg/100 g according to Japanese and US data, **in fish, spices, chocolate, oil or bread, pies and pie crusts, fast food composite dishes** (MK-4 to MK-8 and total menaquinones in EU and US data).

For **dihydrophylloquinone** (Section 2.1.), the highest contents (about 60–165 µg/100 g) are reported in products such as some shortenings, some margarines, some snacks and crackers, some pie crusts and some pop-corns (USDA, 2015).

Currently, phylloquinone (phytomenadione) and menaquinone (menaquinone occurring principally as MK-7 and, to a minor extent, MK-6) may be added to foods[15] and food supplements[16] The vitamin K content of infant and follow-on formulae and of processed cereal-based foods and baby foods for infants and children is regulated.[17]

[15] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, p. 26

[16] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51.

[17] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, p.1. and Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children, OJ L 339, 06.12.2006, p. 16-35.

3.2. Dietary intake in Europe

The Panel aimed at presenting in this Section observed intakes of vitamin K (both forms) or of phylloquinone or (total or individual) menaquinones in Europe estimated using the EFSA Comprehensive European Food Consumption Database (EFSA, 2011b) and the EFSA Food Composition Database compiled during a procurement project (Roe et al., 2013) involving several national food database compiler organisations. However, the EFSA Food Composition Database did not contain data on phylloquinone or menaquinones, most of the involved national food composition databases did not contain any vitamin K data and the estimates for 'total vitamin K' also have limitations as described below (Section 3.2.1.). In view of these limitations, the Panel also collected published data on estimated intake of phylloquinone and menaquinones (Section 3.2.2.).

3.2.1. Dietary intake of 'total vitamin K' estimated by EFSA

3.2.1.1. Methodology

The Panel presents in this section observed 'total vitamin K' intakes in Europe, estimated by EFSA using the EFSA Comprehensive European Food Consumption Database and the EFSA Food Composition Database. Data presented as 'total vitamin K' in the EFSA Food Composition Database were available **originally only from three countries (Denmark, Germany and Sweden)**. Involved food database compiler organisations were allowed to borrow food composition data from other countries in case no original composition data were available in their own national database. As a result, Germany and Sweden borrowed respectively 2.5% and 30% of the 'total vitamin K' values they reported in the composition database, while Finland, Italy, United Kingdom (UK), Netherlands and France borrowed 100% of the values reported. In addition, further research on the websites of the Danish,[18] German[19] and Swedish[20] food composition databases suggests that **only the data originally provided by Sweden may correspond to amounts of both phylloquinone and menaquinones**, while data originally provided by Denmark and Germany concern phylloquinone only. This means that phylloquinone data and vitamin K data (i.e. phylloquinone and menaquinones) may have been listed under the term 'total vitamin K' in the composition data provided to EFSA. For intake estimates of Ireland and Latvia, food composition data from the UK and Germany, respectively, were used by EFSA, because no specific composition data from these countries were available. **The Panel notes** that these methodological limitations induce considerable uncertainty in the 'total vitamin K' intake estimates for the included European countries.

This assessment includes food consumption data from 13 dietary surveys (Appendix B) from nine countries (Finland, France, Germany, Ireland, Italy, Latvia, the Netherlands, Sweden and the UK). Individual data from these nationally representative surveys (except for the Finnish surveys in children) undertaken between 2000 and 2012 were available to EFSA, and classified according to the FoodEx2 food classification system (EFSA, 2011a). Intake calculations were performed only on subjects with **at least two reporting days**. For EFSA's assessment, it was assumed that the best intake estimate would be obtained when both the consumption data and the composition data are provided to EFSA for the same country. EFSA intake estimates are based on consumption of foods, either fortified or not, but without taking dietary supplements into account.

The data covers all age groups from infants to adults. Data on infants 1–11 months old were available from Finland, Germany, Italy and UK. The proportions of breastfed infants were between 21% and 58% according to the survey considered and most breastfed infants were partially breastfed (see table footnotes of Appendices C–D). **The Panel notes** the limitations in the methods used for assessing breast milk consumption in infants (table footnotes of Appendices C–D) and related uncertainties in the intake estimates for infants.

[18] http://www.foodcomp.dk/v7/fcdb_aboutfooddata_vitamins.asp#Vitamin and http://frida.fooddata.dk/CntList.php
[19] https://www.blsdb.de/assets/uploads/BLS_Variablen_3.02.pdf
[20] http://www.livsmedelsverket.se/livsmedel-och-innehall/naringsamne/vitaminer-och-antioxidanter/vitamin-k/

3.2.1.2. Results

Taking into account the uncertainties mentioned above, 'total vitamin K' intake mean estimates ranged between 23 and 61 µg/day in infants, between 36 and 53 µg/day in children aged 1 to < 3 years, between 42 and 93 µg/day in children aged 3 to < 10 years, between 68 and 143 µg/day in children aged 10 to < 18 years (Appendices C–D). 'Total vitamin K' intake mean estimates ranged between 72 and 196 µg/day in adults (≥ 18 years). The main food group contributing to 'total vitamin K' intakes was vegetables and vegetable products (Appendices E–F). Leafy vegetables followed by brassica vegetables were the most important contributors to 'total vitamin K' intakes for all age classes apart from infants, for whom the group 'food products for young population' was the main source of 'total vitamin K'. Also composite dishes were contributors to 'total vitamin K' intakes, probably at least partly due to vegetable based ingredients in the dishes, as well as (to a lower extent) the groups 'animal and vegetable fats and oils' and 'legumes, nuts, oilseeds and spices'.

Mean intake estimates in adults for two countries (Italy, the Netherlands) were generally higher than the others (and higher than about 150 µg/day) in the different age ranges investigated in adults (Section 3.2.2. for other published Dutch intake data). This may be explained by a particular high contribution of leafy vegetables and aromatic herbs (Italy) and brassica vegetables (the Netherlands) compared to the other countries, while composition data for these food categories were generally in line among countries. In the countries investigated, average daily intakes were in most cases slightly higher among males (Appendix C) compared to females (Appendix D).

3.2.1.3. Discussion

EFSA intake estimates were compared with published intake estimates from the same included national surveys. Published data were available for comparison only in **Finland**, i.e. for *children* aged 10 to < 18 years (Hoppu et al., 2010) and *adults* (FINDIET2012 (Helldán et al., 2013)), and in **Germany** for *children* aged 3 to < 18 years (Mensink et al., 2007)).

EFSA mean intake estimates for *Finnish adults* differed by about 5–12% from the published values (Helldán et al., 2013). The comparison of EFSA intake estimates with the published intake estimates of *Finnish children* (Hoppu et al., 2010) (i.e. different by 12–14%) have inherent limitations as they were for two consecutive days of dietary recall, while EFSA data comprised 2 x 48 hour dietary recall. The sources of 'total vitamin K' in the diet were not presented in this publication, and therefore could not be compared with EFSA's estimates. Considering the uncertainties of this intake assessment by EFSA (discussed above), a difference of up to 14% can be considered acceptable.

Difference between the 'total vitamin K' intake calculated by EFSA and the published estimates for *German children* (Mensink et al., 2007) (different by 63–65%, EFSA estimates being lower than the published values) is large. The published intake estimates for children are high even in comparison with intakes reported for older age classes in a different study in Germany (DGE, 2012) (Appendix G). One possible explanation could be a different version of the German food composition database used for this last publication and for the publication on children, which was confirmed by a personal communication.[21] This communication indicated that phylloquinone intake data in children were calculated on the basis of the version II.3 of the German composition table of Bundeslebensmittelschlüssel (BLS) of the Max Rubner Institut (MRI),[22] and were much higher than adult data, calculated with the newer version of the BLS (3.02). The EFSA Food Composition Database contained German data that were also from an earlier version of the German composition table (3.01, but vitamin K data were identical with the current BLS version 3.02). In the newer version of the BLS (3.02), 120 more recent and better data have been introduced. With the introduction of the new data, 77 items had a 74% lower phylloquinone content, and 43 items a 67% higher phylloquinone

[21] From a member of the team in charge of the national food composition data base (Bundeslebensmittelschlüssel, BLS) at the Max Rubner Institute.
[22] https://www.blsdb.de/

content. In conclusion, the 'older' data are too high, but, on the other hand, the new data have flaws and may yield some underestimation, due to the lack of data source (thus the values were considered as 'missing' by the national food database compiler and '0' for intake calculations).

Uncertainties on the nature of the 'total vitamin K' composition data (i.e. phylloquinone only or the sum of phylloquinone and menaquinones) and on the assessment of the intake data in infants (see table footnotes of Appendices C–D) have been discussed above. In addition, uncertainties in the estimates of all countries may be caused by inaccuracies in mapping food consumption data according to the FoodEx2 classification, analytical errors or errors in estimating 'total vitamin K' composition for the food composition table, due to the use of borrowed 'total vitamin K' values from other countries and the replacement of missing 'total vitamin K' values by values of similar foods or food groups in the intake estimation process. These uncertainties may, in principle cause both too high and too low estimates of 'total vitamin K' intake.

3.2.2. Dietary intake of phylloquinone and menaquinones as reported in the literature

The Panel performed an additional literature search on vitamin K intake estimates (i.e. phylloquinone, total or individual menaquinones) in observational studies/surveys undertaken in the EU, mainly in adults (Appendix G). Comparison of EFSA's 'total vitamin K' intake estimates in EU countries with the published intakes of vitamin K from studies undertaken outside Europe (Korea, USA and Japan) (Booth et al., 1996a; Feskanich et al., 1999; Booth et al., 2003b; Kamao et al., 2007a; Kim et al., 2013) was not undertaken, as consumption patterns are significantly different.

Published studies on intake of phylloquinone or menaquinones used different designs, dietary intake assessments and food composition data, which limit direct comparisons between them (Appendix G). However, the intake estimates of 'vitamin K' or phylloquinone of these publications are variable and not completely in line with EFSA's calculations (Section 3.2.1.). This can be explained by difference in the methods to assess intake (dietary recalls or record for at least two reporting days for EFSA's calculations, versus e.g. dietary history or FFQ), the methods of statistical analysis, the sources of composition data, the adjustments of intake values, or the size and characteristics of the samples of subjects that were smaller and/or not nationally representative. These differences make these published values not directly comparable with EFSA's intake estimates.

Five studies estimated the intake of **phylloquinone and menaquinones separately** using FFQs, including one (Geleijnse et al., 2004) being on subjects from the same Dutch prospective cohort as in another study (Schurgers et al., 1999) but considering more publications on composition data; and other Dutch and German prospective cohorts. The individual menaquinones investigated in these studies were not all the same (Appendix G). Estimated median intake of total menaquinones (34.7 µg/day) represented **about one third** of estimated median phylloquinone intake (93.6 µg/day) in Germany (Nimptsch et al., 2008), while estimated mean total menaquinone intake (about 27–31 µg/day) was about **10–13%** of the sum of mean intake of phylloquinone and the mean intake of menaquinone in the Netherlands (about 230–288 µg/day according to sex and study (Schurgers et al., 1999; Geleijnse et al., 2004; Gast et al., 2009; Vissers et al., 2013)).

Among **individual menaquinones**, MK-4, MK-8 and MK-9 had the highest contributions to total menaquinone intakes in one Dutch and one German studies in adults (Nimptsch et al., 2008; Gast et al., 2009). MK-7 is used in the EU for fortification and supplementation (Section 3.2.1.) but no data were available to EFSA to assess its intake via these sources.

In addition, personal communication[23] suggested that 'older' published vitamin K intake data from the Netherlands, like the German data for phylloquinone intake calculated with the older version of the

[23] From members of the National Institute for Public Health and the Environment in the Netherlands, and a member of the team in charge of the national food composition data base (Bundeslebensmittelschlüssel, BLS) at the Max Rubner Institute as mentioned in Section 3.2.1.3.

BLS (II.3) (Sections 3.2.1.3.), are an **overestimate** of the actual vitamin K intake. This may be due to the fact that both Germany and the Netherlands used the same 'old' composition data from Schurgers (in both cases the intakes were 200 µg/day or more), that the current analytical methods may be more precise than in the past, and that different food consumption measurements have been used (FFQ in the Dutch studies mentioned above, versus 2x24 h recall in the recent Dutch food consumption survey). Personal communication also confirmed that the Dutch National Food Composition tables for vitamin K (phylloquinone and MK-4 to MK-10) are being updated, with analytical values from Dutch analysis and new literature values are used (e.g. from the database of the US Department of Agriculture USDA) whenever possible. Vitamin K intake data estimated from the Dutch National Food Consumption Survey 2007–2010 were calculated with **partially updated** composition data from 2013, which cover the most relevant sources of vitamin K but are not complete. This may lead to a possible underestimation of the vitamin K intake. In a recently published memo on this **Dutch National Survey**,[24] the median (mean, IQR) intake estimates for **vitamin K** (**phylloquinone and MK-n)** for children are 62 (70, 43–89) and 72 (80, 51–99) µg/day for girls (n = 857) and boys (n = 856) aged 7-18 years, respectively. For adults aged 19–69 years, these values were 100 (111, 70-140) and 117 (128, 85–159) µg/day in women (n = 1,051) and men (n = 1,055), respectively. Of note, according to the National Nutrition Survey II in **Germany (DGE, 2012)** using a **recently updated** version of the German food composition table (BLS 3.02, MRI, Section 3.2.1.3.), median **phylloquinone** intake, assessed by 2 x 24-h recall, was 76 µg/day (95% CI 75–77) for subjects aged 15 to 80 years (n = 6,160) (mean intake was not reported).

3.2.3. Conclusions on dietary intake in Europe

The Panel notes that 'total vitamin K' mean intake estimated by EFSA for nine EU countries ranged between 72 and 196 µg/day in adults (≥ 18 years). The Panel notes the uncertainties in this intake assessment, in particular with regard to the nature of the 'total vitamin K' composition data (i.e. phylloquinone only or the sum of phylloquinone and menaquinones) and on the assessment of the intake data in infants, and that intake of phylloquinone or menaquinones in these countries could not be estimated by EFSA with the available databases.

Published data on intake of phylloquinone and menaquinones in Europe show that phylloquinone is the major form consumed although the exact proportion of phylloquinone in vitamin K intake remains uncertain, and suggest that MK-4, MK-8 and MK-9 have the highest contributions to the intake of total menaquinones.

The Panel also notes the updated food composition database and intake estimates for the Netherlands (vitamin K (i.e. phylloquinone and menaquinones), in children and adults) and for Germany (phylloquinone, in adults). These updated median intake estimates are in line with the lower bound of the range of mean intakes in adults in nine EU countries estimated by EFSA, mentioned above.

4. Overview of Dietary Reference Values and recommendations

4.1. Adults

D-A-CH (2015) derived an adequate intake (AI) for vitamin K of **1 µg/kg body weight per day** for adults, based on the influence of vitamin K (**phylloquinone**) on blood coagulation (Frick et al., 1967; National Research Council, 1989; Suttie, 1996)[25]. Expressed in µg/day, the AIs were 70 and 60 µg/day for men and women aged 19–50 years, respectively. As a precaution, the AIs for older adults were increased, i.e. 80 µg/day for men and 65 µg/day for women, to take into account possible malabsorption and medication at that age.

[24] http://www.rivm.nl/dsresource?objectid=b96a6448-882a-41c1-bb72-6ece306bc4b2&type=org&disposition=inline

[25] The conclusion of the National Research Council (1989) was mainly based on Frick et al. (1967) and Suttie et al. (1988), which both dealt with phylloquinone.

For the NNR 2012, due to a lack of additional evidence, the Nordic Council of Ministers (2014) kept the previously set recommended intake of **1 µg/kg body weight per day for adults**, taking into account that **phylloquinone** intakes of about 60 to 80 µg/day (i.e. 1 µg/kg body weight per day) are adequate to prevent vitamin K deficiency in healthy subjects (Suttie et al., 1988; National Research Council, 1989; Jones et al., 1991; Bach et al., 1996). The Council considered that the available evidence on the relationship between phylloquinone or menaquinones intake and health consequences (bone health, atherosclerosis, and other health outcomes) could not be used to set reference values for vitamin K. The Council noted the low prevalence of vitamin K deficiency in the general population, the impossibility to induce deficiency symptoms with a vitamin K depleted diet, and the insufficient bacterial synthesis of vitamin K in the intestine to maintain serum concentrations of vitamin K. The Council considered that data on biomarkers (concentration of coagulation factors, plasma/serum concentrations of phylloquinone, degree of carboxylation of vitamin K-dependent proteins, urinary vitamin K metabolites) (Suttie et al., 1988; Ferland et al., 1993; Booth and Suttie, 1998; Booth et al., 2001; Binkley et al., 2002; Booth et al., 2003a; Bugel et al., 2007; Harrington et al., 2007; Schurgers et al., 2007; Booth, 2009; McCann and Ames, 2009) were insufficient to change the previously set reference value.

The World Health Organization (WHO/FAO, 2004) derived a Recommended Nutrient Intake (RNI) of **1 µg/kg body weight per day of phylloquinone**, corresponding to 55 µg/day for adult women and 65 µg/day for adult men. WHO/FAO (2004) set this value considering the function of vitamin K in blood coagulation and the average intakes (mainly of phylloquinone) in adults that are close to UK and US reference values of this period (National Research Council, 1989; DH, 1991; Suttie, 1992; Booth et al., 1996b). WHO/FAO (2004) considered that available data on γ-carboxylation of OC could not be used to set reference values (Sokoll et al., 1997).

The U.S. Institute of Medicine (IOM, 2001) considered that data on biomarkers of vitamin K status, including PT, FVII activity, plasma/serum concentrations of phylloquinone, the degree of carboxylation of vitamin K-dependent proteins (prothrombin, OC) and urinary vitamin K metabolite concentrations could not be used to assess the requirements for vitamin K. The IOM considered that only PT has been associated with adverse clinical effects and that the significance of changes observed in the other biomarkers following changes in vitamin K intake is unclear. The IOM also considered that data on the relationship between vitamin K intake and chronic diseases (osteoporosis, atherosclerosis) could not be used as well. The IOM reported on data showing abnormal PIVKA-II concentrations for intakes (phylloquinone) below 40–60 µg/day and lack of signs of deficiency to intakes above 80 µg/day (Suttie et al., 1988; Jones et al., 1991; Ferland et al., 1993; Bach et al., 1996). IOM (2001) took into account the lack of sufficient dose-response data between vitamin K intake and biomarkers of status, the uncertainty surrounding the interpretation of these biomarkers and the low prevalence of vitamin K deficiency in the general population. Thus, IOM (2001) derived an AI of 120 µg/day for men and of 90 µg/day for women, based on the **highest median intake of dietary 'vitamin K'**[26] in apparently healthy subjects (NHANES III, 1988-1994) (highest intake chosen to take into account possible underestimation by dietary intake assessment methods), rounded up to the nearest five.

The French Food Safety Agency (Afssa, 2001) considered that the requirement for vitamin K in adults is probably low due to the efficient vitamin K recycling in the liver. Afssa (2001) considered that this requirement may be between 0.1 and 1 µg/kg body weight per day based on data on maintenance of normal coagulation reviewed in Shearer et al. (1988), as data on the need for complete γ-carboxylation of vitamin K-dependent protein were insufficient for DRV-setting (Shearer, 1995). Afssa (2001) set a reference value of 45 µg phylloquinone/day for younger adults (< 75 years). For older adults (≥ 75 years), the reference value was set at 70 µg phylloquinone/day, based on data on vitamin K and bone health in older adults or suggesting a role of vitamin K to maintain sufficient concentration of

[26] Assumed by the Panel to be probably phylloquinone.

carboxylated osteocalcin (cOC) in bone tissues (Knapen et al., 1998; Liu and Peacock, 1998; Tamatani et al., 1998; Feskanich et al., 1999; Cynober et al., 2000).

The SCF (1993) did not set an AR or a PRI for vitamin K, but considered that an intake of **1 µg/kg body weight per day**, which would be provided by a usual diet, was **adequate**. To set this value, (SCF, 1993) considered the effect of depletion at about 50 µg phylloquinone/day (with no effect on PT) and supplementation with 50 µg phylloquinone/day, on prothrombin biosynthesis and Gla urinary excretion (Suttie et al., 1988) and a previous review (Suttie, 1987).

The Netherlands Food and Nutrition Council (1992) did not consider vitamin K when setting reference values for the whole population.

DH (1991) concluded that, for adults, **1 µg/kg body weight per day phylloquinone** is 'safe and adequate' (Suttie, 1985), since it maintains vitamin K-dependent coagulation factors. DH (1991) did not derive an AR or a PRI for vitamin K for adults.

An overview of DRVs for vitamin K for adults is presented in Table 1.

Table 1: Overview of dietary reference values for vitamin K (expressed as phylloquinone) for adults

	D-A-CH (2015) [a,b]	NCM (2014) [c]	WHO/FAO (2004) [a,b]	Afssa (2001) [a]	IOM (2001) [a]	SCF (1993) [c]	NL (1992)	DH (1991) [c]
Age (years)	19–50	≥ 18	19–≥ 65	19–74	19–≥ 70	≥ 18	-	≥ 18
Men	70	1	65	45	120	1	-	1
Women	60	1	55	45	90	1	-	1
Age (years)	51– ≥ 65			≥ 75				
Men	80			70				
Women	65			70				

D-A-CH: Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährung; NCM: Nordic Council of Ministers; WHO/FAO: World Health Organization/Food and Agriculture Organization of the United Nations; Afssa: Agence française de sécurité sanitaire des aliments; IOM: Institute of Medicine; SCF: Scientific Committee on Food; NL: Health Council of the Netherlands; DH: Department of Health.
(a): µg/day.
(b): derived considering an intake of 1 µg/kg body weight per day.
(c): µg/kg body weight per day.

4.2. Infants and children

D-A-CH (2015) also set an AI for vitamin K of 1 µg/kg body weight per day (Section 4.1.) for children. Expressed in µg/day, AIs for children range between 10 µg/day in infants 4–12 months, to 60 (girls) and 70 (boys) µg/day in adolescents 15–19 years.

The Nordic Council of Ministers (2014) could not set ARs or PRIs for vitamin K in µg/day for children, due to a lack of sufficient evidence. For children, NNR 2012 kept the previously set recommended intake of 1 µg/kg body weight per day (Section 4.1.). NNR 2012 also reported on prophylactic vitamin K administration to newborns (IOM, 2001; Hansen et al., 2003; Van Winckel et al., 2009).

For infants aged 7–12 months and children, WHO/FAO (2004) set RNIs ranging between 10 µg/day (7–12 months) and 35–55 µg/day (10–18 years), based on an intake of phylloquinone of 1 µg/kg body weight per day as for adults (Section 4.1.). WHO/FAO (2004) also mentioned prophylactic vitamin K administration to newborns.

For infants aged 7–12 months, IOM (2001) set an AI of 2.5 µg/day based on the extrapolation from the phylloquinone intake of infants aged 0–6 months, estimated considering a mean breast milk intake of 0.78 L/day and an average phylloquinone concentration of 2.5 µg/L in human milk (Haroon et al., 1982; von Kries et al., 1987b; Hogenbirk et al., 1993; Greer et al., 1997). This upward extrapolation was done by allometric scaling (body weight to the power of 0.75, using reference body weights). No adverse clinical outcome was observed in older infants at that intake (Greer et al., 1991). Data on vitamin K in weaning foods were lacking and downward extrapolation from adults was not used to set an AI for older infants. AIs for children aged 1–18 years were set on basis of the highest median intake reported (NHANES III, 1988-1994) (and rounding), since age-specific data on vitamin K requirement were lacking. The AIs ranged between 30 and 75 µg 'vitamin K'/day,[27] for children aged 1–3 years and 14–18 years respectively. IOM (2001) noted that the methods used to establish AIs for older infants and children and the increased consumption of vitamin K sources (vitamin K-rich fruits and vegetables) with age may explain the difference in AI values for infants and children.

Afssa (2001) set the reference value for infants at 5 to 10 µg phylloquinone/day, and reference values for children based on an estimated requirement of 1 µg/kg body weight per day, leading to reference values between 15 (children 1–3 years) and 65 (children 16–19 years) µg phylloquinone/day. Afssa (2001) also mentioned prophylactic vitamin K administration to newborns.

SCF (1993) did not discuss specifically the requirement for vitamin K in children, did not set ARs or PRIs, but generally considered the intake of 1 µg/kg body weight per day (Section 4.1.) to be adequate.

The Netherlands Food and Nutrition Council (1992) did not consider vitamin K when setting reference values for the whole population.

After rounding up, the UK COMA (DH, 1991) proposed a 'safe intake' of 10 µg/day for infants (about 2 µg/kg body weight), derived from the highest and rounded phylloquinone concentration in human milk (10 µg/L) in the available data (von Kries et al., 1987b; Canfield and Hopkinson, 1989) and a breast milk consumption of 0.85 L/day. They noted the low hepatic reserves of phylloquinone and the absence of hepatic menaquinones at birth (Shearer et al., 1988), as well as the association between haemorrhagic disease of the newborn and exclusive breastfeeding (von Kries et al., 1988). They supported prophylactic vitamin K administration to all newborns. No specific reference value was mentioned for older children.

An overview of DRVs for vitamin K for infants and children is presented in Table 2.

[27] Assumed by the Panel to be probably phylloquinone.

1734 **Table 2:** Overview of dietary reference values for vitamin K (expressed as phylloquinone) for
1735 infants and children

	D-A-CH (2015) [a,b]	NCM (2014) [c]	WHO/FAO (2004) [a,b]	Afssa (2001) [a,b]	IOM (2001) [a]	SCF (1993) [c]	DH (1991) [a]
Age (months)	4–12	All children	7–12	'Infants'	7–12	All children	'Infants'
Infants (µg/day)	10	1	10	5–10	2.5	1	10
Age (years)	1–< 4		1–3	1–3	1–3		
All (µg/day)	15		15	15	30		-
Age (years)	4–< 7		4–6	4–6	4–8		
All (µg/day)	20		20	20	55		-
Age (years)	7–< 10		7–9	7–9			
All (µg/day)	30		25	30			-
Age (years)	10–< 13			10–12	9–13		
All (µg/day)	40			40	60		-
Age (years)	13–< 15		10–18	13–15	14–18		
All (µg/day)	50		35–55	45	75		-
Age (years)	15–< 19			16–19			
Boys(µg/day)	70			65			-
Girls (µg/day)	60						

1736 D-A-CH: Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für
1737 Ernährung; NCM: Nordic Council of Ministers; WHO/FAO: World Health Organization/Food and Agriculture
1738 Organization of the United Nations; Afssa: Agence française de sécurité sanitaire des aliments; IOM: Institute of
1739 Medicine; SCF: Scientific Committee on Food; NL: Health Council of the Netherlands; DH: Department of Health.
1740 (a): µg/day.
1741 (b): derived considering an intake of 1 µg/kg body weight per day.
1742 (c): µg/kg body weight per day.

1743 ### 4.3. Pregnancy and lactation

1744 D-A-CH (2015) set the same AI for vitamin K for healthy pregnant or lactating women as for other
1745 women, as it is unknown whether pregnant women need additional vitamin K and as the possibly
1746 small additional need in lactation is fully covered by a healthy and balanced diet. WHO/FAO (2004)
1747 and Afssa (2001) also proposed for pregnant or lactating women the same reference value as for other
1748 women (Section 4.1.).

1749 Nordic Council of Ministers (2014), SCF (1993) and DH (1991) mentioned no specific information or
1750 reference values for vitamin K for pregnant or lactating women. The Netherlands Food and Nutrition
1751 Council (1992) did not consider vitamin K when setting reference values for the whole population.

1752 IOM (2001) noted that studies on pregnant women reported no signs of vitamin K deficiency and
1753 comparable blood vitamin K concentrations to those of non-pregnant women (Mandelbrot et al., 1988;
1754 von Kries et al., 1992). There was no data on vitamin K content of foetal tissue, and studies on
1755 vitamin K supplementation in pregnant women (Morales et al., 1988; Kazzi et al., 1990; Anai et al.,
1756 1993; Dickson et al., 1994) could not be used for establishing additional requirements during
1757 pregnancy. Median intakes in pregnant or non-pregnant women ((NHANES III, 1988-1994), TDS
1758 1991-1997) and (Booth et al., 1999a)) were noted. IOM (2001) set the same AI for pregnant
1759 adolescent or women as for other adolescent girls or women, based on median intakes[28] in non-
1760 pregnant women. Data suggested comparable phylloquinone intake in lactating or non-lactating
1761 women and no significant correlation between phylloquinone intake from a usual diet and breast milk

[28] Assumed by the Panel to be probably phylloquinone.

concentration (NHANES III, 1988-1994; Greer et al., 1991). As vitamin K concentration in human milk is low, the AI was the same as for non-pregnant women.

An overview of DRVs for vitamin K for pregnant or lactating women is presented in Table 3.

Table 3: Overview of dietary reference values for vitamin K (expressed as phylloquinone) for pregnant and lactating women

	D-A-CH (2015) [(a)]	NCM (2014)	WHO/FAO (2004)[(a)]	Afssa (2001)[(a)]	IOM (2001)[(a)]	SCF (1993)	DH (1991)
Pregnant women					75 [(b)]		
(µg/day)	60	-	55	45	90 [(c)]	-	-
Lactating women		-			75 [(b)]		
(µg/day)	60		55	45	90 [(c)]	-	-

D-A-CH: Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährung; NCM: Nordic Council of Ministers; WHO/FAO: World Health Organization/Food and Agriculture Organization of the United Nations; Afssa: Agence française de sécurité sanitaire des aliments; IOM: Institute of Medicine; SCF: Scientific Committee on Food; NL: Health Council of the Netherlands; DH: Department of Health.
(a): derived considering an intake of 1 µg/kg body weight per day.
(b): girls aged 14–18 years.
(c): adults.

5. Criteria (endpoints) on which to base Dietary Reference Values

5.1. Indicators of vitamin K requirement

5.1.1. Adults

5.1.1.1. Use of biomarkers

As discussed in Sections 2.2.2.1. and 2.4., vitamin K deficiency leads to an increased PT and eventually associated adverse clinical symptoms. However, PT and the PTT are not sensitive markers of vitamin K intake and status and non-specific indicators of vitamin K deficiency, and symptomatic vitamin K deficiency and impairment of normal haemostatic control in healthy adults may take more than two to three weeks to develop at 'low' phylloquinone intake (i.e. < 10 µg/day) (Sections 2.2.2.1. and 2.4.).

For the other biomarkers investigated (Section 2.4.), even if they may change with changes in vitamin K (phylloquinone or menaquinone) dietary intake, no clear dose-response relationship with phylloquinone or menaquinone intake has been established. The available metabolic studies generally assessed whether the biomarkers returned to baseline values with phylloquinone supplementation/dietary repletion after phylloquinone depletion. However, for these biomarkers, no cut-off value to define adequate vitamin K status is available, so these changes in biomarkers are difficult to interpret. The Panel considers that none of these biomarkers is suitable by itself to assess vitamin K adequacy (Section 2.4.).

The SCF (1993) considered that an intake of phylloquinone of 1 µg/kg body weight per day was adequate, mainly based on the depletion/repletion study in young men (mean ± SD: 72 ± 9 kg body weight) by Suttie et al. (1988), which showed that supplementation with 50 µg phylloquinone/day in addition to a restricted diet (median of about 32–40 µg phylloquinone/day) restored the S:E ratio (a measure of functionally active prothrombin) and urinary Gla concentration to their baseline values (Section 2.4.). **The Panel notes** that phylloquinone intake from diet was analytically measured in duplicate portions of all foods and beverages consumed (and not estimated using a food composition database). The Panel also notes that this study was previously used to support a reference value of 1 µg/kg/body weight, based on a mean body weight of subjects that is slightly higher than the reference body weight for adult men for this Opinion (68.1 kg, Section 6.). The Panel however

considers that the physiological relevance of the changes in biomarkers observed in this study is unclear.

The Panel notes that SCF (1993) set a reference value of 1 µg/kg/day based on data on biomarkers from Suttie et al. (1988). **The Panel considers** that none of the new data on the biomarkers reviewed (Section 2.4.) are suitable as such to derive DRVs for vitamin K.

5.1.1.2. Factorial approach

The maintenance of an adequate body pool of phylloquinone can be considered as a criterion for establishing the requirement for vitamin K, assuming that it is associated with fulfilling the function of vitamin K as cofactor of GGCX in the different target tissues (Section 2.2.1.).

As explained in Section 2.3.4., there is no data on the total body pool of menaquinones and the Panel considers the most accurate values for the total body pool of phylloquinone, obtained from a compartmental analysis of phylloquinone kinetics in adults (46 and 41 µg for men and women) (Novotny et al., 2010), and that can be expressed as 0.53 and 0.55 µg/kg body weight, respectively. The Panel also notes that the study of Olson et al. (2002), when taking into account the value for plasma phylloquinone considered as most accurate by the authors, identifies a body pool of phylloquinone of 0.57 µg/kg body weight, which is a value close to the values obtained from the study by Novotny et al. (2010). The Panel thus considers a body pool of phylloquinone of about 0.55 µg/kg body weight in healthy adults at steady state not to be associated with signs of vitamin K deficiency (Section 2.3.4.). The Panel considers this value as a desirable body pool size for phylloquinone.

Turnover of phylloquinone can be determined from kinetic studies. Based on the 6-day kinetic study by Olson et al. (2002) on seven adults (six men and one woman) consuming 75 µg/day and receiving 0.3 µg isotope-labelled phylloquinone administered intravenously, the authors found that a mean of about 62% of injected phylloquinone is catabolised and excreted as radioactive metabolites in urine (mean of 30%) and faeces through the bile (mean of 31.8%) (Section 2.3.6.).

In view of the fast turnover of phylloquinone in the body (Section 2.3.5.), the Panel applied these percentages to the desirable body pool size of phylloquinone calculated above. Thus, assuming a total body pool of phylloquinone of 0.55 µg/kg body weight in adults, the Panel estimates that 0.340 µg phylloquinone/kg body weight would be excreted in the form of phylloquinone metabolites in urine (30% of 0.55 µg/kg body weight, i.e. 0.165 µg/kg) and in bile (31.8% of 0.55 µg/kg body weight, i.e. 0.175 µg/kg body weight). The Panel assumes that 0.340 µg phylloquinone/kg body weight could be considered as the daily losses via faeces and urine. The Panel notes that the daily losses of menaquinones cannot be estimated.

The Panel considered to estimate the daily dietary intake of phylloquinone required to balance total phylloquinone losses through urine and faeces (bile) and to maintain an adequate body pool of phylloquinone (factorial approach). This approach to derive DRVs for vitamin K would require taking into account phylloquinone absorption. However, as explained in Section 2.3.1., the Panel considers that data on phylloquinone absorption in healthy adults, measured from different food sources and matrices, consumed with or without fat, are widely variable. The Panel also considers that it is not possible from the available data in healthy adults to estimate precisely an average absorption of phylloquinone, menaquinones, and thus vitamin K from the diet that would be valid for all dietary conditions.

The Panel noted in Section 2.3.1. the limitations of the available studies and that the observed mean phylloquinone absorption ranged between about 3-80%. In particular, taking into account the reported absolute value of absorption of phylloquinone from kale and assuming, as reference, maximum reported absorption of 80% for free phylloquinone (as a supplement consumed with fat) to convert the relative absorption observed for other plant foods into absolute values, the range of mean absorption from spinach, kale, broccoli or romaine lettuce (fresh or cooked, with or without fat) would be equivalent to about 3% to 50%.

On the assumption that absorption of phylloquinone from the European diet would be about 35% and that the assumed metabolic losses of phylloquinone mentioned above would be 0.340 µg phylloquinone/kg body weight, an intake of phylloquinone of 1 µg/kg body weight per day would balance the losses.

Although this value agrees with the AI set by the SCF, in view of the limitations associated with deriving the figures for absorption and losses, the Panel considers that the factorial approach cannot be used as such to set DRVs.

5.1.1.3. Intake data

The Panel considers that average/median intakes of vitamin K could be used to estimate an AI. Available data for vitamin K intake mean estimates in adults vary considerably among EU countries (between 72 and 196 ug/day) and suffer from limitations and uncertainties of food composition data with regard to both phylloquinone and menaquinones (Section 3.2.1.). Although two national surveys applied partially updated food composition data, the impact of the remaining uncertainty in the composition data on the results (median intake estimates for adults for vitamin K (phylloquinone and menaquinones) of 100–117 µg/day (Dutch National Survey) and for phylloquinone of 76 µg/day for subjects aged 15 to 80 years (German National Nutrition Survey II) (Section 3.2.2.) is still not entirely clear.

5.1.1.4. Conclusions on indicators of vitamin K requirement for adults

The Panel concludes that available data on biomarkers do not allow to estimate an average requirement (AR) for either phylloquinone or vitamin K.

The Panel also concludes that, due to the limitations of the data on absorption and excretion of phylloquinone and menaquinone, it is not possible to use the factorial approach to derive DRVs for vitamin K.

Due to the uncertainty associated with available data on average daily level of intake, the Panel concludes that an AI established from these data cannot be sufficiently reliable.

5.1.2. Infants and children

The Panel considers that there are no studies in infants aged 7–11 months and children that can be used for deriving the requirement for vitamin K in infants and children.

5.1.3. Pregnant or lactating women

During pregnancy only small quantities of phylloquinone cross the placenta from mother to fetus, and there is no correlation between maternal and cord blood concentrations (Section 2.3.3.). No data are available in relation to placental transfer of menaquinones, as shown in studies on vitamin K supplementation in pregnant women (Morales et al., 1988; Kazzi et al., 1990; Dickson et al., 1994) (Section 4. and Section 2.). Human milk contains 'low' concentrations of vitamin K (mostly phylloquinone) but the concentration of vitamin K in human milk is affected by maternal oral supplementation of phylloquinone (Section 2.3.6.3.).

The Panel considers that there are no studies that can be used for deriving the requirement for vitamin K in pregnant or lactating women and that would suggest that the requirement for vitamin K in pregnant or lactating women is different from non-pregnant non-lactating adults.

5.2. Vitamin K intake and health consequences

The relationship between intake of vitamin K (phylloquinone and/or menaquinones) and chronic disease outcomes has been investigated in RCTs, and also in observational studies where associations between intake and disease outcomes may be confounded by uncertainties inherent to the methodology used for the assessment of vitamin K intake and by the effect of dietary, lifestyle, or

other undefined factors on the disease outcomes investigated. RCTs, as well as prospective cohort studies in populations free of the investigated health outcome/disease(s) at baseline, are discussed in this Section. Taking into account the uncertainty about the relationship between vitamin K intake and biomarkers (Section 2.4.), the Panel only considered studies that include either one or longitudinal assessments of vitamin K intake, whereas studies on the relationship of levels of vitamin K biomarkers and health outcomes with no quantitative data on vitamin K intake are not considered.

A comprehensive search of the literature published between 1990 and 2011 was performed as preparatory work to this assessment in order to identify data on relevant health outcomes upon which DRVs for vitamin K may potentially be based (Heinonen et al., 2012). This provided individual studies that are described below. An additional literature search (in PubMed) was performed to identify more recent data published until 2016 on vitamin K intake and health outcomes.

Since the reports by SCF (1993), more data have become available on the relationship between phylloquinone or menaquinone intake and diabetes mellitus (one observational study (Beulens et al., 2010)), metabolic syndrome (one observational study (Dam et al., 2015)), cancer (two publications from the same observational study (Nimptsch et al., 2008; Nimptsch et al., 2010)), all-cause-mortality, cardiovascular-related outcomes or bone health. The Panel considers that evidence from only one observational study on a particular outcome is not sufficient to provide strong evidence of a relationship and thus cannot be used for setting DRVs for vitamin K. The Panel thus considers that available data on phylloquinone or menaquinones intake and the risk of diabetes mellitus, metabolic syndrome, various types of cancer cannot be used to derive DRVs for vitamin K. The Panel also noted three studies that investigated the relationship between intake of phylloquinone, menaquinones or both and the risk of all-cause mortality (Geleijnse et al., 2004; Juanola-Falgarona et al., 2014; Zwakenberg et al., 2016) with inconsistent results and therefore are not considered to derive DRVs for vitamin K.

5.2.1. Cardiovascular-related outcomes

The seven prospective cohort studies below assessed the association between several cardiovascular-related outcomes and vitamin K intake from food only or from food and supplements as assessed by an FFQ administered mostly solely at baseline, or also repeatedly during follow-up. These studies were undertaken in men and women or in one sex only, mostly included large populations (about 4,800–73,000 subjects) and with a mean follow-up ranging between 7.2 and 16 years, except for one smaller study (Villines et al., 2005) that investigated 807 active-duty army members with a shorter follow-up (less than 1.5 year). Results after adjustments for potential confounders are described below.

In one study, the risk of coronary heart disease (CHD) events (*total CHD, non-fatal myocardial infarction (MI),* or *fatal CHD*) was not significantly associated with quintiles of **phylloquinone** intake, even when comparing quintile Q5 ≥ 249 µg/day to Q1 ≤ 107 µg/day (Erkkila et al., 2007). In another study, the risks of *total CHD* and of *non-fatal MI* were significantly lower only in quintiles Q2 and Q4 of phylloquinone intake compared to Q1 (Q2: 110–144 µg/day, e.g. for total CHD, RR: 0.83 (95% CI: 0.71–0.97); Q4: 183-241 µg/day, e.g. for total CHD, RR 0.82 (95% CI: 0.69–0.96), but p for trend was not statistically significant (Erkkila et al., 2005). In the same study, the risk of *fatal CHD* was not associated with quintiles of phylloquinone intake. In a third study, the risk of coronary events (*incident CHD, non-fatal MI, CHD mortality*) was not associated with energy-adjusted tertiles of phylloquinone intake even when comparing the highest tertile > 278 µg/day to the lowest < 200 µg/day (Geleijnse et al., 2004). In a fourth study, the risk of *CHD* was not significantly associated with phylloquinone intake (per 10 µg/day increment in intake) (Gast et al., 2009).

In a study mentioned above (Geleijnse et al., 2004), only in the upper tertile of energy-adjusted intake of **menaquinone** (MK-4 to MK-10) (> 32.7 µg/day) compared to the lower one (< 21.6 µg/day), there was a significantly reduced risk of *incident CHD* (RR 0.59, 95% CI: 0.40–0.86) and *CHD mortality* (RR 0.43, 95% CI: 0.24–0.77), p trend 0.007 and 0.005, respectively, but no significant association was observed for *non-fatal MI*. In another study mentioned above (Gast et al., 2009), the risk of *CHD* was not significantly associated with phylloquinone intake or menaquinone intake (MK-4 to MK-9) (per 10 µg/day increment in intake).

Thus, there was no significant (linear or non-linear) association with phylloquinone intake and **the risk of CHD events** (four studies); while either a significant non-linear or no significant linear association was reported between menaquinone intake and the risk of CHD events (two studies).

In one study mentioned above (Erkkila et al., 2007), the risk of strokes (total or ischemic) was not significantly associated with quintiles of **phylloquinone** intake, even when comparing Q5 ≥ 249 µg/day to Q1 ≤ 107 µg/day. In another study (Vissers et al., 2013), there was no association between risk of stroke and energy-adjusted phylloquinone or **menaquinone** intake (MK-4 to MK-10), either per 50 µg/day increment in intake or comparing the highest to the lowest quartiles (mean phylloquinone intake: 96.6 µg/day (q1), 332.7 µg/day (q4); mean MK-n intake: 15.6 µg/day (q1), 49.3 µg/day (q4). These results did not change when analysing separately haemorragic and ischemic stroke, or separately total vitamin K or MK-4 through MK-6 and MK-7 through MK-10.

Thus, intakes of phylloquinone (two studies) or menaquinones (one study) were not significantly associated (linearly or non-linearly) **with the risk of stroke**.

The risk of peripheral arterial disease (PAD) (e.g. atherosclerosis, arterial embolism and thrombosis, aortic aneurysm) was not significantly associated with energy-adjusted **phylloquinone** intake, either per 50 µg increment in intake or comparing the highest to the lowest quartiles (mean: 97 µg/day in q1, 333 µg/day in q4) (Vissers et al., 2016). In this study, there was a significant (linear) inverse association between the risk of PAD and intake of **menaquinones** (per 10 µg increment in intake of MK-4 to 10) (hazard ratio (HR), 0.92, 95% CI: 0.85–0.99, p = 0.03). The risk of PAD was also significantly reduced when comparing the highest to the lowest quartiles of energy-adjusted intake of menaquinones, (HR 0.71, 95% CI: 0.53–0.95 (mean: 15.5 µg/day in q1, 49.2 µg/day in q4), but p for trend (0.06) was not significant. Such relationships were not observed among participants without hypertension.

Thus, there was no significant (linear or non-linear) association between intake of phylloquinone or menaquinones and **the risk of PAD** in subjects without hypertension (one study).

In one study, there was no significant association between the presence of coronary artery calcification (CAC) (assessed by computed tomography) and **phylloquinone** intake (either per µg/day increment in intake or comparing quartile q4: > 143.5 µg/day phylloquinone to q1 < 69.5 µg/day phylloquinone) (Villines et al., 2005). In this study, there was no significant linear association of phylloquinone intake with severity of CAC in a bivariate analysis. In another study mentioned above, there was no significant association between energy-adjusted tertiles of phylloquinone intake and moderate or severe aortic calcification (assessed by a lateral radiography) (Geleijnse et al., 2004).

In the same study, there was no association between energy-adjusted tertiles of intake of **menaquinones** (MK-4 to MK-9) and moderate aortic calcification, but an association was observed for severe calcification when comparing the highest to the lowest tertiles of intake (odds ratio (OR) 0.48, 95% CI: 0.32, 0.71, p trend < 0.001) (Geleijnse et al., 2004).

Thus, there was no significant (linear or non-linear) association between phylloquinone intake and **aortic/coronary calcification** (two studies), while a significant (non-linear) association was observed between menaquinone intake and severe (but not moderate) calcification (one study).

The Panel considers that the available data from these prospective cohort studies on associations between the intake of phylloquinone or menaquinones and the risk of cardiovascular-related outcomes cannot be used to derive DRVs for vitamin K.

5.2.2. Bone health

In this Section, the Panel does not report on studies (Cockayne et al., 2006; Knapen et al., 2007; Emaus et al., 2010) using doses much higher (1-10 mg/day phylloquinone, 15–45 µg/day MK-4, 360 µg/day MK-7) than the observed phylloquinone and menaquinone dietary intakes in Europe

(Section 3.2.). Results of two available RCTs and of eight prospective observational studies after adjustments for potential confounders, are described below. These observational studies generally assessed vitamin K (from food only or from food and supplements) through a FFQ at baseline, whereas a few among them assessed intake at different time points or used other methods (three, four or seven day food records). They were all in adults except one in children (Kalkwarf et al., 2004), with follow-up between 2 and 10 years and population size between 200 and about 72,000 subjects.

A 12-months RCT on 173 healthy women (mean age 62 years) investigated the effect on BMD of the intake of **phylloquinone** or MK-7,[29] calcium and vitamin D through fortified milk or yogurt (Kanellakis et al., 2012). The subjects received either 800 mg/day of calcium and 10 µg/day of vitamin D_3 (n = 38), or the same amounts of these nutrients with 100 µg/day of phylloquinone (n = 38) or MK-7 (n = 39), or continued with their usual diet during the study (control group, n = 39). BMD of total body and lumbar spine (LS) were measured at baseline and follow-up with dual-emission X-ray absorptiometry (DXA) and the BMD of other regional skeletal sites was extracted from the total body scans and data analysis was done on the subjects with compliance of at least 75% (n = 115). Baseline mean phylloquinone intake, assessed by three 24-h recalls, was between 80.2 and 121.2 µg/day among groups (not statistically different). After adjustments for 25(OH)D concentrations, dietary calcium intake and physical activity, changes (increases) in **total-body BMD** in the intervention groups were not significantly different from that (decrease) in the control group. However, there was an increase in **BMD of the LS** in the vitamin K-supplemented groups, which was still significantly different, after adjustments, from the change (decrease) observed in control group (p = 0. 002).

In a two-year double-blind RCT of the effect of **phylloquinone** on BMD, 244 healthy women aged ≥ 60 years (Bolton-Smith et al., 2007) were allocated to: (1) placebo, (2) 200 µg/day phylloquinone, (3) 1,000 mg calcium plus 10 µg/day vitamin D_3, or (4) combined supplementation with the three nutrients at the levels in groups 2 and 3. Baseline mean phylloquinone intake (from food and supplements) assessed by FFQ was about 82–87 µg/day among the 209 completers. Bone mineral content (BMC) and BMD were measured by DXA of the femur and radius every six months. After adjustments for potential confounders, there was **no significant difference** of the two-year changes in BMD or BMC between groups at any site.

Thus, two available RCTs with phylloquinone intake at levels comparable to the observed dietary intakes in Europe do not provide consistent results on the effect of phylloquinone intake on BMD and/or BMC in postmenopausal women (Bolton-Smith et al., 2007; Kanellakis et al., 2012).

In one observational study, in either men or women aged 65 years and older, there was no significant association between risk of hip fracture (assessed from hospital records) and energy-adjusted log-transformed **phylloquinone** intake (per SD increment in intake) (Chan et al., 2012). In a second study (Booth et al., 2000b), the risk of hip fracture (assessed from hospital records including X-rays) was also not significantly associated with phylloquinone intake, even when comparing the highest to the lowest quartiles (median intake according to sexes: 60–64 µg/day in q1 and 234–268 µg/day in q4). In the largest observational study (Feskanich et al., 1999) undertaken among women (nurses), only women in quintile Q3 of baseline phylloquinone intake (146–183 µg/day) had a significantly lower RR of hip fractures (self-reported), i.e. 0.67 (95% CI: 0.46–0.99), compared to those in Q1 (< 109 µg/day), and p for trend (= 0.32) was not significant. In this study, the RR of hip fracture was significantly lower in quintiles 2–5 combined of baseline phylloquinone intake (109–> 242 µg/day) compared to quintile 1, with a RR (95% CI) of 0.70 (0.53, 0.93), but this result did not remain statistically significant when using updated dietary data during follow-up (secondary analyses). In a fourth study (Apalset et al., 2011), the risk of hip fracture (assessed from hospital records) was significantly higher in the lowest quartile of phylloquinone intake when compared to the highest (q1: < 42.2 (women) or 52.9 (men) µg/day; q4: > 108.7 (women) or 113.9 (men) µg/day; HR 1.63,

[29] In view of the high dose investigated (100 µg/day MK-7) much higher than observed intakes in Europe (Section 3.2.), the results for MK-7 are not discussed.

95% CI: 1.06–2.49, p for trend: 0.015), but findings were not significant for the intermediate quartiles. In this study, the HR of hip fractures was 0.98 (95% CI: 0.95–1.00, p = 0.030) per 10 µg/day increment in phylloquinone intake.

In the same study (Apalset et al., 2011), the risk of hip fractures (assessed from hospital records) was not significantly associated with intake of **menaquinones** (forms not specified), either per 1 µg increment in intake or comparing the lowest to the highest quartiles (q1: < 7.2 (women) or 8.5 (men) µg/day, q4 > 14.5 (women) or 16.2 (men) µg/day).

Thus, the results on the association between phylloquinone intake and **the risk of hip fractures**, are inconsistent (four studies), while there was no significant (linear or non-linear) association with menaquinone intake (one study).

In either men or women aged 65 years and older from a study mentioned above, there was no significant association between risk of non-vertebral fracture and energy-adjusted log-transformed **phylloquinone** intake (per SD increment in intake) (Chan et al., 2012). In peri-menopausal women (nested case-control study), receiving or not hormonal replacement therapy and some having already sustained a fracture at baseline, there was no significant association between the risk of vertebral fracture (assessed from hospital records and X-rays) and **phylloquinone** intake, even when comparing the highest to the lowest quartiles (> 105 vs < 25 µg/day) or the 95th to the 5th percentiles (> 210 versus < 25 µg/day) (Rejnmark et al., 2006).

Thus, there was no association between phylloquinone intake, and **the risk of either non-vertebral** (one study) or **vertebral fractures** (one study).

In a study mentioned above (Rejnmark et al., 2006), changes in BMD of the LS or femoral neck (FN) (measured by DXA) were not significantly associated with **phylloquinone** intake expressed either continuously or categorically (in quartiles). In another study mentioned above (Booth et al., 2000b), there was also no significant difference in changes in BMD at any site (hip (FN, trochanter, Ward's area), LS and arm, measured by different methods[30]) across quartiles of phylloquinone intake, for either men or women (median intake according to sexes of 60–64 µg/day in q1 and 234–268 µg/day in q4). In a third study (Macdonald et al., 2008), in which phylloquinone intake data was available for 898 women at baseline and final visits and 2,340 only at final visit only, there was again no significant difference in the yearly change in BMD at the FN or LS between quartiles of energy-adjusted phylloquinone at visit 2 (mean intake: 64 (q1) and 181 µg/day (q4)). In this study, energy-adjusted intake of phylloquinone assessed as a continuous variable was not a significant predictor of BMD at LS or FN. In a fourth study (Bullo et al., 2011), 362 participants of the larger PREDIMED trial (Estruch et al., 2013) were enrolled in a parallel study on bone metabolism. At baseline, participants provided a FFQ. After two years of follow-up, 200 participants provided a second dietary assessment and quantitative ultrasound bone-related assessments. The study investigated the relationship between change in phylloquinone intake (between beginning and end of follow-up) and change in BMD or bone structure quality (speed of sound (SOS)), broadband ultrasound attenuation (BUA) and quantitative ultrasound index (QUI) assessed by quantitative ultrasound at the calcaneus. The mean (± SE) phylloquinone intake at baseline was 333.6 ± 17.3 µg/day in men (n = 162) and 299.8 ± 11.6 µg/day in women (n = 200). After two years follow-up, those who increased their phylloquinone intake (mean change ± SD: +104.1 ± 10.9 µg/day, n = 74) had a statistically significant lower loss of BMD (mean change ± SD: -0.009 ± 0.006 g/cm^2) compared to those who decreased their phylloquinone intake (mean change ± SD: -155.8 ± 17.57 µg/day, n = 126) during the follow-up (mean change in BMD ± SD: -0.023 ± 0.004 g/cm^2), p = 0.049. There was no significantly different change in BUA, SOS and QUI. No information was provided on why subjects changed their phylloquinone intake during follow-up.

[30] Dual-photon absorptiometry, single-photon absorptiometry and DXA.

Thus, the results on the association between phylloquinone intake and **changes in BMD** are inconsistent (four studies). For most of the sites investigated, however, the (linear or non-linear) associations were not significant.

In 245 healthy girls aged 3–16 years at baseline (median 9.8 years) (Kalkwarf et al., 2004) (Section 2.4.), BMC (total body, total body minus head, LS, hip, assessed by DXA) was not significantly associated with **phylloquinone** intake, except for the hip (1.0% decrease when increasing from the 10th percentile of phylloquinone intake i.e. 21 µg/day to the 90th percentile i.e. 89 µg/day, $p < 0.01$).

Thus, there was no significant association between phylloquinone intake and **BMC** for most of the sites investigated (one study in children). Menaquinone intake was not investigated.

The Panel considers that the available data on intake of phylloquinone or menaquinones and bone-related health outcomes cannot be used to derive DRVs for vitamin K.

5.2.3. Conclusions on vitamin K intake and health consequences

The Panel considers that the available data on intake of phylloquinone or menaquinones and health outcomes cannot be used to derive DRVs for vitamin K.

6. Data on which to base Dietary Reference Values

The Panel reviewed the recent information on vitamin K (phylloquinone and menaquinones) with the aim of possibly updating the DRV of 1 µg/kg body weight per day of phylloquinone that was previously set by SCF (1993) (Section 4.) based on data on biomarkers and phylloquinone intake (Suttie et al., 1988). The Panel came to the conclusion that the uncertainties pointed out by SCF (1993) have not been resolved.

The Panel considers that all possible approaches investigated to set DRVs (biomarker, factorial approach, intake data) have considerable uncertainties (Sections 5.1.1.1. to 5.1.1.4.). The Panel considers that there is no scientific evidence to update the previous reference value. The Panel notes that there is no indication that 1 µg/kg body weight per day phylloquinone would be associated with a risk of deficiency in the general population and is above the intake at which an increase in PT has been observed in healthy subjects (Sections 2.2.2.1. and 2.4.).

In view of the uncertainties and limited data, the Panel considers that an average requirement (AR) and population reference intake (PRI) cannot be set for vitamin K, but instead set an *adequate intake* (AI), at 1 µg/kg body weight per day *phylloquinone*.

The Panel tried to take *menaquinones* into account in setting DRVs for vitamin K, as this vitamin is defined as phylloquinone and menaquinones (Section 2.1.). The Panel however came to the conclusion that the knowledge on MK-n, i.e. their intake (Section 3.2.), absorption (Section 2.3.1.), function (Sections 2.2.1. and 2.4.) and content in the body or organs (Section 2.3.4.), is limited and highly contradictory. Thus, the Panel considers that, at present, there are not enough data to take menaquinones into account to set DRVs for vitamin K.

The Panel also considers that the available data on intake of phylloquinone or menaquinones and health outcomes cannot be used to derive DRVs for vitamin K (Section 5.2.).

6.1. Adults

The reference body weights of 18 to 79 year-old men and women were calculated by the measured body heights of 16,500 men and 19,969 women in 13 EU Member States and assuming a BMI of 22 kg/m^2 (see Appendix 11 in EFSA NDA Panel (2013b)). Considering these reference body weights and the AI of 1 µg/kg body weight per day of phylloquinone, the daily phylloquinone intake would be 68.1 µg for men and 58.5 µg for women, rounded up to 70 µg/day for all adults.

The Panel notes that the proposed AI is close to the median phylloquinone intake of 76 µg/day (for subjects aged 15 to 80 years, n = 6,160) in the German national survey that used updated phylloquinone composition data (Section 3.2., mean intake not reported). The Panel also considers that there was no evidence of different vitamin K absorption and different losses according to age in adults, thus sets the same AI for 'younger' and 'older' adults.

6.2. Infants aged 7–11 months

The Panel decided to use for infants aged 7–11 months the same AI of 1 µg/kg body weight per day of phylloquinone obtained in adults. Considering the uncertainties associated with the setting of this value, and the small size of the body pool of phylloquinone, the Panel decided not to use growth factors (calculated in EFSA NDA Panel (2014)), considering that the requirement for growth would be covered by such an intake of 1 µg/kg body weight/day.

The Panel calculated averages of the median weights of male and female infants, aged 9 months (8.6 kg) from the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006). Considering a reference body weight of 8.6 kg for infants aged 7–11 months and the AI of 1 µg/kg body weight per day phylloquinone, the daily phylloquinone intake would be 8.6 µg/day, rounded up to 10 µg/day.

The Panel notes that low vitamin K stores at birth may predispose to haemorrhages in healthy neonates and young infants (EFSA NDA Panel, 2013a). The Panel also notes that European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition (Mihatsch et al., 2016) recommends supplementation with phylloquinone of healthy newborn infants, according to national recommendations on the regimen, which may differ between countries.

6.3. Children

As for infants, the Panel decided not to use growth factors, considering that the requirement for growth would be covered by such an intake of 1 µg/kg body weight per day. Considering median body weights of boys and girls according to van Buuren et al. (2012), the daily phylloquinone intake in children is indicated in Table 4.

The Panel notes that the median (mean, IQR) intake estimates for vitamin K (phylloquinone and MK-n) for children are 62 (70, 43–89) and 72 (80, 51–99) µg/day for girls (n = 857) and boys (n = 856) aged 7-18 years, in the Dutch national survey that used updated composition data for phylloquinone and menaquinones (Section 3.2.).

Table 4: Daily phylloquinone intake in boys and girls based on an AI of 1 µg/kg body weight per day and reference body weights

	Boys	Girls	AIs for both sexes (rounded value)
1–3 years	12.2	11.5	12
4–6 years	19.2	18.7	20
7–10 years	29	28.4	30
11–14 years	44	45.1	45
15–17 years	64.1	56.4	65

6.4. Pregnancy

The Panel notes that, during pregnancy, only small quantities of phylloquinone cross the placenta from mother to fetus, that there is no correlation between maternal and cord blood phylloquinone concentrations (Section 2.3.3.), and that no data are available in relation to placental transfer of menaquinones. The Panel considers that the AI of 1 µg/kg body weight per day of phylloquinone set for non-pregnant women also applies to pregnant women.

A mean gestational increase in body weight of 12 kg, for women with a singleton pregnancy and a pre-pregnancy BMI in the range between 18.5 and 24.9 kg/m^2, was also previously considered (EFSA NDA Panel, 2013b). In view of the increase in blood volume during pregnancy, and considering a mean gestational increase in body weight of 12 kg to the reference body weight of 58.5 kg for non-pregnant women, the daily phylloquinone intake would be 70.5 µg/day.

As the Panel set an AI of 70 µg/day for all adults after rounding (Section 6.1.), the Panel concludes that there is no need for a specific AI for vitamin K for pregnant women. The AI for pregnant women is thus the same as for non-pregnant women (i.e. 70 µg phylloquinone/day)

6.5. Lactation

The Panel considers that the AI of 1 µg/kg body weight per day of phylloquinone set for non-lactating women covers the small excretion of vitamin K (mainly phylloquinone) in breast milk, thus that no compensation for this excretion is required in setting DRVs for lactating women. The AI for lactating women is thus the same as for non-lactating women (i.e. 70 µg phylloquinone/day).

CONCLUSIONS

The Panel considers vitamin K as phylloquinone and menaquinones. The Panel concludes that none of the biomarkers of vitamin K intake or status is suitable by itself to derive DRVs for vitamin K and that available data on intake of phylloquinone or menaquinones and health outcomes cannot be used to derive DRVs for vitamin K. The Panel concludes that ARs and PRIs for vitamin K cannot be derived for adults, infants and children, and therefore sets AIs. The Panel also concludes that available evidence on intake, absorption, function and content in the body or organs of menaquinones is insufficient, thus sets AIs for phylloquinone only.

After having considered several possible approaches, based on biomarkers, intake data and the factorial approach, which all are associated with considerable uncertainties, the reference value proposed by the SCF in 1993 is maintained. The same AI for phylloquinone of 1 µg/kg body weight per day is set for all age and sex population groups. For infants and children, the Panel decided not to use growth factors, considering that the requirement for growth would be covered by such an intake. The Panel considers the respective reference body weights for adults, infants and children to set AIs for phylloquinone expressed in µg/day. The Panel notes that the proposed AI in adults (70 µg/day) is close to the median phylloquinone intake of 76 µg/day in the 2012 German national survey that used updated phllyquinone composition data. The mean gestational increase in body weight and the reference body weight of non-pregnant women were taken into account by the Panel in its calculations, but the AI set for pregnant women is finally the same as for non-pregnant women obtained after rounding. In view of the small excretion of vitamin K in breast milk, the AI set for lactating women is the same as the one for non-lactating women obtained after rounding (Table 5).

Table 5: Summary of Dietary Reference Values for vitamin K (based on phylloquinone only)

Age	AI (µg/day)
7-11 months	10
1-3 years	12
4-6 years	20
7-10 years	30
11-14 years	45
15-17 years	65
≥ 18 years[(a)]	70

(a) : including pregnancy and lactation.

RECOMMENDATIONS FOR RESEARCH

The Panel suggests to undertake further research on:

- better phylloquinone and menaquinone composition data.
- the measurement of phylloquinone and menaquinones absorption and/or diffusion in the intestine.
- the intake of menaquinones in the EU and their metabolism and functions in the body.
- the "potency" of different menaquinones in relation to phylloquinone functions.
- studies specifically designed to identify cut-off values for biomarkers for vitamin K status to derive DRVs for vitamin K for infants, children, adults, pregnant and lactating women.
- vitamin K and bone health.

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APPENDICES

Appendix A. Concentrations of phylloquinone and menaquinones in breast milk of healthy mothers

Reference	Number of women (number of samples)	Country	Maternal dietary intake (Mean ± SD)	Maternal serum/plasma (phylloquinone/ menaquinone) concentration yes/n.a.	Stage of lactation	Phylloquinone concentration in breast milk (µg/L) (Mean ± SD)	Menaquinone concentration in breast milk (µg/L) (Mean ± SD)	Comments
Haroon et al. (1982)	20 (unsupplemented)	UK	n.a.	n.a.	n.a.	2.1 (1.1.–6.5)	n.a.	No information was given as to whether infants were full-term or not.
	1 (supplemented)		20 mg (one dose)	n.a.	~ 6 months post-partum	140	n.a.	
Fournier et al. (1987)	10	FR	n.a.	n.a.	21 days post-partum	9.18 (4.85–12.76) (median (range))	n.a.	Full-term infants.
von Kries et al. (1987a)	9 (unsupplemented)	DE	n.a.	a.	8–36 days post-partum	1.2 (median)	n.a.	Full-term infants. The authors considered transitional (days 8–15) and mature (days 22–36) milk as one group (days 8–36) as there were no significant differences in phylloquinone concentration.
	1 (supplemented)		100 µg (one dose)	n.a.		4.9	n.a.	Breast milk phylloquinone concentration of the supplemented woman at baseline (before supplementation) was 2.5 µg/L. Breast milk phylloquinone concentration is given for one supplemented mother for whom phylloquinone administration and milk sampling techniques were standardised.
Canfield et al. (1990)	7 (16)	US	n.a.	n.a.	1 month post-partum	2.94 ± 1.94 (pooled samples)	n.a.	Infants were growing within normal limits and free of illness. No explicit information was given as to whether infants were full-term or not.
	15					3.15 ± 2.87 (mean of individuals)		

Reference	Number of women (number of samples)	Coun-try	Maternal dietary intake (Mean ± SD)	Maternal serum/plasma (phylloquinone/ menaquinone) concentration yes/n.a.	Stage of lactation	Phylloquinone concentration in breast milk (µg/L) (Mean ± SD)	Menaquinone concentration in breast milk (µg/L) (Mean ± SD)	Comments
Canfield et al. (1991)	15 (45)	US	n.a.	n.a.	1–6 months post-partum	2.87 ± 2.40 (mean of all determinations)	n.a.	No explicit information was given as to whether infants were full-term or not. Samples assayed in triplicate at each time point (1, 3 and 6 months).
Greer et al. (1991)	11 (study part 1)	US	supplementation, 20 mg (one dose)	yes	2–6 months post-partum	130 ± 188	n.a.	No information was given as to whether infants were full-term or not. Breast milk phylloquinone concentration at baseline (before supplementation) was 1.11 ± 0.82 µg/L. Maternal intakes of phylloquinone exceeded the DRV of 1 µg/kg body weight per day.
	23 (study part 2)		unsupplemented (µg/day) 302 ± 361 296 ± 169 436 ± 667	yes	weeks post-partum 6 12 26	 0.86 ± 0.52 1.14 ± 0.72 0.87 ± 0.5	n.a.	Full-term infants.
Pietschnig et al. (1993)	20 (supplemented)	AT	mean (range) from food and supplement (µg/day) 442 (226–778) 386 (223–687) Supplementation (µg/day) 88 ± 40 (from 4 through 91 days post-partum)	n.a.	days post-partum 27–29 89–91	 1.36 (0.40–3.81) 1.67 (0.56–8.61)	n.a.	Full-term infants. Average mother intake exceeded the DRV for lactating women (55 µg/day) by 670%. The supplemental intake of 88 ± 40 µg/day was calculated on average over the whole study period.
	16 (unsupplemented)		mean (range) (µg/day) 417 (134–1,224) 391 (209–695)	n.a.	days post-partum 25–29 87–91	 1.68 (0.64–2.91) 1.78 (0.80–4.11)	n.a.	Full-term infants.

Reference	Number of women (number of samples)	Coun-try	Maternal dietary intake (Mean ± SD)	Maternal serum/plasma (phylloquinone/ menaquinone) concentration yes/n.a.	Stage of lactation	Phylloquinone concentration in breast milk (µg/L) (Mean ± SD)	Menaquinone concentration in breast milk (µg/L) (Mean ± SD)	Comments
Greer et al. (1997)		US						Full term infants.
	phase 1- preliminary investigation)		supplementation (daily for 6 weeks, starting within 3 days of delivery)	yes	weeks post-partum		n.a.	
			2.5 mg		2	27.12 ±12.18		Breast milk phylloquinone concentration at baseline (before supplementation) was 0.63 ± 0.58 µg/L (2.5 mg group) and 0.92 ± 0.62 µg/L (5 mg/day)
	10				6	22.43 ± 16.62		
			5 mg		2	58.96 ± 25.39		
	10				6	44.1 ± 24.10		
	phase 2 (supplementation study)		supplementation (daily for 12 weeks (starting time not reported))	yes	weeks post-partum		n.a.	No information was given as to whether infants were full-term or not.
			0 (placebo)					Breast milk phylloquinone concentration at baseline (before supplementation) was 0.69 ± 0.39 µg/L (5 mg group) and 1.10 ± 0.75 µg/L (placebo)
	11				2	1.17 ± 0.7		
					6	1.14 ± 0.46		
					12	1.17 ± 0.40		
					weeks post-partum			
	11		5 mg		2	76.53 ± 26.98		
					6	75.27 ± 46.23		
					12	82.10 ± 40.10		

Reference	Number of women (number of samples)	Coun-try	Maternal dietary intake (Mean ± SD)	Maternal serum/plasma (phylloquinone/ menaquinone) concentration yes/n.a.	Stage of lactation	Phylloquinone concentration in breast milk (µg/L) (Mean ± SD)	Menaquinone concentration in breast milk (µg/L) (Mean ± SD)	Comments
Thijssen et al. (2002)		NL	(dietary intake not reported) daily supplementation (from day 4 to day 16 post-partum)				MK-4	Full-term infants.
	8		0 (control)	yes	days post-partum			
					16	2.2 ± 0.64	0.96 ± 0.4	
					19	2.2 ± 1.33	0.79 ± 0.28	
	8		0.8 mg	yes	days post-partum			
					16	11.05 ± 4.57	1.55 ± 1.15	
					19	5.57 ± 5.64	1.44 ± 1.14	
	8		2 mg	yes	days post-partum			
					16	27.33 ± 14.24	2.46 ± 1.5	
					19	5.44 ± 2.09	1.34 ± 0.6	
	7		4 mg	yes	days post-partum			
					16	62.93 ± 20.66	7.33 ± 4.07	
					19	20.23 ± 17.95	4.40 ± 2.30	
Kojima et al. (2004)	(416)	JP	n.a.	n.a.	days post-partum		MK-4	No explicit information was given as to whether infants were full-term or not. Infants with birth weight higher than 2.5 kg.
					21–89	1.95 ± 0.88	1.85 ± 0.41	
					90–179	2.21 ± 4.29	1.35 ± 0.35	
					180–365	1.55 ± 0.88	1.28 ± 0.31	

Reference	Number of women (number of samples)	Coun-try	Maternal dietary intake (Mean ± SD)	Maternal serum/plasma (phylloquinone/ menaquinone) concentration yes/n.a.	Stage of lactation	Phylloquinone concentration in breast milk (µg/L) (Mean ± SD)	Menaquinone concentration in breast milk (µg/L) (Mean ± SD)		Comments
Kamao et al. (2007b)		JP	n.a.	n.a.	Days post-partum		MK-4	MK-7	No information on the health status of the infants or if they were born at term or not.
	43				11–30	3.94 ± 2.45	1.80 ± 0.66	1.67 ± 2.73	
	18				31–90	3.53 ± 1.45	1.78 ± 0.55	0.80 ± 0.75	
	8				91–180	2.30 ± 1.22	1.19 ± 0.34	1.36 ± 1.29	
	5				181–270	3.41 ± 1.46	1.51 ± 0.42	0.92 ± 0.92	

AT: Austria; DE: Germany; DRV: dietary reference value; FR: France; JP: Japan; MK: menaquinone; n.a.: not applicable; NL: the Netherlands; SD: standard deviation; UK: United Kingdom; US: United States.
Molecular masses: phylloquinone: 450.7 g/mol; MK-4: 444.7 g/mol; MK-7: 648.9 g/mol.

Appendix B. Dietary surveys in the EFSA Comprehensive European Food Consumption Database included in EFSA's nutrient intake calculation for 'total vitamin K'

Country	Dietary survey (year)	Year	Method	Days	Age (years)	Number of subjects						
						Infants [a] < 1 year	Children 1–< 3 years	Children 3–< 10 years	Adolescents 10–< 18 years	Adults 18–< 65 years	Adults 65–< 75	Adults ≥ 75 years
Finland/1	NWSSP	2007–2008	48-hour dietary recall [b]	2x2[b]	13–15				306			
Finland/2	FINDIET2012	2012	48-hour dietary recall [b]	2[b]	25–74					1 295	413	
Finland/3	DIPP	2000–2010	Dietary record	3	0.5-6	499	500	750				
France	INCA2	2006–2007	Dietary record	7	3–79			482	973	2 276	264	84
Germany/1	EsKiMo	2006	Dietary record	3	6–11			835	393			
Germany/2	VELS	2001–2002	Dietary record	6	< 1–4	158	348[c]	296[c]				
Ireland	NANS	2008–2010	Dietary record	4	18–90					1 274	149	77
Italy	INRAN-SCAI 2005–06	2005–2006	Dietary record	3	< 1–98	16 [d]	36 [d]	193	247	2 313	290	228
Latvia	FC_PREGNANT WOMEN 2011	2011	24-hour dietary recall	2	15–45				12 [d]	991 [c]		
Netherlands	DNFCS2007_2010	2007–2010	24-hour dietary recall	2	7–69			447	1142	2 057	173	
Sweden	RISKMATEN	2010–2011	Dietary records (Web)[e]	4	18–80					1 430	295	72
UK/1	DNSIYC-2011	2011	Dietary record	4	0.3–1.5	1 369	1 314					
UK/2	NDNS Rolling Programme (Years 1–3)	2008–2011	Dietary record	4	1–94		185	651	666	1 266	166	139

DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungsstudie als KIGGS-Modul; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale des Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione – Studio sui Consumi Alimentari in Italia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): Infants 1–11 months of age.

(b): A 48-hour dietary recall comprising two consecutive days.

(c): Four children from the VELS study (one aged 1–< 3 and three aged 3–< 10 years) and one adult from the Latvian study were not considered in the assessment as only one 24-hour dietary recall day was available.

(d): 5th or 95th percentile intakes calculated from fewer than 60 subjects require cautious interpretation as the results may not be statistically robust (EFSA, 2011b) and, therefore, for these dietary surveys/age classes, the 5th and 95th percentile estimates are not presented in the intake results.

(e): The Swedish dietary records were introduced through the Internet.

3069 **Appendix C. 'Total vitamin K' intakes in males in different surveys, estimated by EFSA according to age class and country**

Age class	Country	Survey	Intakes[b] expressed in µg per day					Intakes[b] expressed in µg per MJ			
			n[c]	Average	Median	P5	P95	Average	Median	P5	P95
< 1 year [a]	Finland	DIPP	247	34	35	4	67	18[d]	16[d]	7[d]	34[d]
	Germany	VELS	84	43	39	7	111	13	12	2	34
	Italy	INRAN_SCAI_2005_06	9	23	14	-[b]	-[b]	8	4	-[b]	-[b]
	United Kingdom	DNSIYC_2011	699	61	56	20	116	18	17	6	31
1–< 3 years	Finland	DIPP	245	42	39	15	74	12	11	4	20
	Germany	VELS	174	51	36	12	137	11	8	3	30
	Italy	INRAN_SCAI_2005_06	20	51	41	-[b]	-[b]	11	9	-[b]	-[b]
	United Kingdom	NDNS-Rolling Programme Years 1–3	107	51	45	19	106	12	11	4	21
	United Kingdom	DNSIYC_2011	663	53	43	18	106	11	10	5	25
3–< 10 years	Finland	DIPP	381	45	40	21	81	8	7	4	13
	France	INCA2	239	62	52	17	139	10	8	3	26
	Germany	EsKiMo	426	67	51	21	157	9	6	3	21
	Germany	VELS	146	47	36	16	122	9	6	3	21
	Italy	INRAN_SCAI_2005_06	94	91	68	30	235	13	9	4	37
	Netherlands	DNFCS2007	231	93	54	19	364	11	6	3	39
	United Kingdom	NDNS-Rolling Programme Years 1–3	326	68	60	20	144	11	9	4	26
10–< 18 years	Finland	NWSSP07_08	136	73	70	29	129	9	8	4	15
	France	INCA2	449	80	62	22	183	10	8	3	24
	Germany	EsKiMo	197	69	55	21	171	9	7	3	21
	Italy	INRAN_SCAI_2005_06	108	143	85	43	367	16	9	4	45
	Netherlands	DNFCS2007	566	112	69	28	377	11	6	3	35
	United Kingdom	NDNS-Rolling Programme Years 1–3	340	80	66	26	178	10	8	4	22
18–< 65 years	Finland	FINDIET2012	585	92	81	30	180	10	9	3	22
	France	INCA2	936	103	89	28	228	12	10	4	27
	Ireland	NANS_2012	634	84	71	26	182	9	7	3	19
	Italy	INRAN_SCAI_2005_06	1068	161	115	40	440	18	13	5	53
	Netherlands	DNFCS2007	1023	157	93	35	637	14	8	3	56
	Sweden	Riksmaten 2010	623	91	77	31	184	9	8	4	20
	United Kingdom	NDNS-Rolling Programme Years 1–3	560	103	84	32	244	12	9	4	28
65–< 75 years	Finland	FINDIET2012	210	94	81	32	200	12	10	4	26
	France	INCA2	111	130	116	42	240	16	14	5	30
	Ireland	NANS_2012	72	96	84	23	212	11	9	4	24
	Italy	INRAN_SCAI_2005_06	133	196	152	52	531	24	15	7	74
	Netherlands	DNFCS2007	91	155	89	43	553	17	11	4	53
	Sweden	Riksmaten 2010	127	92	80	37	167	11	10	5	19

Age class	Country	Survey	Intakes[b] expressed in µg per day					Intakes[b] expressed in µg per MJ			
			n[c]	Average	Median	P5	P95	Average	Median	P5	P95
	United Kingdom	NDNS-Rolling Programme Years 1–3	75	119	104	39	230	15	13	5	26
≥ 75 years	France	INCA2	40	135	104	-[b]	-[b]	18	16	-[b]	-[b]
	Ireland	NANS_2012	34	72	57	-[b]	-[b]	9	9	-[b]	-[b]
	Italy	INRAN_SCAI_2005_06	69	157	110	52	360	18	13	5	42
	Sweden	Riksmaten 2010	42	104	87	-[b]	-[b]	12	10	-[b]	-[b]
	United Kingdom	NDNS-Rolling Programme Years 1–3	56	88	82	-[b]	-[b]	12	11	-[b]	-[b]

DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungsstudie als KIGGS-Modul; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale des Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione – Studio sui Consumi Alimentari in Italia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): Infants between 1 and 11 months. The proportions of breastfed infants were 58% in the Finnish survey, 40% in the German survey, 44% in the Italian survey, and 21% in the UK survey. Most infants were partially breastfed. The consumption of breast milk was taken into account if the consumption was reported as human milk (Italian survey) or if the number of breast milk consumption events was reported (German and UK surveys). For the German study, the total amount of breast milk was calculated based on the observations by Paul et al. (1988) on breast milk consumption during one eating occasion at different age groups: the amount of breast milk consumed on one eating occasion was set to 135 g/eating occasion for infants between 6-7 months of age and to 100 g/eating occasion for infants between 8–12 months of age (Kersting and Clausen, 2003). For the UK survey, the amount of breast milk consumed was either directly quantified by the mother (expressed breast milk) or extrapolated from the duration of each breastfeeding event. As no information on the breastfeeding events were reported in the Finnish survey, breast milk intake was not taken into consideration in the intake estimates of Finnish infants.

(b): 5th or 95th percentile intakes calculated from fewer than 60 subjects require cautious interpretation as the results may not be statistically robust (EFSA, 2011b) and, therefore, for these dietary surveys/age classes, the 5th and 95th percentile estimates are not presented in the intake results.

(c): n, number of subjects.

(d): The intake expressed as µg/MJ is referring to 245 male subjects of the Finnish DIPP study as energy intake was not reported for two subjects.

3087 **Appendix D. 'Total vitamin K' intakes in females in different surveys, estimated by EFSA according to age class and country**

Age class	Country	Survey	Intakes[b] expressed in µg/day					Intakes[b] expressed in µg/MJ			
			n[c]	Average	Median	P5	P95	Average	Median	P5	P95
< 1 year[a]	Finland	DIPP	253	33	32	5	69	22[e]	17[e]	8[e]	45[e]
	Germany	VELS	75	36	33	10	77	12	12	3	24
	Italy	INRAN_SCAI_2005_06	7	31	32	-[b]	-[b]	10	9	-[b]	-[b]
	United Kingdom	DNSIYC_2011	670	53	50	11	100	17	17	4	31
1–< 3 years	Finland	DIPP	255	36	34	12	72	11	10	4	20
	Germany	VELS	174	46	37	12	120	11	8	3	29
	Italy	INRAN_SCAI_2005_06	16	50	37	-[b]	-[b]	10	7	-[b]	-[b]
	United Kingdom	NDNS-Rolling Programme Years 1–3	78	52	47	18	103	12	11	5	22
	United Kingdom	DNSIYC_2011	651	50	44	16	102	13	11	4	26
3–< 10 years	Finland	DIPP	369	42	37	19	84	8	7	4	15
	France	INCA2	243	63	50	19	160	11	9	4	28
	Germany	EsKiMo	409	65	50	19	166	10	7	3	23
	Germany	VELS	147	50	37	14	137	10	7	3	28
	Italy	INRAN_SCAI_2005_06	99	85	65	20	223	12	9	3	30
	Netherlands	DNFCS2007	216	70	49	22	164	9	6	3	21
	United Kingdom	NDNS-Rolling Programme Years 1–3	325	65	57	22	139	11	10	4	23
10–< 18 years	Finland	NWSSP07_08	170	71	68	34	115	11	10	6	18
	France	INCA2	524	70	57	19	178	12	9	3	30
	Germany	EsKiMo	196	74	56	20	200	10	8	3	29
	Italy	INRAN_SCAI_2005_06	139	111	79	30	322	15	10	4	51
	Latvia[d]	FC_PREGNANTWOMEN_2011	12	88	67	-[b]	-[b]	9	7	-[b]	-[b]
	Netherlands	DNFCS2007	576	95	60	26	336	12	7	3	42
	United Kingdom	NDNS-Rolling Programme Years 1–3	326	68	57	24	140	10	9	4	22
18–< 65 years	Finland	FINDIET2012	710	90	80	27	176	13	11	4	28
	France	INCA2	1 340	105	86	27	244	17	14	5	41
	Ireland	NANS_2012	640	81	68	25	187	11	9	4	25
	Italy	INRAN_SCAI_2005_06	1 245	157	114	40	432	23	15	6	64
	Latvia[d]	FC_PREGNANTWOMEN_2011	990	88	76	32	171	11	9	4	20
	Netherlands	DNFCS2007	1 034	135	78	26	516	17	10	3	60
	Sweden	Riksmaten 2010	807	98	82	33	213	13	11	5	28
	United Kingdom	NDNS-Rolling Programme Years 1–3	706	101	86	27	218	16	13	5	36
65–< 75 years	Finland	FINDIET2012	83	75	32	154	14	12	6	25	83
	France	INCA2	125	105	44	268	21	17	9	43	125
	Ireland	NANS_2012	96	81	24	200	15	12	4	33	96
	Italy	INRAN_SCAI_2005_06	169	120	38	392	25	17	7	62	169

Age class	Country	Survey	Intakes[b] expressed in µg/day					Intakes[b] expressed in µg/MJ			
			n[c]	Average	Median	P5	P95	Average	Median	P5	P95
	Netherlands	VCPBasis_AVL2007_2010	151	82	22	505	23	12	4	66	151
	Sweden	Riksmaten 2010	89	75	39	186	13	12	6	25	89
	United Kingdom	NDNS-Rolling Programme Years 1–3	107	97	28	240	18	15	5	42	107
≥ 75 years	France	INCA2	44	120	102	-[b]	-[b]	20	17	-[b]	-[b]
	Ireland	NANS_2012	43	89	76	-[b]	-[b]	14	12	-[b]	-[b]
	Italy	INRAN_SCAI_2005_06	159	164	121	33	466	25	16	6	74
	Sweden	Riksmaten 2010	30	111	108	-[b]	-[b]	16	16	-[b]	-[b]
	United Kingdom	NDNS-Rolling Programme Years 1–3	83	88	79	32	177	15	13	6	31

DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungsstudie als KIGGS-Modul; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale des Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione - Studio sui Consumi Alimentari in Italia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): Infants between 1 and 11 months. The proportions of breastfed infants were 58% in the Finnish survey, 40% in the German survey, 44% in the Italian survey, and 21% in the UK survey. Most breastfed infants were partially breastfed. The consumption of breast milk was taken into account if the consumption was reported as human milk (Italian survey) or if the number of breast milk consumption events was reported (German and UK surveys). For the German study, the total amount of breast milk was calculated based on the observations by Paul et al. (1988) on breast milk consumption during one eating occasion at different age groups: the amount of breast milk consumed on one eating occasion was set to 135 g/eating occasion for infants between 6–7 months of age and to 100 g/eating occasion for infants between 8–12 months of age (Kersting and Clausen, 2003). For the UK survey, the amount of breast milk consumed was either directly quantified by the mother (expressed breast milk) or extrapolated from the duration of each breastfeeding event. As no information on the breastfeeding events were reported in the Finnish survey, breast milk intake was not taken into consideration in the intake estimates of Finnish infants.

(b): 5^{th} or 95^{th} percentile intakes calculated from fewer than 60 subjects require cautious interpretation as the results may not be statistically robust (EFSA, 2011b) and, therefore, for these dietary surveys/age classes, the 5^{th} and 95^{th} percentile estimates are not presented in the intake results.

(c): n, number of subjects.

(d): Pregnant women only.

(e): The intake expressed as µg/MJ is referring to 251 female subjects of the Finnish DIPP study as energy intake was not reported for two subjects.

3107 **Appendix E. Minimum and maximum percentage contributions of different food groups (FoodEx2 level 1) to 'total vitamin K' intake**
3108 **estimates in males**

Food groups	Age						
	< 1 year	**1–< 3 years**	**3–< 10 years**	**10–< 18 years**	**18–< 65 years**	**65–< 75 years**	**≥ 75 years**
Additives, flavours, baking and processing aids	0	0	0	0	0	0	0
Alcoholic beverages	0	0	0	0	0	0	0
Animal and vegetable fats and oils	1–12	3–15	5–31	5–36	5–26	6–28	5–13
Coffee, cocoa, tea and infusions	0	0	< 1	< 1	< 1	< 1	< 1
Composite dishes	< 1–6	< 1–10	< 1–10	< 1–13	< 1–34	< 1–34	< 1–34
Eggs and egg products	< 1	< 1–1	< 1–1	< 1–2	< 1–4	< 1–5	< 1–4
Fish, seafood, amphibians, reptiles and invertebrates	0	< 1	< 1	< 1	< 1–2	< 1–3	< 1–2
Food products for young population	48–62	5–30	< 1–1	< 1	< 1	-	-
Fruit and fruit products	3–14	5–12	4–10	3–9	2–6	3–8	3–8
Fruit and vegetable juices and nectars	< 1–1	< 1–2	1–4	< 1–3	< 1–2	< 1–1	< 1–1
Grains and grain-based products	< 1–3	3–8	3–9	2–9	1–12	1–13	1–18
Human milk	0	0	-	-	-	-	-
Legumes, nuts, oilseeds and spices	< 1–6	2–24	1–23	1–21	1–18	2–13	3–15
Meat and meat products	0–1	< 1–2	< 1–5	1–5	1–4	1–3	1–3
Milk and dairy products	< 1–2	1–6	2–4	1–3	< 1–3	< 1–2	1–2
Products for non-standard diets, food imitates and food supplements or fortifying agents	0	0	0	< 1	< 1	0	0
Seasoning, sauces and condiments	0	0–2	< 1–2	< 1–3	< 1–7	< 1–2	< 1–2
Starchy roots or tubers and products thereof, sugar plants	< 1–2	1–4	1–5	1–7	1–5	1–4	1–4
Sugar, confectionery and water-based sweet desserts	0	< 1–1	< 1–1	< 1–1	< 1	< 1	< 1
Vegetables and vegetable products	12–37	25–62	32–64	31–71	25–75	22–77	23–73
Water and water-based beverages	0	0	0–1	< 1–1	< 1	0	0

3109 '-' means that there was no consumption event of the food group for the age and sex group considered, while '0' means that there were some consumption events, but that the food group does
3110 not contribute to the intake of the nutrient considered, for the age and sex group considered.
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3113 **Appendix F. Minimum and maximum percentage contributions of different food groups (FoodEx2 level 1) to 'total vitamin K' intake**
3114 **estimates in females**

Food groups	Age						
	< 1 year	1–< 3 years	3–< 10 years	10–< 18 years	18–< 65 years	65–< 75 years	≥ 75 years
Additives, flavours, baking and processing aids	0	0	0	0	0	0	0
Alcoholic beverages	0	0	0	0	0	0	0
Animal and vegetable fats and oils	1–10	3–17	4–31	5–31	4–21	3–21	4–9
Coffee, cocoa, tea and infusions	0	< 1	< 1	0	0	0	< 1
Composite dishes	< 1–2	0–11	< 1–12	< 1–15	< 1–32	< 1–34	< 1–35
Eggs and egg products	< 1	< 1–1	< 1–1	< 1–1	< 1–4	< 1–5	< 1–4
Fish, seafood, amphibians, reptiles and invertebrates	0	< 1	< 1	< 1	< 1–1	< 1–2	< 1–2
Food products for young population	38–61	5–28	< 1–1	< 1	< 1	-	< 1
Fruit and fruit products	3–14	5–11	6–11	4–11	3–9	4–11	4–9
Fruit and vegetable juices and nectars	< 1–1	< 1–2	1–4	< 1–5	< 1–2	< 1–1	< 1–1
Grains and grain-based products	0–2	3–8	3–9	2–8	1–12	1–12	1–13
Human milk	0	0	-	-	-	-	-
Legumes, nuts, oilseeds and spices	1–5	2–23	2–20	2–18	1–15	1–12	2–9
Meat and meat products	0	< 1–3	1–4	< 1–4	< 1–2	< 1–3	< 1–2
Milk and dairy products	< 1–4	1–6	2–3	1–4	1–2	< 1–2	1–2
Products for non-standard diets, food imitates and food supplements or fortifying agents	0	0	0	0	< 1	0	0
Seasoning, sauces and condiments	0	< 1–2	< 1–3	< 1–4	< 1–6	< 1–3	< 1–2
Starchy roots or tubers and products thereof, sugar plants	1–2	1–4	1–5	1–8	1–4	1–3	1–3
Sugar, confectionery and water-based sweet desserts	0	< 1–1	< 1–1	< 1–1	< 1	< 1	< 1
Vegetables and vegetable products	26–28	25–59	32–64	34–68	33–76	27–76	30–77
Water and water-based beverages	0	0	0–1	0–2	< 1	0	< 1

3115 '-' means that there was no consumption event of the food group for the age and sex group considered, while '0' means that there were some consumption events, but that the food group does
3116 not contribute to the intake of the nutrient considered, for the age and sex group considered.

Appendix G. Estimated dietary intakes of phylloquinone and menaquinones in EU countries as reported in the literature

Reference	Type of study	Country	Subjects	n	Source of the vitamin K composition data	Intake assessment method	Value of intake (µg/day)	Mean/median/range/ IQR
Phylloquinone								
Jie et al. (1995)[31]	Case-control study	NL	Postmenopausal women	113 79 females without aortic calcifications 34 females with aortic calcifications	Shearer et al. (1980); Booth SL et al. (1993)	FFQ	243.6 (women without aortic calcifications, n = 79) 189.9 (women with aortic calcifications, n = 34)	Mean
Schurgers et al. (1999)	Prospective cohort	NL	Adults (≥ 55 years)	5,435	Ferland and Sadowski (1992); Booth SL et al. (1993); Shearer et al. (1996) and unpublished data	FFQ	249 ± 2 (all) 257 ± 3 (men) 244 ± 2 (women)	Mean ± SE
Thane et al. (2002)	Cross-sectional, nationally representative sample	UK	Adults (≥ 65 years)	1,152	Bolton-Smith et al. (2000) and unpublished data	Four-day-weighed food record	88 (men) 78 (women)	Mean
Geleijnse et al. (2004)	Prospective cohort (same cohort as in Schurgers et al. (1999))	NL	Adults (≥ 55 years)	4,807 (after exclusion of 613 subjects with a history of myocardial infarction diagnosed at baseline, from the 5,435 investigated in Schurgers et al. (1999)	Suttie (1992); Ferland et al. (1992); Booth SL et al. (1993); Olson (1994); Booth et al. (1995); Ferland et al. (1992); Shearer et al. (1996); data from the laboratory analysed following Schurgers and Vermeer (2000) and Gijsbers et al. (1996)	FFQ	257.1 ± 116.1 (men) 244.3 ± 131.9 (women)	Mean ± SD

[31] Presented as 'vitamin K' in the reference by Jie et al., but assumed to be phylloquinone based on the two references cited as source of composition data.

Reference	Type of study	Country	Subjects	n	Source of the vitamin K composition data	Intake assessment method	Value of intake (µg/day)	Mean/median/range/ IQR
Prynne et al. (2005)	On-going prospective cohort	UK	Adults	5,362 included initially (in 1946); data analysis on 1,253	Bolton-Smith et al. (2000) and unpublished data	Five-day diary (data analysis on subjects with at least three reporting days)	59–81 (women, 81 µg/day in year 1999) 72–77 (men; 77 µg/day in year 1999)	Range of means (adjusted for social class and region of residence) for the years 1982, 1989 and 1999.
Rejnmark et al. (2006)	Prospective cohort, four study centers	DK	Perimenopausal women (43–58 years)	2,016	Danish Food composition tables (Moller, 1989)	Four-day or seven-day food record	67 (45–105)	Median ± SD
Nimptsch et al. (2008)	Prospective cohort	DE	Men (40–65 years)	11,319	Bolton-Smith et al. (2000) and unpublished data	Semi-quantitative FFQ	93.6 (70.9–123.5)	Median (IQR)
Gast et al. (2009)	Prospective cohort	NL	Postmenopausal women (49-70 years)	16,057	Mainly Schurgers and Vermeer (2000); also: Ferland and Sadowski (1992); Suttie (1992); Booth SL et al. (1993); Booth et al. (1995); Shearer et al. (1996)	FFQ	211.7 ± 100.3 (9.1 ± 991.1)	Mean ± SD
Bullo et al. (2011)	Prospective cohort	ES	Adults (55–80 years)	200	USDA (2009)	Semi-quantitative FFQ	333.6 ± 17.3 (men) 299.8 ± 11.6 (women)	Mean ± SE
DGE (2012)	National survey, Cross-sectional	DE	Adults (15–80 years)	6,160	German food composition database (BLS 3.02) (MRI)	Two 24-h recalls	76	Median
Elmadfa et al. (2012)	National survey, cross-sectional	AT	Children (7–14 years)	332 (children)	Elmadfa et al. (1994) (using the German food composition database BLS 2.1. (MRI) completed with food composition tables of typical Austrian dishes and nutrient enriched foods)	Three-day dietary record	59–75 (children)	Range of means depending on sex and age-range
			Adults (18–80 years)	380 (18–64 years) 176 (65–80 years)	Jakob and Elmadfa (1996)	Two 24-h recalls	89–117 (adults)	Range of means depending on sex and age-range
Vissers et al. (2013)	Prospective cohort	NL	Adults (49 ± 12 years), including the cohort of women investigated by Gast et al. (2009)	35,476	Mainly Schurgers and Vermeer (2000); also: Ferland and Sadowski (1992); Suttie (1992); Booth SL et al. (1993); Booth et al. (1995); Shearer et al. (1996)	FFQ	199 ± 97.8	Mean ± SD

Reference	Type of study	Country	Subjects	n	Source of the vitamin K composition data	Intake assessment method	Value of intake (µg/day)	Mean/median/range/ IQR
Ortega Anta et al. (2014)[32]	Cross-sectional, nationally representative sample	ES	Mostly adults (17–60 years)	1,068	Spanish database: Ortega et al. (2010)	Three-day food record	174.2 (males), 166.4 (females) 170.2 (all)	Mean (adjusted for energy intake)
Weber et al. (2014)[33]	Prospective cohort	DE	Children (8–12 years)	268	German food composition database BLS II.3 (MRI)	Dietary history over four weeks	292.3	Median
Hayes et al. (2016)	National survey, cross-sectional	IE	Adults (18–90 years)	1,500	Mainly UK food composition table (FSA, 2002), which vitamin K data are largely based on Bolton-Smith et al. (2000), and data from the previous version of the UK table; also recipe calculations, and USDA (2015)	Four day semi-weighted food diary	85.2 ± 59.1 (all) 86.0 ± 57.4 (men) 84.4 ± 60.7 (women)	Mean ± SD
				Menaquinones				
Schurgers et al. (1999)	Prospective cohort	NL	Adults (≥ 55 years)	5,435	Unpublished data	FFQ	**Total menaquinones (MK-4 to MK-10)** 28.4 (all) **MK-4** 6.8 ± 0.04 (all) 7.5 ± 0.1 (men) 6.3 ± 0.1 (women) **MK-5 to MK-10** 21.6 ± 0.2 (all) 22.9 ± 0.3 (men) 20.6 ± 0.3 (women)	 Mean Mean ± SE Mean ± SE

[32] Presented as 'vitamin K' in the reference, but personal communication from one of the authors confirmed that composition data were on phylloquinone.
[33] Presented as 'vitamin K' in the reference, but assumed to be phylloquinone, based on information from Section 3.2.1.

Reference	Type of study	Country	Subjects	n	Source of the vitamin K composition data	Intake assessment method	Value of intake (µg/day)	Mean/median/range/ IQR
Geleijnse et al. (2004)	Prospective cohort	NL	Adults (≥ 55 years)	4,807	Data from the laboratory analysed following Schurgers and Vermeer (2000) and Gijsbers et al. (1996)	FFQ	**Total menaquinones (MK-4 to MK-10)** 30.8 ± 18 (men) 27 ± 15.1(women) **MK-4** 7.7 ± 3.4 (men) 6.3 ± 2.8 (women) **MK-5 to MK-10** 23.1 ± 16.3 (men) 20.7 ± 13.8 (women)	Mean ± SD Mean ± SD Mean ± SD
Nimptsch et al. (2008)	Prospective cohort	DE	Men (40–65 years)	11,319	Hirauchi et al. (1989); Schurgers and Vermeer (2000)	FFQ	**Total menaquinones (MK-4 to MK-14)** 34.7 (25.7–45.7) **MK-4** 14.4 (10.9–18.7) **MK-5** 0.3 (0.2–0.5) **MK-6** 0.3 (0.2–0.5) **MK-7** 0.8 (0.5–1.1) **MK-8** 4.6 (3.1–6.7) **MK-9** 11.9 (7.4–18.4) **MK-10** 0.06 (0.01–0.13) **MK-11** 0.12 (0.03–0.27) **MK-12** 0.20(0.04–0.42) **MK-13** 0.40 (0.08–0.85) **MK-14** 0.02 (0.00–0.05)	Median (IQR)

Reference	Type of study	Country	Subjects	n	Source of the vitamin K composition data	Intake assessment method	Value of intake (µg/day)	Mean/median/range/ IQR
Gast et al. (2009)	Prospective cohort	NL	Postmenopausal women (49–70 years)	16,057	Schurgers and Vermeer (2000)	FFQ	**Total menaquinones (MK-4 to MK-9)** 29.1 ± 12.8 (0.9–128) **MK-4** 7.1 ± 2.1 (0.5–28.2) **MK-5** 0.3 ± 0.2 (0–2.1) **MK-6** 0.3 ± 0.2 (0–1.5) **MK-7** 0.3 ± 0.2 (0–2.2) **MK-8** 6.0 ± 3.4 (0–32.8) **MK-9** 14.7 ± 8.1 (0–81.9)	Mean ± SD (range)
Vissers et al. (2013)	Prospective cohort	NL	Adults (49 ±12 years) including the cohort of women investigated by Gast et al. (2009)	35,476	Schurgers and Vermeer (2000)	FFQ	**Total menaquinones (MK-4 to MK-10)** 30.7 ± 13.8	Mean ± SD

(a): presented as 'vitamin K' in the reference, but assumed to be phylloquinone, based on information from Section 3.2.1.

AT: Austria; BLS: Bundeslebensmittelschlüssel; DE: Germany; ES: Spain; FFQ: food frequency questionnaire; IQR: interquartile range; MK: menaquinone; MRI: Max Rubner Institut; NL: the Netherlands; SD: standard deviation; SE: standard error; USDA: US Department of Agriculture; UK: United Kingdom.

3121 **ABBREVIATIONS**

$1,25(OH)_2D$	1,25-hydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
%ucOC	percentage of undercarboxylated osteocalcin
Afssa	Agence française de sécurité sanitaire des aliments
AI	adequate intake
ApoE	apolipoprotein E
APTT	activated partial thromboplastin time
AR	average requirement
AU	arbitrary unit
AUC	area under the curve
BAC	breast artery calcification
BLS	Bundeslebensmittelschlüssel
BMC	bone mineral content
BMD	bone mineral density
BUA	broadband ultrasound attenuation
CAC	coronary artery calcification
CHD	coronary heart disease
CI	confidence interval
cOC	carboxylated osteocalcin
COMA	Committee on Medical Aspects of Food Policy
CVD	cardiovascular disease
CYP4F2	cytochrome P450 4F2
D-A-CH	Deutschland- Austria- Confoederatio Helvetica
DH	UK Department of Health
DIPP	Type 1 Diabetes Prediction and Prevention survey
DNA	deoxyribonucleic acid
DNFCS	Dutch National Food Consumption Survey

DNSIYC	Diet and Nutrition Survey of Infants and Young Children
dp-ucMGP	desphospho-uncarboxylated MGP
DRV	dietary reference values
DXA	dual-emission X-ray absorptiometry
EC	European Commission
EsKiMo	Ernährungsstudie als KIGGS-Modul
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FAO	Food and Agriculture Organization
FC_PREGNANTWOMEN	Food consumption of pregnant women in Latvia
FFQ	food frequency questionnaire
FINDIET	National dietary survey of Finland
FN	femoral neck
FVII	factor VII
GAS6	growth arrest-specific protein 6
GC/MS	gas chromatography/mass spectrometry
GGCX	γ-glutamyl carboxylase
Gla	γ-carboxyglutamic acid
Glu	glutamic acid
HDL	high-density lipoproteins
HPLC	high performance liquid chromatography
HR	hazard ratio
HSPG	heparan sulfate proteoglycans
IDL	intermediate-density lipoprotein
INCA	Étude Individuelle Nationale de Consommations Alimentaires
INRAN-SCAI	Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione – Studio sui Consumi Alimentari in Italia
IOM	US Institute of Medicine of the National Academy of Sciences
IQR	interquartile range

IU	international units
LDL	low-density lipoproteins
LS	lumbar spine
MGP	matrix Gla-protein or matrix γ-carboxyglutamic protein
MK	menaquinone
MRI	Max Rubner Institut
NANS	National Adult Nutrition Survey
NDNS	National Diet and Nutrition Survey
NHANES	National Health And Nutrition Examination Survey
NNR	Nordic Nutrition Recommendations
NWSSP	Nutrition and Wellbeing of Secondary School Pupils
OC	osteocalcin
OR	odds ratio
PAD	peripheral arterial disease
PIVKA-II	protein induced by vitamin K absence or antagonism-II
PRI	population reference intake
PT	prothrombin time
PTT	partial thromboplastin time
Q	quintile
q	quartile
QUI	quantitative ultrasound index
QUS	quantitative ultrasound
RCT	randomised controlled trial
RNI	recommended nutrient intake
RR	relative risk
SCF	Scientific Committee for Food
SD	standard deviation
SEM	standard error of the mean

SNP	single nucleotide polymorphism
SOS	speed of sound
TG	triglyceride
TRL	triglyceride-rich lipoproteins
UBIAD1	enzyme UbiA prenhyltransferase domain-containing protein 1
ucOC	undercarboxylated osteocalcin
UK	United Kingdom
UL	tolerable upper intake level
USDA	United States Department of Agriculture
VELS	Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln
VKDB	vitamin K deficiency bleeding
VKOR	vitamin K epoxide reductase
VKORC1	vitamin K epoxide reductase complex subunit 1
VLDL	very low-density lipoproteins
WHO	World Health Organization

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