FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting document 1

Risk and technical assessment – Application A1300

A1300 – Vitamin K2 (as Menaquinone-7) as a permitted form of Vitamin K in FSMP

Executive summary

Food Standards Australia New Zealand (FSANZ) has assessed an application to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of vitamin K_2 (as menaquinone-7) as a permitted form of vitamin K in food for special medical purposes (FSMP).

The method of production of menaquinone-7 (MK-7) is via a standard submerged fermentation, using the bacterial strain *Bacillus paralicheniformis*. MK-7 can be added and incorporated in a uniform manner into food products in the same way as other lipid soluble vitamins, including vitamin K_1. It has good stability at both standard and accelerated storage conditions. There is a specification for MK-7 in the Code, which data provided by the applicant demonstrated it is able to comply with.

In order to determine whether MK-7 is an equivalent source of vitamin K in the diet, FSANZ considered human studies that measured the absorption of MK-7 and the effect of MK-7 supplementation on biomarkers of vitamin K status. Following supplementation, blood MK-7 concentrations increased compared to placebo or baseline in all studies, with greater levels of absorption compared to vitamin K_1 at similar intake. Supplementation with MK-7 also resulted in an improvement in biomarkers for vitamin K status at doses of 90 to 360 µg/day. FSANZ concludes that based on the available evidence in humans MK-7 is a bioavailable form of vitamin K which would be expected to support normal physiological function at doses of 90 to 360 µg/day. Due to a lack of human studies that compare the bioavailability of MK-7 with vitamin K_1, at current recommended levels, FSANZ cannot determine to what extent MK-7 would support essential requirements for vitamin K at the current Adequate Intake, when it is the only form of vitamin K in the diet. However, FSMP are used under the supervision of a medical practitioner and can be modified as required.

No evidence was identified to indicate that MK-7 would inhibit the absorption of other nutrients.

There is a history of safe human consumption of MK-7 from the diet, and MK-7 is also produced endogenously by gastrointestinal bacteria. No adverse effects of MK-7 were identified in toxicity studies in laboratory animals and clinical studies in humans. Toxicity studies with the structurally related compound MK-4 were also considered as supporting

evidence. A comparison of estimated dietary intakes of MK-7 to the no observed adverse effect level (NOAEL) in a chronic toxicity study with MK-4 resulted in a large margin of exposure (< 5400), indicating no safety concerns.

There is a potential for interaction between MK-7 and vitamin K antagonist (VKA) anticoagulant drugs, but patients on anticoagulant therapy receive medical advice about the risk of an interaction with vitamin K supplements, and individuals consuming FSMP are under medical supervision.

The assessment concluded there are no public health and safety concerns associated with the use of MK-7 as a permitted form of vitamin K in FSMP.

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1 Introduction

FSANZ received an application from Novozymes Australia Pty Ltd to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of vitamin K_2 (as menaquinone-7 (MK-7)) as a permitted form of vitamin K in food for special medical purposes (FSMP). FSMP partially or totally replace the daily diet and are recommended to be used under medical supervision.

This application requests permission to use a new form of an already permitted vitamin. It does not propose any amendment to the mandatory compositional, labelling or other requirements for FSMP.

2 Food technology assessment

The primary purpose of the food technology section is to assist in assessing whether the requested form of vitamin K, being vitamin K_2, is an appropriate form of vitamin K to be added to food consistent with the only currently permitted form, vitamin K_1. Therefore it is important to consider how the different forms of vitamin K compare to each other.

Specifically, are the two forms technologically equivalent for the proposed purpose, by considering whether:

- vitamin K_2 is chemically and physically equivalent or similar to vitamin K_1;
- it can be incorporated into the relevant food matrices;
- it has the same performance such as stability and shelf life as the currently permitted form;
- it is produced in a similar way;
- there are any safety concerns or impurities produced during its manufacture;
- there are relevant internationally recognised identity and purity specifications for this form of vitamin K, and if so does the applicant provide evidence indicating that its form of vitamin K_2 is able to meet such specifications.

2.1 Chemical and physical properties of vitamin K_2

Vitamin K captures a group of fat soluble vitamins which includes two distinct main forms; vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinone); as well as vitamin K_3 (menadione).

The applicant's form of vitamin K_2 is also called menaquinone-7 (MK-7) with a specific chemical structure. There are fourteen (14) specific forms of vitamin K_2 which are differentiated by the size of the length of the side-chain of the chemical composition. MK-7 has seven (7) linked isoprene units, and has been identified as the most biologically active form of vitamin K_2. All the vitamin K structures have the same ring structure of 2-methyl-1,4-naphthoquinone unit with different side chains. The structural differences between vitamin K_1 and examples of various forms of K_2 (MK-4 and MK-7) are provided in Figure 1.

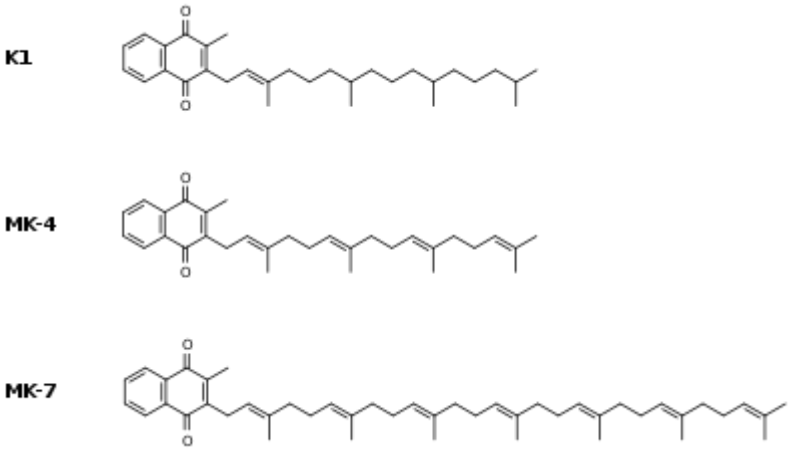


Figure 1. Chemical structure of vitamin K_1 compared to two types of vitamin K_2, being MK-4 and MK-7

A comparison of the main chemical and physical properties of MK-7 compared to the currently permitted form of vitamin K in the Code, vitamin K_1, is provided in Table 1.

Table 1. Chemical and physical properties of vitamin K_2 (MK-7) (the applicant's product) compared to vitamin K_1

Parameter	Description	
Common name	MK-7	Vitamin K_1
Alternative names	Vitamin MK7, menaquinone K7, MK7, Vitamin K_2	Phylloquinone, phytonadione
Systematic name	1,4-Naphthalenedione, 2-(3,7,11,15,19,23,27-heptamethyl-2,6,10,14,18,22,26-octacosahept-aenyl)-3-methyl-, (all-E)-	N/A
IUPAC[1] systematic name	2-(3,7,11,15,19,23,27-heptamethyloctacosa-2,6,10,14,18,22,26-heptaenyl)-3-methylnaphthalene-1,4-dione	2-methyl-3-[(E)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione
Chemical formula	$C_{46}H_{64}O_2$	$C_{31}H_{46}O_2$
Molecular weight (g/mol)	649.0	450.7
CAS[2] No.	2124-57-4	12001-79-5
Microbial source	*Bacillus paralicheniformis*	N/A
Appearance	Off-white to pale yellow powder, or oil	Pale yellow oil

Notes: 1 = International Union of Pure and Applied Chemistry; 2 = Chemical abstracts service; N/A = Not applicable

2.2 Manufacturing process

The manufacturing process to produce the applicant's MK-7 is via standard submerged fermentation using the bacterial strain *Bacillus paralicheniformis*. It is stated by the applicant

to be well characterised and is a non-toxicogenic and non-pathogenic bacterial strain. The substances used as sources for the fermentation are gram flour from chickpeas and dextrin.

After fermentation the fermentation broth is separated, spray dried and MK-7 is extracted using hexane and purified. The final commercial MK-7 preparation can be provided either as the original oil or made into a powder. To prepare a powder, the oil is ground to a fine paste with glycerol monostearate and milled to the required mesh size. For the liquid preparation the concentrated oil is blended with vegetable oil to the standardised concentration.

As noted in section 2.4, MK-7 preparations produced by fermentation also contain small amounts of MK-6 (with the smaller side chain length) and the biologically inactive cis isomer of MK-7 (Marles et al. 2017). Purity limits of these substances are provided in the international specification of MK-7 (see section 2.4). These limits ensure the safety and quality of the preparation. MK-6 is not as biologically active as MK-7, which has been identified as the most biologically active form of vitamin K_2.

2.3 Incorporation into food matrices and stability of MK-7

The product is prepared and sold as a commercial nutritive substance preparation, either as an oil or it can be processed into a powder (as noted in section 2.2). It is a lipid-soluble vitamin and it will be added and incorporated in a uniform manner into food products in the same way as other lipid soluble vitamins. This is also no different to how vitamin K_1 is used and incorporated into food. Likewise MK-7 is permitted and added into different food matrices in other countries with no issues.

The applicant provided a number of stability studies specific to the oil version of their MK-7. The conclusions of these studies were that it is stable for 30 months at storage temperatures of 30°C ± 2°C and relative humidity of 65% ± 5%; 30 months at storage temperature of 30°C ± 2°C and relative humidity of 75% ± 5%; and 6 months at storage temperature of 40°C ± 2°C and relative humidity of 75% ± 5%. These results show MK-7 is stable at standard temperatures, as well as higher storage temperatures and relative humidity.

2.4 Specifications

The Code (Section 1.1.1—15) requires that a substance that is used as a nutritive substance must comply with any relevant specification set out in Schedule 3.

The applicant notes that there is a specification for MK-7 in one of the secondary sources of specifications within subsection S3—3(b), being the United States Pharmacopeia (USP)[1]. The applicant provided a confidential copy of the most recent version of this specification since there is a cost to subscribing and purchasing copies of specifications. The title of the specification is 'Menaquinone-7 Preparation' with the date of 1 December 2019, though the printed date was 23 January 2024.

The applicant provided non confidential analytical results in the application from three batches of their preparation demonstrating they are able to comply with the requirements of the USP specification.

It is noted that the specification requires that MK-6 is less than 10% of the labelled amount of MK-7 in the preparation (either on the dried basis for the powder or on the liquid preparation). Another purity requirement is that the cis isomer of MK-7 is less than 2% of the vitamin MK-7 concentration. The applicant provided non confidential results that their product complies with these two purity parameters.

[1] Menaquinone-7 Preparation (usp.org)

2.5 Analytical methods

There are analytical methods available that can be adapted to quantify the concentration of MK-7 in food matrices (for this application, FSMP). These are the Association of Official Analytical Chemists (AOAC) method numbers 992.27 and 999.15, modified. These methods can be used to analyse for vitamin K in general, including vitamin K_1 and MK-7.

The methods are based on reverse phase high-performance liquid chromatography (HPLC) with fluorescence detection. Samples are digested with enzymes to metabolise all fat, extracted with organic solvents and injected into the HPLC system.

2.6 Food technology conclusion

FSANZ concludes that MK-7 is a different form of vitamin K to the currently permitted vitamin K_1. The manufacturing process to produce MK-7 is via a standard submerged fermentation, using the bacterial strain *Bacillus paralicheniformis*. The final commercial preparation can be provided either as the original oil or dried to a powder. It is a lipid-soluble vitamin that can be added and incorporated in a uniform manner into food products in the same way as other lipid soluble vitamins, including vitamin K_1. MK-7 is stable at both standard (30°C) and higher storage temperatures (40°C) and relative humidity (65% and 75%).

There is a specification for MK-7 in the Code under subsection S3—3(b), the United States Pharmacopeia (USP). Data provided by the applicant demonstrated it is able to comply with the requirements of this specification.

3 Nutrition assessment

3.1 Objectives for the nutrition risk assessment

Objectives for the nutrition risk assessment were to:

- determine whether vitamin K_2 (as menaquinone-7) is an equivalent source of vitamin K to the currently permitted form of vitamin K (vitamin K_1 as phylloquinone (phytonadione)).

- determine the ability of vitamin K_2 (as menaquinone-7) to inhibit or modify the absorption of other nutrients.

3.2 Introduction

Vitamin K is a group of fat soluble vitamins that includes phylloquinone (vitamin K_1) and the menaquinones (vitamin K_2). Vitamin K is a co-factor for an enzyme that activates vitamin K-dependent proteins (VKDPs), which have several roles including blood coagulation and cell cycle regulation, vascular repair and prevention of calcification, and bone metabolism and turnover (Vermeer et al. 2004; Booth 2009; Bus and Szterk 2021). All forms of vitamin K contain a 2-methyl-1,4-naphthoquinone structure (also known as menadione) and an isoprenoid side chain at the 3-position, but differ in the length of side chain and degree of saturation (Shearer and Newman 2008; Booth 2009; Bus and Szterk 2021). Menaquinone-7 (MK-7) contains an isoprenyl side chain with seven unsaturated prenyl units with the chemical name 2-methyl-3-farnesylgeranyl-geranyl-1,4-naphthoquinone[2] (Figure 1), while vitamin K_1 contains a phytyl side chain with one unsaturated isoprenoid residue (Schurgers and Vermeer 2000; Shearer and Newman 2008).

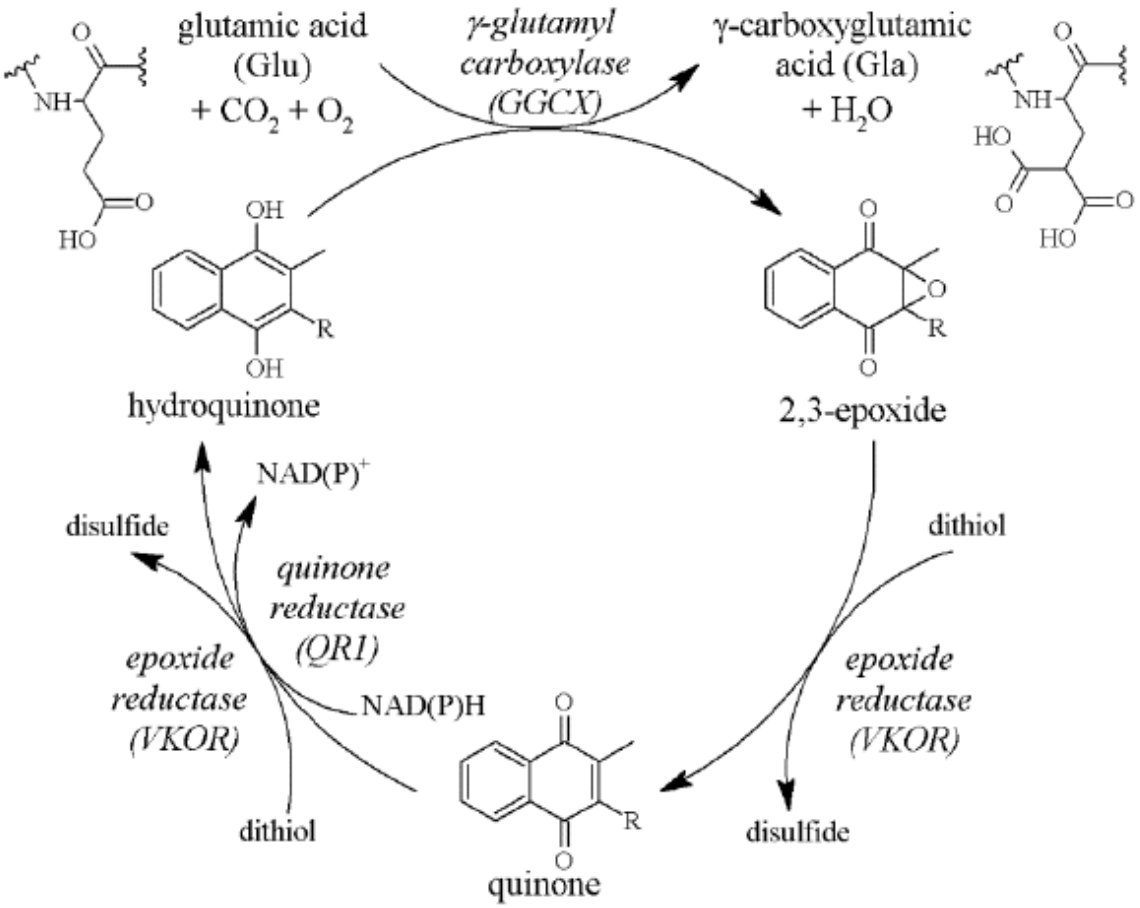
Structural differences in the isoprenyl side chain of differing forms of vitamin K affect metabolism including how they are transported, absorbed, and excreted (Shearer and Newman 2008). After intestinal absorption, vitamin K_1 and MK-7 are packaged into chylomicrons and transported into circulation to target tissues by lipoproteins (Schurgers and Vermeer 2002; Shearer et al. 2012). Circulating vitamin K_1 is present in very low density triglyceride-rich lipoprotein fractions (Kohlmeier et al. 1996) and rapidly accumulates in the liver (IOM 2001). Less is known about MK-7 lipoprotein transport, however it is thought they may be repackaged into low density lipoproteins and delivered from the liver to extrahepatic cells including in the bone and vascular system (Schurgers and Vermeer 2002; Schurgers et al. 2007; Shearer et al. 2012).

Absorbed vitamin K (quinone) is converted to hydroquinone (KH_2) by quinone reductase (QR1) or vitamin K epoxide reductase (VKOR) (Bus and Szterk 2021). KH_2 is a co-factor for the enzyme gamma (γ)-glutamyl carboxylase (GGCX), that modifies glutamyl residues (Glu) to γ-carboxyglutamic acid (Gla) in a limited number of VKDPs (IOM 2001; Stafford 2005). During the carboxylation process, KH_2 is converted to vitamin K 2,3-epoxide (KO) that is continually recycled by VKOR to its quinone and KH_2 forms in a process known as the vitamin K cycle (Stafford 2005; Schurgers et al. 2007; Bus and Szterk 2021) (Figure 2). All forms of vitamin K share this electron donor function in the vitamin K cycle (Schurgers et al. 2007; Shearer and Newman 2008; Rishavy and Berkner 2012; Beulens et al. 2013; Simes et al. 2020; Cirilli et al. 2022).

Figure 2. The vitamin K cycle. Vitamin K (quinone) is reduced to hydroquinone by vitamin K epoxide reductase (VKOR) or quinone reductase (QR1). During carboxylation of glutamyl

[2] Menaquinone-7 | C46H64O2 | CID 5287554 - PubChem (nih.gov)

residues of vitamin K-dependent proteins (Glu) to γ-carboxyglutamic acid (Gla), vitamin K is an electron donor and undergoes oxidation to 2,3-epoxide, which is converted back to quinone by VKOR. (Reproduced from Bus et al. 2021 under the Creative Commons Attribution 4.0 Licence http://creativecommons.org/licenses/by/4.0/)



VKDPs include factors VII, IX, X, prothrombin, and proteins Z, S and C involved in blood coagulation, and bone-related proteins osteocalcin and matrix Gla protein (IOM 2001; Stafford 2005). The degree of carboxylation of serum osteocalcin (undercarboxylated osteocalcin (ucOC) into carboxylated osteocalcin (cOC)) and dephosphorylated-undercarboxylated matrix Gla protein (dp-ucMGP) concentration are considered sensitive biomarkers of vitamin K status[3] (Sokoll et al. 1997; Booth and Al Rajabi 2008; Rennenberg et al. 2010; Shea et al. 2011).

3.3 Dietary sources and bioavailability of vitamin K

The major dietary source of vitamin K in most diets is as vitamin K_1 from plant sources, particularly green vegetables (for example broccoli, spinach and cabbage) and vegetable oils, with lower concentrations found in fruits, grains and dairy products (Shearer and Newman 2008; Bus and Szterk 2021). Dietary sources of MK-7 are primarily from bacteria-fermented foods including some cheeses and the fermented soybean product natto which is most commonly consumed in Japan (Schurgers and Vermeer 2000; Shearer and Newman 2008). In Western diets, approximately 90% of dietary vitamin K is from vitamin K_1 with approximately 10% from menaquinones (Schurgers et al. 1999; Beulens et al. 2013). In addition, menaquinones can be synthesised by intestinal bacteria (Booth and Al Rajabi 2008;

[3] A decrease in ucOC and dp-ucMGP and an increase in cOC concentrations indicate improved vitamin K status. A decrease in ucOC:cOC and an increase in cOC:ucOC is considered an improvement.

Shearer et al. 2012; Walther et al. 2013).

The absorption of vitamin K_1 varies depending on the food. When compared to absorption of vitamin K_1 in supplement form, vitamin K_1 from spinach was 4 to 13% bioavailable depending on whether it was cooked with or without butter (Gijsbers et al. 1996). Vitamin K_1 from fresh spinach, broccoli and romaine ranged from 9% to 17% and cooked broccoli was 23% as available in serum as from supplement (Garber et al. 1999). Absorption of MK-7 is less well studied, however *in vitro* testing suggests it is approximately 55 ± 2.1% (standard deviation (SD)) bioaccessible from natto and approximately 58 ± 12% from eight month ripened blue cheese (Jensen et al. 2021; Jensen et al. 2022).

3.4 Bioavailability of menaquinone-7 (MK-7)

The applicant provided 10 relevant human studies in support of MK-7 as a permitted form of vitamin K in FSMP (Schurgers and Vermeer 2000; Schurgers et al. 2007; van Summeren et al. 2009; Emaus et al. 2010; Brugè et al. 2011; Dalmeijer et al. 2012; Theuwissen et al. 2012; Knapen et al. 2013; Knapen et al. 2015; Møller et al. 2016). FSANZ undertook a literature search on 05 June 2024 to identify any additional relevant human studies[4]. Forty-six studies were identified of which one was included in the body of evidence (Kanellakis et al. 2012).

In cases of publications where data were only available in graphs, webplotdigitizer was used to extract information (WebPlotDigitizer (utk.edu)).

FSANZ conducted a literature search on 20 June 2024 to identify any relevant evidence on whether MK-7 would modify or inhibit the absorption of other nutrients[5]. No studies were identified.

3.4.1 Summary of studies

Open-label non-randomised controlled study of absorption of vitamin K_1 and MK-7 in healthy adults (Schurgers and Vermeer 2000)

A non-randomised, open-label absorption study using six healthy male volunteers with normal serum lipid profiles (mean age 33.5 ± 2.57 standard error (SE) years) from the Netherlands was undertaken in which detergent-solubilised vitamin K_1 (3.5 µmol vitamin K_1; 1577.4 µg) or 400 g spinach (3.5 µmol vitamin K_1; 1577.4 µg) and 200 g natto (3.1 µmol MK-7; 2011.9 µg) or control breakfast were ingested. Following an overnight fast subjects received either a breakfast low in vitamin K (control) or a similar meal containing 400 g spinach and 200 g natto supplemented with corn oil to 30 g total fat content or a single detergent-solubilised vitamin K_1 supplement, with a one week washout period between test meals. On study days participants were permitted to consume a lunch low in vitamin K and to drink orange juice and water ad libitum. Vitamin K-rich foods were restricted for the duration of the experiment. Serum vitamin K_1 and MK-7 levels were measured following the collection of blood samples at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 24, 48, and 72 hr for each study phase. Mean values and SE for each test arm were calculated, with mean serum vitamin K concentrations from the control arm subtracted.

Peak serum vitamin K_1 and MK-7 concentrations occurred 6 hr after spinach and natto consumption. The peak vitamin K_1 serum concentration was approximately 8 nmol/L (3.6 µg/L), whereas the peak MK-7 serum concentration was 80 nmol/L (51.9 µg/L). Peak

[4] Search terms: “phylloquinone or phytonadione or vitamin K1” and “menaquinone-7 or MK-7 or vitamin K2-7” and “bioavailability or availability or equivalence or bioequivalence or comparison or osteocalcin or carboxylation or matrix Gla protein”

[5] Search terms: "vitamin K2-7 or MK-7 or menaquinone-7 or menaquinone 7" and “inhibit or inhibition or modify or modification or inhibitory or modification”

serum vitamin K_1 concentrations from the K_1 supplement occurred 4 hr after ingestion and peaked at approximately 45 nmol/L (20.3 µg/L). Between 6 and 8 hr after the meal, the half-life for both forms of vitamin K was 1.5 hr. The second phase of MK-7 absorption had a half-life of approximately 50 hr. MK-7 was still detected in serum at the end of the study timeframe (72 hr).

To ensure that absorption of one vitamin did not compete with the other, the experiment was repeated with 400 g spinach and 200 g natto given in two separate meals with a one week washout period. All other study parameters remained constant. Serum vitamin K levels were similar to the mixed meal study, with serum concentrations of both forms of vitamin K peaking 6 hr post-meal. Peak concentration for vitamin K_1 was approximately 8 nmol/L (3.6 µg/L) and MK-7 was approximately 75 nmol/L (48.7 µg/L).

The post-meal peak serum concentrations of MK-7 from natto were more than 10x higher than those of vitamin K_1 from spinach from similar molar concentration doses. Both forms peaked at 6 hr post-meal, but the half-life of MK-7 was much higher with serum concentrations still detected 72 hr post-meal. The authors concluded that MK-7 is more bioavailable than vitamin K_1.

Three studies of the absorption and function of vitamin K_1 and MK-7 in healthy adults (Schurgers et al. 2007)

Study one: non-randomised single dose absorption study of vitamin K_1 and MK-7 in healthy adults.

A non-randomised, single dose absorption study using 15 healthy volunteers (reported as equal numbers of men and women, aged 25 to 35 years) from the Netherlands was undertaken in which a 30 mL solution (20 mL orange juice mixed with 10 mL of corn oil fortified with 1 mg vitamin K_1 and 1 mg MK-7) was ingested. The solution was consumed with a standard breakfast containing 30 g fat. For two days prior to commencement and for the duration of the study, participants were requested to avoid consuming vitamin K-rich foods. Serum vitamin K_1 and MK-7 levels were measured from non-fasting blood samples at 0, 2, 4, 6, 8, 24, 48, 72, and 96 hr. Pre-dose corrected areas under the curve ($AUC_{0\text{-}24h}$ and $AUC_{0\text{-}96h}$ (µg/L)) were calculated for both forms of vitamin K.

Baseline serum concentration of vitamin K_1 and MK-7 was 0.45 µg/L and < 0.05 µg/L respectively, with peak concentrations of 14 µg/L and 17 µg/L observed 4 hours after consumption. Both vitamin K_1 and MK-7 serum levels declined rapidly 4 to 8 hr after consumption, with an 86% decline observed for vitamin K_1 to return to near baseline levels. For MK-7, the estimated half-life was 68 hr, based on the slope of the second phase decline. MK-7 was detected in serum at the end of the study timeframe (96 hr). The authors reported that the $AUC_{0\text{-}24hr}$ and $AUC_{0\text{-}96hr}$ for MK-7 was 2.5x and 6x higher for MK-7 respectively compared to vitamin K_1, indicating that MK-7 is at least 2.5x more bioavailable than vitamin K_1.

Study two: non-randomised study of dose-response absorption of vitamin K_1 and MK-7 in healthy adults.

A non-randomised, dose-escalation study using 10 healthy female and male volunteers (reported as equal numbers of men and women, aged 25 to 35 years) from the Netherlands was undertaken in which a fortified oil solution of increasing vitamin K_1 and MK-7 doses (50, 100, 150, 200, 250, 300, and 500 µg of each form of vitamin K) was consumed with a standard breakfast. Participants were requested to avoid vitamin K-rich foods for two days prior to commencement and for the duration of the study. Consumption of each dose was separated by a two week washout period. Serum vitamin K_1 and MK-7 levels were measured

following collection of blood samples at 0, 4 and 24 hr after breakfast. Mean values and SD were calculated at 4 and 24 hr.

At 4 hr, serum vitamin K_1 and MK-7 concentrations showed a dose-response relationship, with a steeper incline for MK-7 compared to vitamin K_1. At 24 hr, serum vitamin K_1 concentrations were close to baseline at doses up to 200 µg while serum MK-7 was 1 µg/L (1.5 nmol) following a dose of 100 µg.

Study three: randomised control cross-over study of osteocalcin carboxylation during prolonged intake of vitamin K_1 and MK-7 in healthy adults.

A randomised, placebo-controlled crossover study using 18 healthy volunteers (reported as equal numbers of men and women, aged 25 to 35 years) was undertaken in which either 0.22 µmol/day of vitamin K_1 (99.2 µg/day in a tablet) or MK-7 (142.8 µg/day in a natto extract capsule) or placebo was ingested for six weeks at dinner time, separated by 12-week washout periods. Participants were asked to avoid consuming vitamin K-rich foods for two days prior to commencement and for the duration of the study. Serum vitamin K concentrations and cOC:ucOC ratio were measured in non-fasting blood samples collected at 8 am on day 0, 3, 7, 14, 21, 28, 35 and 42. Mean values and SD were calculated for each treatment.

Serum MK-7 concentration increased in the first two weeks to a maximum of 6 µg/L (10 nmol/L), and the mean vitamin K_1 concentration was 0.8 µg/L by day 3 and remained at a similar level for the remainder of the study. This was a small increase compared to placebo (0.2 µg/L). At comparable (equimolar) intakes, serum MK-7 concentration was approximately 8 times greater than vitamin K_1 by day 14. At baseline, the mean cOC:ucOC ratio was 1.74, 1.8 and 1.7 for MK-7, vitamin K1 and placebo respectively. The baseline-corrected cOC:ucOC ratio increased by 0.5 by day 3 for both forms of vitamin K. The cOC:ucOC ratio following vitamin K_1 supplementation remained at + 0.5 for the duration of the study. After MK-7 supplementation the cOC:ucOC ratio increased, to a peak of approximately + 1.5 at day 42, three times greater than vitamin K_1.

From the three studies, the authors concluded that serum MK-7 is available for longer than vitamin K_1 and is more effective at osteocalcin carboxylation.

Randomised controlled trial of 12-month consumption of dairy products enriched with calcium, vitamin D_3, and vitamin K_1 or MK-7 in healthy postmenopausal women (Kanellakis et al. 2012)

A randomised, placebo-controlled four-arm parallel study using 115 healthy postmenopausal women (mean age 62 ± 5.8 (SD) years) from Greece was undertaken in which participants received fortified low-fat yoghurt and milk products with either i) 800 mg calcium and 10 µg vitamin D_3 (n = 26; control), ii) 800 mg calcium, 10 µg vitamin D_3, and 100 µg vitamin K_1 (0.221 µmol; n = 26), iii) 800 mg calcium, 10 µg vitamin D_3, and 100 µg MK-7 (0.154 µmol; n = 24) or reference group (no dietary intervention; n = 39) daily for 12 months. The three intervention groups replaced regular dairy products with the fortified dairy products. No other dietary changes were reported. Subjects were excluded if they had osteoporosis markers, were taking medications that modify bone metabolism, had chronic degenerative disease, smoked more than 5 cigarettes/day, calcium intake greater than 800 mg/day, or less than 5 years post menopause. Serum ucOC levels were measured as a marker of vitamin K status following the collection of fasting blood samples at baseline and 12 months.

Repeated measures ANOVA was used to evaluate differences between groups at baseline and follow up (p treatment effect) and the effect of treatment x time interaction (p treatment x time) for serum ucOC concentration. All p values were two-tailed and at a statistical

significance level of $p \leq 0.05$.

Dietary intake of vitamin K_1 and MK-7 increased in the vitamin K_1 and MK-7 intervention groups respectively compared to control and reference groups with supplementation ($p = 0.001$). Vitamin K_1 intake did not increase in the MK-7 group after 12 months. Serum ucOC concentration decreased by 23.6% (95% confidence interval (CI): [-56.4, 9.2]) and 13.3% (95% CI: [-44.8, 18.3]) compared to baseline for the MK-7 and vitamin K_1 groups while the concentration increased by 12.8% (95% CI: [18.8, 44.3]) and 28.7% (95% CI: [2.9, 54.5]) in the control and reference groups respectively. However a significant treatment x time effect was observed for ucOC, which were significantly lower in the vitamin K_1 and MK-7 intervention groups compared to both control and reference groups ($p < 0.05$).

One study limitation was that 173 women were recruited but supplementation compliance for any of the key nutrients was less than 75% for 58 participants (final sample size of 115). Analysis was performed on the per-protocol population. The authors report that appropriate sample size was still achieved with statistical power > 90% and Type I error probability < 0.05.

The authors concluded that supplementation with vitamin K in postmenopausal women can significantly reduce serum levels of ucOC.

Randomised double-blind controlled parallel study on the functionality of synthetic and fermentation-derived MK-7 in healthy adults (Møller et al. 2016)

A randomised, double-blind, five-arm parallel study using 46 healthy adults (30 women and 16 men, mean age 28.0 ± 2.15 (SD) years) from Norway was undertaken in which participants received a 45 µg (n = 10), 90 µg (n = 11) or 180 µg (n = 9) capsule of synthetic MK-7, a 90 µg (n = 11) capsule of fermentation-derived MK-7, or a placebo (n = 5) daily with breakfast that was requested to contain some fat for 43 days. Subjects were excluded if they used vitamin K supplements or antagonists, had chronic disease or long-term drug treatment, were pregnant or lactating, had a history of drug abuse, or high levels of C-reactive protein, creatinine, alanine aminotransferase, or total cholesterol. Serum MK-7, cOC and ucOC concentrations were measured from blood samples collected on day 1, 4 (MK-7 only), 8, 22, 36, and 43(+2) before dosing. Differences between $AUC_{(1–43d)}$ (calculated using the linear trapezoidal rule) for each MK-7 dose were compared to placebo using the Wilcoxon two-sample test. Changes in cOC and ucOC from baseline to day 43 for each MK-7 dose were compared using the Wilcoxon signed rank test.

Supplementation with synthetic MK-7 generally resulted in a dose-dependent increase in serum MK-7 concentration. MK-7 $AUC_{(1–43d)}$ was 17.3 ± 3.5 (SE), 64.1 ± 20.3, 125.6 ± 48.6, 159.6 ± 28.6, and 111.8 ± 36.9 ng-h/mL for placebo, 45, 90, 180 µg synthetic MK-7 and 90 µg fermentation-derived MK-7 groups respectively. The difference versus placebo for all treatments was statistically significant ($p = 0.035$, 0.027, 0.035 and 0.031 for the 45, 90, 180 µg synthetic MK-7 and 90 µg fermentation-derived MK-7 groups respectively). The difference between $AUC_{(1–43d)}$ for 90 µg synthetic and fermentation-derived MK-7 was not statistically significant ($p = 0.703$). The steady-state MK-7 serum concentration for both 90 µg groups was approximately 2.5 ng/mL (~0.03 ng/mL/µg MK-7 consumed).

Serum cOC concentration increased by 29% from baseline to day 43 in the 180 µg synthetic MK-7 group ($p = 0.021$). Non-statistically significant increases were observed in other treatment groups. Serum ucOC concentrations decreased from baseline by 21% ($p = 0.021$) and 29% ($p = 0.013$) in the 90 µg and 180 µg synthetic MK-7 groups respectively. Non-statistically significant decreases were observed in other treatment groups. The differences in cOC and ucOC levels from baseline to day 43 were not statistically significant between the 90 µg synthetic and 90 µg fermentation-derived MK-7 groups.

The authors concluded that the synthetic MK-7 is bioequivalent to fermentation-derived MK-7 with respect to absorption, and that both forms have a similar ability to increase osteocalcin carboxylation compared to placebo.

Randomised double-blind placebo controlled trial of MK-7 supplementation on osteocalcin carboxylation in healthy prepubertal children (Van Summeren et al. 2009)

A randomised, double-blind, placebo-controlled parallel study using 55 healthy prepubertal children (33 females and 22 males, mean age 8.3 ± 1.2 (SD) years) of normal height and weight from the Netherlands was undertaken in which they ingested either a capsule of 45 µg MK-7 (n = 28) or placebo (n = 27) daily for eight weeks with dinner. Participants maintained a normal diet for the duration of the study. Participants were excluded if they had current or previous metabolic, gastrointestinal or chronic inflammatory disease or were taking systemic corticoid treatments, vitamin K supplements or anticoagulants. Serum MK-7, ucOC and cOC levels were measured following collection of non-fasting blood samples at 0 and 8 weeks. Differences from baseline to follow-up within each group were examined with Wilcoxon signed-rank tests, and differences between the placebo and treatment groups were examined with Mann-Whitney tests.

Following supplementation, serum MK-7 concentration increased in the MK-7 group by 328.5% ($p < 0.001$) but decreased by 7.1% in the placebo group ($p = 0.244$). Serum ucOC concentration decreased by 25.6% in the MK-7 group ($p < 0.001$) but increased in the placebo group by 12.2% ($p = 0.228$). Serum cOC increased in the MK-7 and placebo groups by 12.2% ($p = 0.067$) and 1.2% ($p = 0.657$), but this was not a significant change for either group. The ucOC:cOC ratio decreased in the MK-7 group by 33.3% ($p < 0.001$) but remained unchanged in the placebo group (0.0%, $p = 0.451$). The change between the treatment and placebo groups was significant for MK-7, ucOC, and ucOC:cOC ($p = 0.001$, < 0.001, and < 0.001 respectively), but was not significant for cOC ($p = 0.067$).

The authors concluded that daily supplementation with 45 µg MK-7 improves vitamin K status in healthy prepubertal children.

Randomised, double-blind, placebo controlled parallel study of 3 year MK-7 supplementation on arterial stiffness and bone loss markers in healthy postmenopausal women (Knapen et al. 2013; Knapen et al. 2015)

A randomised, double-blind, placebo-controlled two-arm parallel study using 244 healthy postmenopausal women (mean age 59.5 ± 3.3 (SD) years) from the Netherlands was undertaken in which subjects received a capsule containing 180 µg MK-7 (n = 120) or a placebo (n = 124) daily for three years with either breakfast or dinner, with no additional dietary restrictions during the intervention period. Exclusion criteria included being less than 2 years post menopause, having a high body mass index (BMI, >30 kg/m^2), osteoporosis, coagulation disorder, chronic, metabolic or gastrointestinal disease, taking medication that affects vitamin K or coagulation or vitamin K supplements, and taking corticosteroids, bisphosphonates or hormone replacement therapy. Data was collected on multiple biomarkers of arterial stiffness and bone loss including the markers for vitamin K status plasma dp-ucMGP (Knapen et al. 2015) and serum ucOC and cOC (Knapen et al. 2013). Fasting blood samples were collected annually. The ucOC:cOC ratio was not normally distributed and was log-transformed prior to analysis. Values are presented as mean and SD.

After one year of supplementation, plasma dp-ucMGP concentrations decreased significantly compared to placebo (-192 ± 137 (SD) versus +48 ± 157 pmol/L, $p < 0.0001$). Similar results were observed at three years (-188 ± 157 versus +74 ± 182 pmol/L, $p < 0.0001$). After one

year of supplementation, serum ucOC decreased (-48 ± 27% versus +11 ± 56%)[6] and cOC increased (+19 ± 18% versus -1 ± 12%)[5] significantly compared to placebo ($p < 0.0001$). Similar results were observed at three years for ucOC (-51 ± 21% versus +4 ± 49%, $p < 0.001$) and cOC (+21 ± 19% versus +3 ± 16%, $p < 0.001$), with an improvement to the ucOC:cOC ratio compared to placebo (+58 ± 18% versus +2 ± 47%, $p < 0.001$).

One study limitation was that 244 women were recruited to the study but 21 participants did not complete the study (9 in treatment and 12 in placebo groups). Analysis was conducted on the intention-to-treat population in Knapen et al. 2015 and was not reported in Knapen et al. 2013.

The authors concluded that long-term daily MK-7 supplementation significantly improves osteocalcin and matrix Gla protein carboxylation compared to placebo.

Randomised double-blind placebo controlled trial on effect of menaquinone-7 supplementation on dephosphorylated uncarboxylated matrix Gla protein concentration and osteocalcin carboxylation (Dalmeijer et al. 2012)

A randomised, double-blind, placebo-controlled three-arm parallel study using 60 healthy men and postmenopausal women (24 men and 36 women, mean age 59.5 ± 3.0 (SD) years) from the Netherlands was undertaken in which subjects received either 360 µg MK-7 (n = 18), 180 µg MK-7 (n = 22), or a placebo (n = 20) daily with dinner for 12 weeks. Participants maintained usual food consumption habits for the study duration. Participants were excluded if they took vitamin K antagonists or cardiovascular disease medication, used hormone replacement therapy, had a history of coagulation issues, were a current smoker, vegan or had more than 90 µg/day vitamin K_2 intake. Plasma dp-ucMGP and serum ucOC:cOC ratio were measured as markers of vitamin K status following collection of fasting blood samples at 0, 4 and 12 weeks. A linear mixed model was used to estimate the effect of MK-7 supplementation and biomarker outcomes with treatment arm as the between-subjects factor and time as the within-subjects factor.

Baseline dp-ucMGP concentration in the 360 µg, 180 µg and placebo groups was 391 ± 26 (SE), 401 ± 27 and 448 ± 27 pmol/L. The dp-ucMGP concentration in the 360 µg, 180 µg and placebo groups was 209 ± 25, 294 ± 27 and 452 ± 27 pmol/L at four weeks and 210 ± 23, 276 ± 27 and 462 ± 27 pmol/L at 12 weeks. The baseline ucOC:cOC ratios in the 360 µg, 180 µg and placebo groups were 0.42 ± 0.05, 0.44 ± 0.05 and 0.47 ± 0.05 respectively. The ucOC:cOC ratios in the 360 µg, 180 µg and placebo groups were 0.12 ± 0.05, 0.21 ± 0.05 and 0.47 ± 0.05 after four weeks and 0.11 ± 0.05, 0.19 ± 0.05 and 0.53 ± 0.05 after 12 weeks. Changes over time between all treatment arms for both biomarkers were significant ($p < 0.001$).

The authors concluded that supplementation with MK-7 for 12 weeks can improve carboxylation of matrix Gla protein and osteocalcin in healthy adults.

Pilot randomised double-blind controlled study on the effect of MK-7 dose to increase carboxylation of osteocalcin and matrix Gla protein in healthy adults (Theuwissen et al. 2012)

A randomised, double-blind, placebo-controlled parallel study using 42 healthy men (n = 20) and women (n = 22) (mean age 28 ± 7 (SD) years) from the Netherlands was undertaken in which six subjects per group received either a capsule of 10, 20, 45, 90, 180 or 360 µg MK-7 or placebo daily with breakfast or dinner for twelve weeks. Study subjects were requested to consume what the authors defined as normal amounts of green vegetables (< 200 g/day), and curd cheese (< 50 g/day) and no natto during the study, and to abstain from consuming

[6] Mean value and SD (calculated from SE) extracted using webplotdigitizer, WebPlotDigitizer (utk.edu)

these foods or alcohol for 24 hr prior to blood sampling. Subjects were excluded if they had coagulation disorders, metabolic, chronic or gastrointestinal disease, used vitamin K supplements or medications that interfere with vitamin K or coagulation, or had a high BMI (> 30 kg/m^2). Serum ucOC and cOC and plasma MK-7, vitamin K_1 and dp-ucMGP concentrations were measured following collection of fasting blood samples on day 0, 1, 3, 7, 14, 28, 42, 56, 70, and 84. The Mann-Whitney test was used to assess differences in circulating MK-7 concentrations.

Data were reported as median and range, indicating that data may have been non-normally distributed. At 12 weeks plasma vitamin K_1 concentrations did not differ significantly between intervention groups. Serum MK-7 concentration in most cases increased with increasing dose (0.4 (range 0.0 - 0.5), 0.6 (0.4 - 2.1), 0.5 (0.0 - 1.6), 1.4 (0.4 - 4.6), 1.5 (0.5 - 4.2), 3.4 (1.1 - 6.5) and 5.5 (4.1 - 10.6) ng/mL in the placebo, 10, 20, 45, 90, 180, and 360 µg MK-7 groups respectively). Serum concentration changes were statistically significant compared to placebo for doses ≥ 90 µg/day MK-7 ($p < 0.008$).

At 12 weeks there was wide variation in the data for biomarker values at each MK-7 dose with no significant results reported for disaggregated data. ucOC concentration generally decreased with increasing MK-7 doses (360 µg MK-7 was lower compared to placebo (0.7 (0.3 - 2.2) versus 3.7 (1.8 - 10.1) ng/mL)), cOC concentration was similar across all doses including 360 µg MK-7 compared to placebo (6.7 (3.5 – 13.8) versus 5.2 (2.2 – 8.3) ng/mL), and dp-ucMGP generally decreased with increasing doses (360 µg MK-7 lower compared to placebo (171 (144 - 198) versus 508 (271 - 841) pmol/L)).

The authors concluded that three months of daily supplementation with ≥ 90 µg/day of MK-7 improves MK-7 concentration compared to placebo and while not statistically significant there is a general trend for decreasing concentrations of ucOC and dp-ucMGP with increasing MK-7 doses.

Randomised double-blind placebo-controlled trial of vitamin K_2 supplementation on bone loss in healthy early menopausal women (Emaus et al. 2010)

A randomised, double-blind, placebo-controlled parallel study using 334 healthy early menopausal women (mean age 54.5 ± 2.5 (SD) years) from Norway was undertaken in which participants received 360 µg/day MK-7 as natto extract capsules (n = 167) or placebo (n = 167) daily with meals for 12 months. No other dietary changes were reported. Subjects were excluded if they were more than 5 years past menopause, used anticoagulants, bone remodelling medications or hormone replacement therapy. Serum cOC and ucOC concentrations were measured as markers of vitamin K status following collection of non-fasting blood samples at 0 and 12 months.

Baseline serum cOC concentrations were 13.50 (95% CI: [12.48, 14.52]) and 13.20 (95% CI: [12.20, 14.20]) ng/mL in the treatment and placebo groups. At 12 months, serum cOC concentration increased in the treatment group compared to placebo (19.06 ng/mL (95% CI: [18.04, 20.08] versus 14.90 (95% CI: [13.98, 15.82]). Baseline serum ucOC concentrations were 4.14 (95% CI: [3.69, 4.59]) and 4.11 (95% CI: [3.70, 4.51]) ng/mL in the treatment and placebo groups. Serum ucOC concentration decreased in the treatment group compared to placebo (2.22 ng/mL (95% CI: [1.90, 2.55] versus 3.87 ng/mL (95% CI: [3.46, 4.28]). Independent sample *t* test was used to measure differences in serum baseline-corrected cOC and ucOC concentrations between groups, with serum cOC concentration increasing by 3.8 ng/mL (95% CI: [2.6, 5.1]; $p < 0.001$) and ucOC concentration decreasing by 1.8 ng/mL (95% CI: [-2.4, -1.2]; $p < 0.001$) in the MK-7 group compared to placebo.

One study limitation was that 35 out of the 334 women recruited into the study did not complete the study (21 in treatment and 14 in placebo groups). Biomarker analyses were

performed on participants with two valid repeated measurements (per-protocol population).

The authors concluded that 12 months of MK-7 supplementation resulted in a statistically significant increase in cOC concentration and decrease in ucOC concentration compared to placebo, indicating that the supplement was effectively absorbed and active.

Non-randomised dose-escalation study of MK-7 enriched olive oil on osteocalcin carboxylation in healthy adults (*Brugè et al. 2011)*

A non-randomised dose-escalation study using 12 healthy male (n = 4) and female (n = 8) adults (aged 37 ± 3 (SD) years) was undertaken in Italy in which participants consumed 20 mL extra virgin olive oil enriched with vitamins E, B_6 and CoQ_{10} daily for two weeks (day 1 to 14; placebo), and the same oil containing 45 µg MK-7 from day 15 to 28. This was followed by a washout period from day 29 to 42, and oil containing 90 µg MK-7 was consumed from day 43 to 56. Participants were requested to limit their intake of vitamin K-rich foods for the duration of the study. Plasma MK-7, cOC and ucOC concentrations were measured after the collection of fasting blood samples at five time points between experimental phases. No further details on sample collection were provided. Student's *t* test was used to evaluate the difference between means at study entry and with each intervention.

Supplementation with oil containing 45 or 90 µg MK-7 significantly increased plasma MK-7 concentrations compared to baseline (1.28 ± 0.24 (SD), 2.47 ± 0.23 and 0.42 ± 0.17 ng/mL respectively, $p < 0.001$). Plasma cOC concentration increased in both MK-7 arms compared to placebo, reaching statistical significance in the high dose arm only (16.9 ± 2.2 (SE), 21.1 ± 2.8 ($p \leq 0.01$) and 17.0 ± 2.2 ng/mL in the low, high dose and placebo groups respectively). Similarly, plasma ucOC concentration decreased in the two MK-7 arms compared to placebo, with the high dose arm significantly greater than placebo (2.15 ± 0.38 (SE), 2.05 ± 0.33 ($p \leq 0.05$) and 2.49 ± 0.39 ng/mL in the low, high dose and placebo groups respectively). The cOC:ucOC ratio increased with increasing doses of MK-7, also reaching statistical significance in the high dose group (9.96 ± 2.01 (SE), 13.5 ± 2.85 ($p \leq 0.01$) and 8.54 ± 1.59 in the low, high dose and placebo groups respectively).

The authors concluded that daily supplementation with MK-7 significantly increased plasma MK-7 concentration. However, only higher doses of MK-7 increased osteocalcin carboxylation.

3.5 Discussion and conclusion

Vitamin K functions as a co-factor for the carboxylation of glutamyl residues in VKDPs. Vitamin K_1 (as phylloquinone) is currently permitted to be added to certain foods, including FSMP. Several studies have noted that MK-7 performs the same function as a co-factor in the vitamin K cycle as vitamin K_1 (Schurgers et al. 2007; Shearer and Newman 2008; Rishavy and Berkner 2012; Beulens et al. 2013; Simes et al. 2020; Cirilli et al. 2022).

To determine whether MK-7 is equivalent to vitamin K_1 as a source of vitamin K, FSANZ considered the body of evidence on the effect of MK-7 supplementation on serum or plasma MK-7 concentrations and on biomarkers of vitamin K status. Eleven human studies measured the effect of oral MK-7 supplementation (as food, fortified food or oil, or in capsule/tablet form) in healthy individuals (male children aged 6 to 10 years and male and female adults with mean ages ranging from 28 to 62 years) on MK-7, cOC, ucOC, ucOC:cOC ratio, or dp-ucMGP concentration in blood fractions compared to placebo, pre-dose levels, or varying concentrations of MK-7 (Schurgers and Vermeer 2000; Schurgers et al. 2007; van Summeren et al. 2009; Emaus et al. 2010; Brugè et al. 2011; Dalmeijer et al. 2012;

Kanellakis et al. 2012; Theuwissen et al. 2012; Knapen et al. 2013; Knapen et al. 2015; Møller et al. 2016). Three studies also compared the absorption of MK-7 to vitamin K_1 (Schurgers and Vermeer 2000; Schurgers et al. 2007; Kanellakis et al. 2012), with the latter two studies also comparing the effect of MK-7 and vitamin K_1 on biomarkers. The duration of supplementation ranged from two weeks to three years, with two studies measuring MK-7 concentrations at several time points up to 72 hr or 96 hr following supplementation (Schurgers and Vermeer 2000; Schurgers et al. 2007).

Five studies examined serum or plasma MK-7 concentrations in healthy populations after oral supplementation (Schurgers et al. 2007; van Summeren et al. 2009; Brugè et al. 2011; Theuwissen et al. 2012; Møller et al. 2016). Duration of supplementation varied from two to twelve weeks. MK-7 concentration in blood fractions[7] increased compared to placebo or baseline in all studies and were significantly greater than placebo or baseline for doses ≥ 45 µg in three studies (van Summeren et al. 2009; Brugè et al. 2011; Møller et al. 2016) and for doses ≥ 90 µg/day in one study, however some limitations in the study reporting were noted (Theuwissen et al. 2012).

Nine human studies measured the effect of oral MK-7 supplementation on the carboxylation of osteocalcin, measuring ucOC concentration, with eight studies also measuring either cOC or osteocalcin ratio (Schurgers et al. 2007; van Summeren et al. 2009; Emaus et al. 2010; Brugè et al. 2011; Dalmeijer et al. 2012; Kanellakis et al. 2012; Theuwissen et al. 2012; Knapen et al. 2013; Knapen et al. 2015; Møller et al. 2016). Blood ucOC concentration decreased in all studies following doses of 45 to 360 µg/day with statistically significant differences reported in six studies at doses of 45 µg/day in children and 90 to 360 µg/day in adults (van Summeren et al. 2009; Emaus et al. 2010; Brugè et al. 2011; Kanellakis et al. 2012; Knapen et al. 2013; Møller et al. 2016). Similarly, cOC concentration and osteocalcin ratio improved in seven studies at 90 to 360 µg/day (45 µg in children), reaching statistical significance in five studies at doses of 90 µg/day (Brugè et al. 2011) or 180 to 360 µg/day (Emaus et al. 2010; Dalmeijer et al. 2012; Knapen et al. 2013; Møller et al. 2016). In children, only the ucOC:cOC ratio change was statistically significant at 45 µg/day (van Summeren et al. 2009). Blood dp-ucMGP concentration decreased in all three studies with a statistically significant difference in two studies at doses of 180 and 360 µg/day (Dalmeijer et al. 2012; Knapen et al. 2015).

Three human studies compared the effects of MK-7 supplementation to vitamin K_1 supplementation on serum MK-7 concentrations and vitamin K status biomarkers (Schurgers and Vermeer 2000; Schurgers et al. 2007; Kanellakis et al. 2012). Two studies reported that similar molar doses of MK-7 or vitamin K_1 increased serum vitamin K concentrations, with maximum serum concentration (C_{max}) ~10x greater and total systemic exposure (24 hour and 96 hour area under the curve) 2.5x and 6x greater respectively following MK-7 supplementation (Schurgers and Vermeer 2000; Schurgers et al. 2007). MK-7 and vitamin K_1 serum concentrations peaked 4 to 6 hr after consumption. Two studies reported greater osteocalcin carboxylation following lower or equimolar doses of MK-7 compared to vitamin K_1 supplementation (Schurgers et al. 2007; Kanellakis et al. 2012).

The applicant requests the use of MK-7 as a source of vitamin K in FSMP. FSMP are provided under the supervision of a medical practitioner to manage the diet of people with certain disorders, medical conditions, or diseases (FSANZ 2016). The body of evidence to support the effect of MK-7 on serum or plasma concentrations and biomarkers of vitamin K status in humans relates to healthy individuals. However, in cases where vitamin malabsorption is suspected, FSMP formulations can be modified by the supervising medical practitioner. FSANZ notes that FSMP are not therapeutic goods used to treat or cure medical

[7] Reported as mean difference in all studies except Theuwissen et al. 2012, which was reported as median and range

conditions.

The doses of MK-7 used in human trials ranged from 10 µg/day to 2012 µg/day for absorption studies and 10 to 360 µg/day for biomarker studies. A dose of 90 µg/day MK-7 is equivalent on a molar basis to 62.5 µg/day of vitamin K_1, which is close to the Adequate Intake for women (60 µg/day) and men (70 µg/day). Because of a difference in the molar mass of MK-7 and vitamin K_1 consumption of the Adequate Intake (AI) of vitamin K as MK-7 would result in a lower intake compared to vitamin K_1. FSANZ did not identify any studies in adults that compared the bioavailability of MK-7 and vitamin K_1 at doses that are equivalent to the Adequate Intake for vitamin K_1 (60 µg/day for women and 70 µg/day for men). Therefore no conclusions can be made regarding whether the lower intake would support essential requirements if it was the only form of vitamin K in the diet, however FSMP are used under the supervision of a medical practitioner and can be modified as required.

No studies were identified by FSANZ to indicate that MK-7 supplementation would inhibit the absorption of other nutrients.

4 Safety assessment

4.1 Objectives of the safety assessment

The objectives of the safety assessment were to:

- Evaluate any potential health and safety concerns that may arise from the use of MK-7 as a form of vitamin K in FSMP.
- Determine the maximum safe level of intake of MK-7, if any, for all ages/sub-populations.
- Determine what side effects may be expected from supplementation of FSMP with MK-7.

The applicant submitted evidence on the safety of MK-7, including toxicity studies in laboratory animals, human clinical studies and exposure to Vitamin K_2 from other dietary sources. Studies with the structurally related vitamin K_2 homologue MK-4 were also provided as supporting evidence. A literature search was also conducted to identify any additional relevant information. The data are considered suitable to assess the safety of MK-7.

4.2 History of safe consumption

As noted in the nutrition assessment, there is a history of consumption of MK-7 from the diet. Dietary intake of MK-7 is primarily from bacteria-fermented foods including some cheeses and the fermented soybean product natto. MK-7 has also been found in margarine and thickened cream purchased from Australian supermarkets (Palmer et al. 2021). Menaquinones including MK-7 are also produced endogenously by intestinal bacteria.

4.3 Toxicological data

Three papers reporting the results of a series of toxicity and genotoxicity studies of MK-7 are available in the scientific literature (Pucaj et al. 2011; Ravishankar et al. 2015; Hwang et al. 2024). The test item in the studies by Ravishankar et al. was MK-7 produced by the applicant (MenaquinGold, Viridis Biopharma Pvt Ltd, Mumbai, India, purity not reported). The test item in the studies by Pucaj et al. was synthetic all-trans MK-7 (purity 95 – 75%; produced by Synthetica AS, Oslo, Norway). The studies by Hwang et al. were conducted with MK-7 (purity > 96%) extracted from *Bacillus subtilis* var. natto culture, produced through fermentation and subsequent purification (MediQ7, GF Fermentech, South Korea).

Kinetics and metabolism of MK-7 are reviewed in section 3.

Acute toxicity of MK-7 in animals

Acute toxicity study of MK-7 in BomTac:NMRI mice (Pucaj et al. 2011) Regulatory status: performed in line with GLP principles; conducted in accordance with OECD TG 425

Five female BomTac:NMRI mice were administered a single dose of 2000 mg/kg bw MK-7 by oral gavage. The vehicle was sunflower oil. Two untreated mice were also included as a reference. Clinical signs were monitored for 14 days following treatment and body weights were recorded. Mice were killed at the end of the study. It is not reported whether mice underwent gross pathological examination.

All animals survived to the end of the 14-day observation period. No clinical signs of toxicity were observed and body weight gain was not adversely affected. It was concluded that the oral median lethal dose (LD50) of MK-7 in BomTac:NMRI mice is > 2000 mg/kg bw.

Acute oral toxicity study of MK-7 in Wistar rats (Ravishankar et al. 2015) Regulatory status: GLP status not reported; conducted in compliance with test laboratory's modification of OECD Test Guidelines

Fasted Albino Wistar rats (4/sex) were administered a single dose of 2000 mg/kg bw MK-7 in propylene glycol by oral gavage and monitored for 14 days. Body weights and body weight gain were recorded. At the end of the study animals were killed and subjected to gross necropsy.

All animals survived to the end of the study. No treatment-related effects on clinical signs, body weight, body weight gain or gross pathology observations were reported. It was concluded that the oral LD_{50} of MK-7 in Wistar rats is > 2000 mg/kg bw.

Acute oral toxicity study of MK-7 in Sprague-Dawley rats (Hwang et al. 2024) Regulatory status: GLP; conducted in accordance with OECD TG 420

Five fasted female Sprague-Dawley rats were administered 5000 mg/kg bw MK-7 in medium chain triglyceride (MCT) oil by oral gavage and monitored for 14 days. Body weights were recorded. At the end of the study animals were subjected to gross necropsy.

All animals survived to the end of the study and no clinical signs of toxicity were recorded. There were no adverse effects on body weight and no abnormal findings were observed at necropsy. It was concluded that the acute oral LD_{50} of MK-7 in Sprague-Dawley rats is > 5000 mg/kg bw.

Short-term toxicity of MK-7 and MK-4 in animals

14-day oral toxicity study of MK-7 in Wistar rats (Ravishankar et al. 2015) Regulatory status: GLP status not reported; No guideline reported

MK-7 was administered to fasted Wistar rats (4/sex/group) by oral gavage at doses of 0, 0.5, 1.0, 10 or 20 mg/kg bw/day for 14 days. The vehicle control was propylene glycol. Animals were monitored for clinical signs. At the end of the study animals were killed and examined for gross changes in vital organs.

No mortality occurred during the study and no clinical signs of toxicity were observed. Mild irritability was noted in 2/8 animals given 1.0 mg/kg bw/day, but was not seen in the higher dose groups. There were no treatment-related effects on body weight gain, water or food consumption. The no observed adverse effect level (NOAEL) in this study was 20 mg/kg bw/day, the highest dose tested.

28-day toxicity study of MK-7 in Sprague-Dawley rats (Hwang et al. 2024); Regulatory status: GLP; No guideline reported

Groups of five male and five female Sprague-Dawley rats (age approximately 7 – 8 weeks) were administered 0, 500, 1500 or 4500 mg/kg bw/day MK-7 by oral gavage for 28 days. The vehicle was MCT oil. An additional negative control group (administered water) was included to investigate any potential changes caused by the vehicle. Clinical signs, body weight and food consumption were recorded. At the end of the study blood samples were collected for haematology and clinical chemistry. Animals were killed, organ weights were determined and animals were examined for gross pathological changes.

No mortalities were recorded. There were no treatment-related effects on clinical signs, body weight, food consumption, haematology, clinical chemistry, organ weights or gross pathology observations. The NOAEL in this study was 4500 mg/kg bw/day, the highest dose tested.

90-day toxicity study of MK-7 in Wistar rats (Ravishankar et al. 2015) Regulatory status: GLP status not reported; conducted to a protocol modified from OECD TG 408

MK-7 was administered to Wistar rats (age 8 – 9 weeks; 5/sex/group, rather than 10/sex/group as recommended in OECD TG 408) by oral gavage for 90 days. Doses tested were 0, 0.1, 0.5 or 1 mg/kg bw/day. The vehicle control was propylene glycol. Mortality, clinical signs, body weight and food consumption were recorded. Blood and urine samples were collected on study days 15, 45 and 91 for haematology, clotting time, clinical chemistry and urinalysis. Animals were killed on day 91, organ weights were recorded and histopathology examination of the brain, pituitary, thymus, lymph node, heart, lungs, spleen, seminal vesicles, uterus, skin, trachea, liver, stomach, jejunum, kidney, testis, prostate and ovary was performed.

All animals survived to the end of the study. There were no treatment-related effects on clinical observations, body weight, food consumption, organ weights, haematology, clotting time, clinical chemistry and urinalysis parameters. No adverse histopathological changes were reported in treated animals. Histopathological analysis indicated an increase in the size and number of mature follicles in the ovaries at 0.5 and 1 mg/kg bw/day females compared with controls. In the uterus, increased size of the muscular layer and proliferation of the epithelial layer were observed at 0.5 and 1 mg/kg bw/day. In males, increased spermatogenesis in the testis was observed at 0.5 and 1 mg/kg bw/day compared with controls. These changes were not considered adverse by the study authors.

The NOAEL in this study was 1 mg/kg bw/day, the highest dose tested.

90-day toxicity study of MK-7 in Sprague-Dawley rats (Pucaj et al. 2011) Regulatory status: GLP; conducted in accordance with OECD TG 408

MK-7 was administered to Sprague-Dawley rats (age 7 – 8 weeks; 10/sex/group) at doses of 0, 2.5, 5 or 10 mg/kg bw/day for 90 days by oral gavage. Corn oil was used as the vehicle control. Additional recovery groups of five male and five female rats were administered 0 or 10 mg/kg bw/day MK-7 for 90 days followed by a treatment-free period of 31 days. The high dose was selected based on anticipated daily doses in humans (typically 25 – 100 µg/day). Clinical signs, body weights and food consumption were monitored throughout the study. A functional observational battery (FOB) assessment of motor activity, grip strength and sensory activity to visual, auditory and proprioceptive stimuli was conducted on all animals during the last week of dosing and on recovery animals during the last week of the study. Ophthalmoscopy was performed on all animals prior to treatment and on study day 87. Blood samples were collected from 5 rats/sex/group on study day 44 for haematology and clinical chemistry analysis. Blood samples were also collected from all animals on the day of necropsy for analysis of haematology, coagulation and clinical chemistry parameters. Urinalysis samples were collected overnight during the last weeks of dosing and recovery. The main study animals were killed on day 91 (males) or 92 (females) and recovery animals were killed on day 31 of the recovery period. Animals were subjected to gross necropsy and organ weights were recorded. Histopathology was performed on tissues from control and high-dose animals, as well as tissues with abnormal findings in the low- and mid-dose groups.

All animals survived to the end of the study and no clinical signs of toxicity were observed. There were no treatment-related effects on body weight, food consumption or ophthalmological examinations. No adverse effects in the FOB assessments were reported. There were no treatment-related effects on haematology, coagulation, clinical chemistry or urinalysis parameters. A significant increase in activated partial thromboplastin time (APTT) was observed in mid- and high-dose group females compared with controls at the end of the dosing period. This finding was not considered treatment-related as it was attributed to increased APTT times in two females in the mid-dose group and a single female in the high-dose group, similar changes were not observed in males, and an increase in coagulation

time is inconsistent with the known role of vitamin K in blood coagulation. There were no treatment-related changes in organ weights, macroscopic or histopathological observations.

The NOAEL in this study was 10 mg/kg bw/day, the highest dose tested.

90-day toxicity study of MK-7 in Sprague-Dawley rats (Hwang et al. 2024); Regulatory status: GLP; Conducted in accordance with OECD TG 408

Groups of 10 male and 10 female Sprague-Dawley rats (aged 6 – 7 weeks) were administered MK-7 by oral gavage at doses of 0, 500, 1500 or 4500 mg/kg bw/day for 91 days. The vehicle control was MCT oil. An additional negative control group was administered water. Additional recovery groups (5/sex/group) for the negative control, vehicle control and high-dose groups were included. Clinical signs, body weight and food and water consumption were recorded. A FOB was performed after 11 weeks of treatment. Ophthalmology examinations were conducted in the negative, vehicle and high-dose groups prior to dosing, during the last week of administration and in the last week of the 28-day recovery period. Blood samples were collected for haematology, coagulation parameters and clinical chemistry analyses prior to necropsy. Urine samples for urinalysis were collected from 5 rats/sex/group during the last week of administration and the last week of the recovery period. At the end of the treatment or recovery period, animals were killed, organ weights recorded and gross pathology and histopathological examinations were performed. Sperm and vaginal smears were also examined. The report states that hormones were also assessed but only thyroxine is specifically mentioned in the paper.

All animals survived to the end of the study. Salivation, lower abdominal staining, soft stool, mucous stool and diarrhoea were observed in the vehicle control, low-dose and medium-dose groups, and were considered likely to have been caused by the vehicle. No treatment-related effects on body weight or body weight gain were observed. Feed consumption was decreased sporadically in the vehicle control and treatment groups, which was attributed to the vehicle. There were no treatment-related effects on FOB, ophthalmology, haematology, coagulation, clinical chemistry, hormone measurement, sperm examination, vaginal smear, organ weights, gross pathology or histopathology examinations. Prothrombin time was increased in the high-dose female recovery group, however as this was not observed in the main study animals and is not consistent with the known effects of vitamin K, it was not considered to be treatment-related.

The NOAEL in this study was 4500 mg/kg bw/day, the highest dose tested.

13-week oral toxicity study of MK-4 in Crj:CD(SD) rats (Doi et al. 1995) Regulatory status: GLP status and test guideline followed not reported

Groups of Crj:CD(SD) rats (age 5 weeks; 10/sex/group) were administered 0 or 30 mg/kg bw/day MK-4 by oral gavage for 13 weeks. The vehicle control was a 5% rubber arabic suspension in water. Clinical signs, body weight and food consumption were recorded. Ophthalmology examinations were performed on five males and five females from each group before treatment and on treatment day 78. At the end of the study blood samples were collected from all animals (fasted) for haematology and clinical chemistry analyses. Urinalysis samples were collected over a 4 hour period on the 25th and 85th days of dosing from 5 fasted rats/sex/group. At the end of the study animals were killed, organ weights recorded and organs and tissues subjected to gross and microscopic pathological examination.

All animals survived to the end of the study. There were no treatment-related effects on clinical signs, body weight, food consumption and ophthalmology. Statistically significant increased reticulocyte counts were observed in treated females compared with controls, and

increased platelet counts and calcium levels were observed in males. However the magnitude of these changes was small and within the range of physiological variability. No adverse effects were observed on urinalysis, organ weights, macroscopic or histopathological observations.

The NOAEL in this study was 30 mg/kg bw/day, the highest dose tested.

3-month oral toxicity study of MK-4 in dogs (Goldsmith and Keller 1995) Regulatory status: GLP status and test guideline followed not reported

Groups of male and female Beagle dogs (aged 23 – 30 weeks; number/sex/group not reported) were administered 0, 20, 200 or 2000 mg/kg bw/day MK-4 in gelatine capsules for three months. Two males and two females from the control and high-dose groups were maintained without dosing for a 1-month recovery period. Clinical signs and food consumption were monitored throughout the study. Ophthalmological examinations were performed on all animals on treatment days 31 and 90. Electrocardiograms (ECGs) were performed on all animals on weeks 7 and 13 of treatment, and in the final week of the recovery period. Blood and urine samples were collected from all animals on weeks 7 and 13, and before necropsy at the end of the recovery period. At the end of the study animals were killed, organ weights recorded and organs and tissues were subjected to macroscopic and microscopic pathological examination.

No deaths were observed during the study. The only treatment-related clinical sign was a yellow tinge in the coats of males and females given 2000 mg/kg bw/day. Body weight gain was reduced at the high dose in males during the first week of the study and in females during weeks 1 – 5. There were no treatment-related effects on ophthalmoscopy or ECG examinations. A significant increase in platelet counts was observed in high-dose males and females at 7 and 13 weeks of treatment compared with controls, and also following the recovery period. Males in the high dose group had decreased red blood cells, haemoglobin and haematocrit on weeks 7 and 13. The study authors considered this change was very slight, as no animals appeared anaemic in their general condition, and similar changes were not observed in the 12-month oral toxicity study in dogs. Females given 2000 mg/kg bw/day had significantly increased serum glutamate oxalate transaminase (SGOT), lactate dehydrogenase (LDH) and alkaline phosphatase levels on study weeks 7 and 13, but the magnitude of these changes (< 50%) was not considered of toxicological significance. These changes were no longer observed following the recovery period. Serum glutathione levels were reduced in mid- and high-dose females at the end of the treatment period, but not on week 7 or following the recovery period. Yellow striations were observed in the renal cortex of some animals in all treatment groups at necropsy at the end of the treatment period, and a yellow tone was observed in the intestinal contents and coat of the high-dose group animals. These changes were not observed following the treatment-free period. Uterus weights were significantly lower than controls in females given 2000 mg/kg bw/day at the end of the treatment period, but not following the recovery period. No adverse macroscopic or microscopic observations were recorded. Slight enlargement of the smooth endoplasmic reticulum was observed in the liver of males and females in the 200 and 2000 mg/kg bw/day.

The study authors concluded that the NOAEL in this study was 200 mg/kg bw/day, based on increased platelets in both sexes and reduced red blood cells in males at 2000 mg/kg bw/day. FSANZ notes that the changes observed at 2000 mg/kg bw/day were slight and not reproduced in the 12-month dog study with MK-4. However, as only limited study details are available in the paper reporting this study (e.g. number/sex/group not reported), it is not possible to robustly conclude that the NOAEL value should be revised.

Chronic toxicity/carcinogenicity studies in animals

No chronic toxicity or carcinogenicity studies of MK-7 were submitted in the application or located from other sources. Such studies are not considered to be necessary because the results of genotoxicity assays are negative (see section 4.3.5) and there is no evidence from subchronic studies of lesions that could lead to neoplasia through non-genotoxic mechanisms.

Several long-term toxicity studies of MK-4 are available and were reviewed as supporting information.

52-week dietary toxicity study of MK-4 in rats (Hosokawa et al. 1995) Regulatory status: GLP status and test guideline followed not reported

MK-4 was administered to F344/DuCrj rats (age 6 weeks; 20/sex/group) via the diet at concentrations of 0, 0.04, 0.2 or 1.0% (equivalent to 0, 20, 100 or 500 mg/kg bw/day) for 52 weeks. The vehicle control was Ceasol 228, and an additional untreated control group received only the basal diet. Clinical signs, body weight and food consumption were monitored throughout the study. Four animals/sex/group underwent ophthalmological examination before the study and on weeks 26 and 52 of treatment. Blood samples for haematology, coagulation and clinical chemistry parameters were collected from all animals at the end of the study. Urine samples were collected from all animals at 25 weeks and at the end of the treatment period. Animals were killed at the end of the study, organ weights recorded and pathological and histological examinations were performed on organs and tissues.

No deaths were observed in animals treated with MK-4. Food consumption was similar in all groups. Body weights were lower in high-dose females compared with vehicle controls, but were similar to the naïve controls. There were no treatment-related effects on ophthalmological examinations. Increased platelet counts were observed in treated females at all doses, with increases > 20% compared with vehicle controls in the mid- and high-dose groups. Increased platelet counts (~ 20%) were also observed in high-dose males. Significant, dose-related reductions in erythrocytes, haematocrit and haemoglobin were observed in mid- and high-dose females, but the study authors considered the reductions were slight (generally < 10%) (Table 2). Mean corpuscular volume was increased in treated females but no treatment-related changes in reticulocyte counts were observed.

Table 2. Selected haematology findings (mean ± standard error (SE)) in rats treated with MK-4 for 52 weeks (Hosokawa et al. 1995)

Dose (%)	**Red Blood Cells ($x10^4/mm^3$)**	**Haematocrit (%)**	**Haemoglobin (%)**	**Mean Corpuscular Volume (μm^3)**	**Platelets ($x10^4/mm^3$)**
	Males				
0 (vehicle control)	984.6 ± 19.3	49.22 ± 0.78	16.79 ± 0.11	50.09 ± 0.32	64.92 ± 1.64
0.04	981.3 ± 5.8	49.02 ± 0.27	16.50 ± 0.08*	49.97 ± 0.15	64.33 ± 2.04
0.2	921.5 ± 33.2	46.63 ± 1.67	15.93 ± 0.25**	50.64 ± 0.15	68.76 ± 2.23
1.0	932.3 ± 6.5*	47.64 ± 0.34	16.08 ± 0.09**	51.11 ± 0.18#	76.77 ± 1.37##
0 (naïve control)	990.6 ± 9.3	49.51 ± 0.42	16.47 ± 0.12	49.98 ± 0.25	61.66 ± 1.86

	Females				
0 (vehicle control)	874.9 ± 5.1	48.07 ± 0.25	16.29 ± 0.08	54.95 ± 0.11	56.57 ± 1.30
0.04	858.6 ± 4.7*	47.43 ± 0.23	16.12 ± 0.10	55.25 ± 0.10*	61.83 ± 1.29**
0.2	806.7 ± 9.8**	45.32 ± 0.73**	15.24 ± 0.32**	56.12 ± 0.34**	70.47 ± 2.21**
1.0	780.2 ± 5.0**	43.97 ± 0.27**	14.61 ± 0.09**	56.38 ± 0.15**	78.83 ± 1.41**
0 (naïve control)	861.8 ± 5.5	47.38 ± *	15.94 ± 0.12	54.98 ± 0.12	56.00 ± 1.26

* $p < 0.05$ compared with vehicle control (Welch t-test)
** $p < 0.01$ compared with vehicle control (Welch t-test)
# $p < 0.05$ compared with vehicle control (Student t-test)
$p < 0.01$ compared with vehicle control (Student t-test)

Dose-dependent decreases in prothrombin time were observed in males given ≥ 0.04% MK-4, and activated partial thromboplasin times were decreased in males at 0.2 and 1% (Table 3). Similar changes were not observed in females. No evidence of thrombus formation was observed and the study authors reported that the changes were considered to be within the 'normal range'.

Table 3. Coagulation parameters (mean ± SE) in male rats treated with MK-4 for 52 weeks (Hosokawa et al. 1995)

Dose (%)	Prothrombin time (seconds)	Activated partial thromboplastin time (seconds)
0 (vehicle control)	15.31 ± 0.39	28.41 ± 1.16
0.04	12.80 ± 0.17**	25.60 ± 1.04
0.2	12.69 ± 0.15**	23.02 ± 0.81##
1.0	12.53 ± 53**	21.48 ± 0.65##
0 (naïve control)	14.91 ± 0.34	25.09 ± 0.77#

** $p < 0.01$ compared with vehicle control (Welch t-test)
# $p < 0.05$ compared with vehicle control (Student t-test)
$p < 0.01$ compared with vehicle control (Student t-test)

No treatment-related effects on urinalysis parameters were observed. Increased absolute and relative liver weights were observed in females, but there were no accompanying histological changes.

Increased absolute and relative spleen weights were observed in both sexes at 0.2 and 1.0% MK-4. Histological changes in the spleen consisted of increased incidence and severity of extramedullary haematopoiesis and haemosiderin deposition in both sexes, starting from the lowest dose (Table 4). The study authors considered that as the spleen changes at 0.04% were not accompanied by changes in haematology parameters they were likely to be adaptive rather than adverse. Overall, the study authors considered that the effects on the erythrocyte system observed at ≥ 0.2% were mild and did not worsen over prolonged administration.

Table 4. Histopathological changes in rats treated with MK-4 for 52 weeks (Hosokawa et al. 1995)

Organ findings		Vehicle control	0.04%	0.2%	1.0%	Naïve control
Males						
Extramedullary haematopoiesis	Negative	13	2	0	0	15
	Slight	5	8	10	2	3
	Moderate	1	10	10	18	1
	Marked	0	0	0	0	0
Increase of haemosiderin	Negative	13	6	1	0	16
	Slight	5	12	12	4	2
	Moderate	1	2	4	11	0
	Marked	0	0	3	5	1
Females						
Extramedullary haematopoiesis	Negative	5	4	0	0	14
	Slight	11	8	5	1	4
	Moderate	4	8	15	17	2
	Marked	0	0	1	2	0
Increase of haemosiderin	Negative	5	2	1	0	12
	Slight	8	7	4	3	7
	Moderate	7	11	15	14	1
	Marked	0	0	0	3	0

The study authors concluded that 0.04% (equivalent to 20 mg/kg bw/day) represented a clear NOAEL for this study based on changes in haematology parameters, increased spleen weights and histopathological changes in the spleen at 0.2% (equivalent to 100 mg/kg bw/day). However, they concluded that no serious effect were observed up to 1.0% (equivalent to 500 mg/kg bw/day).

FSANZ notes that the haematological changes were relatively small and generally only reached a magnitude that may suggest an adverse effect at the highest dose. It is possible that the extramedullary haematopoiesis may represent an adaptive response to the reduced red blood cell counts. However, as a conservative approach FSANZ concurs with the NOAEL identified by the study author.

12-month oral toxicity study of MK-4 in dogs (Vanatta et al. 1995) Regulatory status: GLP status and test guideline followed not reported

Groups of six male and six female Beagle dogs (age 4 – 5 months) were administered 0, 20, 200 or 2000 mg/kg bw/day MK-4 in gelatine capsules for 12 months. Two animals/sex/group were randomly assigned to a 3-month recovery period following the end of treatment. Clinical signs, body weight and food consumption were recorded. Ophthalmological examinations were performed on all animals before and at 3, 6 and 12 months of treatment. ECGs were performed on all animals before treatment, at 3, 6 and 12 months of treatment and at 3 months after withdrawal of treatment. Blood and urine samples were collected for haematology, coagulation parameters, clinical chemistry and urinalysis analyses at 3, 6 and 12 months of treatment and at the end of the recovery period. At the end of the treatment or recovery period animals were killed, organ weights recorded and organs and tissues were subjected to macroscopic and microscopic examination.

No treatment-related deaths occurred during the study. One female in the high-dose group showed marked weight loss and mucous and haematochezia (blood in stool) and was killed after 41 weeks of treatment. Histopathological examination showed no relationship to treatment. Treatment-related clinical signs were limited to yellow faeces and yellow stains on the coats of high-dose animals. There were no treatment-related effects on ophthalmology,

ECG or haematology parameters. Increased reticulocytes were observed in high-dose females at 6 months and 12 months of treatment but there were no accompanying haematology or histopathological changes. No treatment-related effects were observed on coagulation parameters, clinical chemistry, urinalysis or organ weights. Gross necropsy findings were yellow body fat in two high-dose males at the end of the treatment period. There were no treatment-related microscopic changes.

The NOAEL in this study was 2000 mg/kg bw/day, the highest dose tested.

Genotoxicity of MK-7

In vitro studies

Bacterial reverse mutation assay with MK-7 (Ravishankar et al. 2015) Regulatory status: GLP status and Test Guideline not reported

The *Salmonella enterica* ser. Typhimurium strains TA1535, TA97a, TA98, TA100 and TA102 were exposed to MK-7 in triplicate cultures at concentrations of 0, 30, 60, 200, 600 or 2000 µg/plate in the presence or absence of metabolic activation (S9). The vehicle control was dimethyl sulfoxide. The plate incorporation method was followed. Concurrent positive controls (identity not reported) were also included.

No increase in the number of revertant colonies compared with vehicle control was observed following treatment with MK-7. The positive controls produced the expected response demonstrating the sensitivity of the test system. It was concluded that MK-7 was not mutagenic under the conditions of this assay.

Bacterial reverse mutation assay with MK-7 (Hwang et al. 2024) Regulatory status: GLP; conducted in accordance with OECD TG 471

Test strains used in this study were *S. enterica* ser. Typhimurium TA98, TA100, TA1535 and TA1537, as well as *Escherichia coli* WP2 *uvrA* (pKM101). Based on the results of a preliminary cytotoxicity assay, triplicate cultures were treated with MK-7 at concentrations of 0, 315.5, 625, 1250, 2500 or 5000 µg/plate in the presence or absence of metabolic activation (rat liver S9). The vehicle control was tetrahydrofuran. Positive control substances were 2-aminoanthracene or benzo[a]pyrene in the presence of S9 and 2-nitrofluorene (TA98), sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537) and Furylfuramide (WP2 *uvrA* (pKM101)). The study was conducted twice.

No cytotoxicity or precipitation of the test item were observed. There were no increases in the number of revertant colonies compared with vehicle controls in any strain following treatment with MK-7 in the presence or absence of metabolic activation. Significant increases in the number of revertant colonies were observed following treatment with the positive controls, confirming the validity of the test system. It was concluded that MK-7 was not mutagenic under the conditions of this study.

Chromosomal aberration test in mammalian cells (Hwang et al. 2024) Regulatory status: GLP; conducted in accordance with OECD TG 473

In preliminary cytotoxicity studies, MK-7 was administered to Chinese hamster lung cells at concentrations ranging from 31.3 – 2000 µg/mL. Short-term treatment assays (6 hours exposure) were performed in the presence and absence of S9 and a continuous treatment assay (24 hours exposure) was conducted in the absence of S9. No growth inhibition was observed in the preliminary studies, but precipitation was observed at concentrations ≥ 250 µg/mL in the short-term treatment groups and at ≥ 125 µg/mL in the continuous treatment

group.

Based on these findings concentrations of 62.5, 125 and 250 µg/mL MK-7 were tested in the short-term treatment assays with and without metabolic activation, and 31.3, 62.5 and 125 µg/mL MK-7 were tested in the continuous treatment assay without metabolic activation. A negative control (water) and vehicle control (acetone) were also included. Positive controls were benzo[a]pyrene and mitomycin C (MMC) in the presence and absence of S9, respectively. Two plates/concentration were evaluated.

Treatment with MK-7 did not result in any increase in the frequency of structural or numerical chromosomal aberrations compared with the negative or vehicle controls in the short-term or continuous treatment assays in the presence or absence of S9. The positive controls induced a significant increase in the frequency of structural chromosomal aberrations, confirming the validity of the test system. It was concluded that MK-7 was not clastogenic under the conditions of this assay.

In vivo studies

Combined chromosomal aberration, micronucleus and comet assay in Wistar rats (Ravishankar et al. 2015) Regulatory status: GLP status and Test Guideline not reported

Wistar rats (age 8 – 9 weeks) were divided into six groups of five males and five females. Two groups were administered 0.1 mg/kg bw/day MK-7 and two groups were administered 1 mg/kg bw/day MK-7 by oral gavage for 28 days. Two positive control groups were administered the vehicle (Tween-20; 2 drops/20 mL) for 28 days, then administered cyclophosphamide (40 mg/kg bw) intraperitoneally on day 29. One set of control and treated groups were used for a chromosomal aberration and micronucleus test, the other set were used for a comet assay. Animals maintained for the chromosomal aberration and micronucleus test were killed on day 30 and femur bones collected for analysis of chromosomal aberration and micronuclei. The control and test groups maintained for the comet assay were administered 4 mg/kg bw colchicine on study day 30 and blood was collected 2 hours later.

No clinical signs of toxicity were observed in MK-7-treated animals. There were no significant differences in the frequency of chromosomal aberrations or micronuclei, or in olive tail movement/cell between the groups administered 0.1 and 1 mg/kg bw/day MK-7. Positive control animals showed signs of clinical toxicity and genotoxicity. It was concluded that MK-7 was not genotoxic under the conditions of this study.

FSANZ notes that the design of this study did not follow OECD test guidelines and has a number of limitations including the lack of a negative control group. As such it is not suitable for regulatory purposes.

Mammalian micronucleus test in ICR mice (Hwang et al. 2024) Regulatory status: GLP; conducted in accordance with OECD TG 474

Groups of 5 male ICR mice were administered 0, 500, 1000 or 2000 mg/kg bw/day MK-7 by oral gavage for three days. Negative control and vehicle control groups were administered water or MCT oil, respectively. A positive control was administered 1 mg/kg bw/day MMC intraperitoneally. Animals were monitored for clinical signs and body weights were recorded. Animals were killed 24 hours after the final treatment and bone marrow samples were collected for analysis of frequencies of polychromatic erythrocytes (PCEs), normochromatic erythrocytes (NCEs) and micronucleated PCEs. Four thousand PCEs were scored for each animal.

No treatment-related effects on clinical signs or body weight were observed. Treatment with MK-7 did not result in an increase in the frequency of micronucleated PCEs compared with negative controls and vehicle controls. The frequency of micronucleated PCEs was significantly increased in the positive control group compared with the negative and vehicle controls. It was concluded that MK-7 was not clastogenic or aneugenic under the conditions of this study.

Developmental and reproductive studies in animals

No studies of developmental and reproductive toxicity of MK-7 are available. Several studies of MK-4 are available and were reviewed as supporting information.

Effects of MK-4 on development of fetuses and offspring in mice (Suzuki et al. 1971)
Regulatory status: Non-GLP, non-guideline

MK-4 was administered to groups of mated female ICR-JCL mice (18-25 per group) on gestation days (GD) 7 – 12 by oral gavage at doses of 0, 10, 500 or 1000 mg/kg bw/day. The vehicle control was cottonseed oil. Clinical signs and maternal food consumption were monitored. On GD 18, 16 – 18 mice per group were killed and the internal organs and uterine contents examined. Pups were weighed after removal and external morphology and the skeletons were examined for variations, abnormalities and the progress of osteogenesis. Groups of 7 – 8 mice treated with 0, 10 or 1000 mg/kg bw/day were allowed to calve spontaneously and post-natal development was monitored. One neonate/sex from each group was selected during the weaning period (21 days post-parturition) for examination of internal organs. The remaining pups were killed at the time of sexual differentiation and examined for development of the reproductive system and the presence of morphological abnormalities in other internal organs.

Maternal animals gained body weight throughout the study and there was no evidence of maternal toxicity during the study or at necropsy. There were no treatment-related effects on implantations, resorptions, numbers of live/dead fetuses, birth weight or sex ratio. An increased frequency of cleft palate, club food and/or kinky tail were observed at the high dose, but the incidence was considered to be similar to the background rate for this strain. The incidence of non-ossified forelimbs was increased at 500 and 1000 mg/kg bw/day compared with controls, but a clear dose-response was not observed and similar changes were not observed in other bones. No treatment-related skeletal variations or malformations were observed. Observation of neonates following parturition showed no differences in timing of parturition, viability index at birth or weaning. There were no treatment-related effects on post-natal development, sensory function, skeletal structure and reproductive or other internal organs.

The NOAELs for maternal toxicity and embryo-fetal toxicity were 1000 mg/kg bw/day, the highest dose tested.

Effects of MK-4 on development of fetuses and offspring in rats (Suzuki et al. 1971)
Regulatory status: Non-GLP, non-guideline

MK-4 was administered to groups of mated female Wistar rats (17-27 per group) on gestation days (GD) 7 – 12 by oral gavage at doses of 0, 10, 500 or 1000 mg/kg bw/day. The vehicle control was cottonseed oil. Clinical signs and maternal food consumption were monitored. On GD 18, 17 – 20 rats per group were killed and the internal organs and uterine contents examined. Pups were weighed after removal and external morphology and the skeletons were examined for variations, abnormalities and the progress of osteogenesis. Groups of 7 – 8 rats treated with 0, 10 or 1000 mg/kg bw/day were allowed to calve spontaneously and

post-natal development was monitored. One neonate/sex from each group was selected during the weaning period (21 days post-parturition) for examination of internal organs. The remaining pups were killed at the time of sexual differentiation and examined for development of the reproductive system and the presence of morphological abnormalities in other internal organs.

Maternal animals gained body weight throughout the study and there was no evidence of maternal toxicity during the study or at necropsy. There were no treatment-related effects on implantations, resorptions, numbers of live/dead fetuses, birth weight or sex ratio. No treatment-related anomalies, or effects on skeletal ossification, variations or malformations were observed. Observation of neonates following parturition showed no differences in timing of parturition, viability index at birth or weaning. There were no treatment-related effects on post-natal development, sensory function, skeletal structure and reproductive or other internal organs.

The NOAELs for maternal toxicity and embryo-fetal toxicity were 1000 mg/kg bw/day, the highest dose tested.

Effects of MK-4 exposure before and during early pregnancy in rats (Mikami et al. 1981)
Regulatory status: GLP status not reported; study conducted in accordance with Pharmaceutical Affairs Law No. 529

Groups of 24 male and 24 female Sprague Dawley rats were administered 0, 10, 100 or 1000 mg/kg bw/day MK-4 by oral gavage before and during early pregnancy. The vehicle control was 5% gum arabic solution. Males were treated from 7 weeks of age for 90 days prior to mating, during mating and until fertility was confirmed. Females were administered from prior to mating until day 7 of pregnancy after conception was established. Clinical signs, body weight and food consumption were monitored throughout the study. Vaginal smears were collected for 14 days before and during the treatment period until mating was confirmed. Males were killed and subjected to gross necropsy at the end of the male treatment period. Females were killed on GD 20, the uterus was removed and the number of implantations, corpora lutea, dead and resorbed embryos and placenta were observed. The liver, kidneys, spleen, ovaries and uterus of non-pregnant female rats were examined histopathologically. All pups were examined for external malformations, two thirds were subjected to visceral examination and the remaining third were examined for skeletal morphology.

One male in the control group died at week 12 and one male in the 100 mg/kg bw/day group died at week 5. These deaths were attributed to dosing errors. There were no treatment-related changes in clinical signs, body weight and food consumption in the parental animals. The oestrus cycle was similar in control and MK-4-treated females. No treatment-related effects on mating or fertility were observed. No adverse effects were found in histopathological examination of organs from non-pregnant females.

There were no treatment-related effects on corpora lutea, implantations, resorptions, live or dead fetuses, fetal weight, placental weight and sex ratio. No external anomalies were observed in any group. No treatment-related anomalies or effects on bone ossification were observed in the visceral and skeletal examinations.

The NOAELs for parental toxicity and embryo-fetal in this study were 1000 mg/kg bw/day, the highest dose tested.

Effects of MK-4 exposure during perinatal and lactation periods in rats (Mikami et al. 1981)
Regulatory status: GLP status not reported; study conducted in accordance with Pharmaceutical Affairs Law No. 529

Groups of 24 pregnant female rats were administered 0, 10, 100 or 1000 mg/kg bw/day MK-4 in 5% gum arabic solution by oral gavage from GD 17 to postnatal day (PND) 21. Maternal animals were observed for clinical signs, delivery and maternal behaviours. At the end of the treatment periods maternal animals were killed, blood samples for haematology, clotting time and clinical chemistry were collected from approximately 50% of each group, and liver, kidneys, spleen, adrenal glands and brain were weighed and subjected to histopathological examination.

Neonatal rats were observed daily for mortality and general condition, and litters were culled to 8 pups (4/sex/litter where possible) on PND 4. Animals were observed for physical and functional development (ear unfolding, eyelid opening, incisors eruption, grasping reflex, acoustic startle response and gait). On PND 21, 50% of pups were killed for haematology, clotting time, skeletal and visceral examinations, and the remaining animals were maintained on study for behavioural (open field, rotor rod and pole climbing tests) and physiological (respiratory rate, heart rate and systolic blood pressure) observations. Descent of testes and vaginal opening were monitored and animals that reach 11 weeks of age were mated. Females confirmed to have mated were separated from males, allowed to give birth and F2 pups were nursed until PND 4.

There were no treatment-related mortalities or clinical signs in maternal animals. No treatment-related effects on body weight, food consumption, gestation length, number of pups born, haematology, clotting time, clinical chemistry or histopathology were observed.

There were no treatment-related effects on physical development of pups, and no adverse effects on haematology, clotting time, visceral or skeletal examinations were observed on PND 21. Among rats maintained post-weaning, there were no adverse effects on behavioural tests, physiological measurements and reproductive performance. Body weights of F2 pups on PNDs 0 and 4 were similar in controls and treated groups.

The NOAEL was 1000 mg/kg bw/day, the highest dose tested.

4.4 Human studies

MK-7 and other menaquinones have been investigated in a range of human clinical studies. While these studies were generally designed to assess the potential beneficial effects of vitamin K_2 rather than specifically assessing safety, several also report details relating to tolerance and/or adverse events. Details relating to safety from these studies are summarised in Table 5.

In these studies MK-7 was administered at up to 360 µg/day for 12 months, up to 180 µg/day for 3 years and up to 1080 µg/day three times/week for 8 weeks. MK-7 was well tolerated in these studies with no significant adverse events reported. Adverse effects were limited to mild gastrointestinal effects and complaints regarding the product's smell.

Two studies assessed the known potential interaction between vitamin K consumption and vitamin K antagonists (VKA) used as anticoagulant therapies. Schurgers et al. (2007) reported that a dose of 130 µg/day MK-7 reduced the International Normalised Ratio (INR; a measure of how long it takes blood to clot) from 2.0 to 1.5 among individuals receiving acenocoumarol, a VKA treatment. In the same study, a dose of 315 µg/day vitamin K_1 reduced the INR from 2.0 to 1.5. The study authors suggested that the reason for the higher potency of MK-7 may be its longer half-life in the circulation and its reported 6-fold higher

cofactor activity *in vitro*. The authors proposed that an upper safety limit of 50 µg/day MK-7 may be appropriate for patients on oral anticoagulant treatment.

In a study of individuals receiving acenocoumarol treatment, daily intakes of 10 or 20 µg MK-7 were independently judged by two haematologists to cause a clinically relevant lowering of the INR in at least 40% and 60% of subjects, respectively, and to significantly increase endogenous thrombin generation by ~ 20% and ~ 30%, respectively (Theuwissen et al. 2013). The study authors recommended that use of MK-7 supplements should be avoided in patients receiving VKA therapy.

In contrast to these findings, no effects on coagulation parameters were reported in clinical studies of individuals not taking VKA anticoagulant therapy.

Table 5. Human studies with MK-7 reporting on safety and tolerance parameters

Study design	Study population	Dose and duration	Findings related to safety	Reference
1) Single-dose oral bioavailability 2) Escalating dose–response 3) Randomised crossover 4) Nonrandomised drug interaction study (interaction with oral anticoagulants)	Healthy men and women; In trial 4, subjects (n = 12) were treated with individualised dose of acenocoumarol to reach target internationalised normal ratio (INR) value of 2.0 within 3 wks, then maintained at stabilising dose of acenocoumarol while treated with escalating doses of MK-7 or K_1	1) MK-7 and K_1 at 1000 µg each (single dose) 2) MK-7 and K_1 at 50, 100, 150, 200, 250, 300, and 500 µg each (single doses) 3) MK-7 at 143 µg/d, K_1 at 99 µg/d for 6 weeks 4) MK-7 at 97.4 µg/d with weekly increment of 97.4 µg and K_1 at 49.6 µg/d with weekly increment of 49.6 µg	1) – 3) No information on safety reported 4) Doses of K_1 at 315 µg/d and MK-7 at 130 µg/d caused significant decrease in INR from 2.0 to 1.5. Study authors proposed an upper safety limit of 50 µg/d for long-chain menaquinones (including MK-7) in patients on oral anticoagulant treatment.	(Schurgers et al. 2007)
Randomised, double-blind, placebo-controlled trial	Healthy children aged 6 – 10 years (n = 55; 28 administered MK-7)	MK-7 (0 or 45 µg/d) for 8 weeks	No side-effects reported. No changes in coagulation parameters over time or compared with placebo controls.	(van Summeren et al. 2009)
Randomised, double-blind, placebo-controlled trial	Healthy women (n=334)	MK-7 (0 or 360 µg/day) for 12 months	Fractures sustained by 5 participants in each group Treatment group: 2 participants had increased nocturnal hot flushes and abdominal pain; 1 had increased palpitations that ceased at study	(Emaus et al. 2010)

			end Placebo group: 4 reports of muscular pain and general unwell feeling; 2 reports of itching	
Randomised, double-blind, placebo-controlled, prospective, longitudinal study	Lung transplant patients (n=35; 16 administered MK-7) and heart transplant patients (n=59; 30 administered MK-7)	MK-7 (0 or 180 µg/d) for 12 months	No treatment-related adverse events reported. One patient given MK-7 died early; autopsy did not reveal any connection to the study.	(Forli et al. 2010)
Open label trial	Patients with muscle cramps (n=19)	MK-7 (100 µg/day) for 3 months	Intervention was well tolerated. Patients occasionally reported mild constipation. Complete blood count, biochemistry and organ function tests (Liver function, serum creatinine, fasting and post-prandial plasma glucose, serum TSH and LDH) in normal limits pre- and post- intervention.	(Mehta et al. 2010)
Randomised, double-blind, placebo-controlled trial	Healthy adults (n=60)	MK-7 (0, 180 or 360 µg/day) for 12 weeks	No adverse events No treatment-related effects on prothrombin time and other risk factors of cardiovascular disease (blood lipid profile and blood pressure)	(Dalmeijer et al. 2012)
Randomised, double-blind, placebo-controlled exploratory pilot study	Healthy adults (n=42)	MK-7 (0, 10, 20, 45, 90, 180 or 360 µg/day) for 12 weeks	No adverse effects on thrombin generation	(Theuwissen et al. 2012)
Randomised, double-blind, placebo-controlled trial	Healthy women (n=244)	MK-7 (0 or 180 µg/day) for 3 years	Placebo group: complaints reported were (hair loss and/or brittle nails (n=2), hot	(Knapen et al. 2013)

			flashes (n=1), knee pain (n=1), numb sensation in arms and legs, washed-out (n=1), and weight gain (n=2); 4 women withdrew due to complaints. Treatment group: complaints reported were bone pain (n=1), hot flashes (n=1), rash around eyes and ears (n=1), smelly capsules (n=1), and weight gain (n=1); 1 woman withdrew due to complaints.	
Open label clinical study	Patients with peripheral neuropathy due to vitamin B_{12} deficiency and/or diabetes mellitis (n=30)	MK-7 (200 µg /day) for 8 weeks	No treatment related adverse events. Haematology (including prothrombin time), biochemical investigations and liver and kidney function tests were in normal limits at baseline, week 4 and week 8.	(Kulkarni et al. 2013)
Prospective non-randomised pilot study	Paediatric patients with thalassemic osteopathy (n=20)	MK-7 (50 µg/day) and calcitriol (5 µg/day) for 12 months	No adverse events or side effects reported. No effect on coagulation parameters (prothrombin time and activated partial thromboplastin time)	(Ozdemir et al. 2013)
Dose escalation study	Healthy adults given vitamin K antagonist (VKA; acenocoumarol) anticoagulation therapy; with an International Normalised Ratio (INR) of 2.0 (n=15)	MK-7 (10, 20 and 45 µg/day; doses increased over successive 2-week intervals followed by a 1-week washout period [acenocoumarol also stopped in final week])	45 µg/day MK-7 significantly decreased mean INR and under-carboxylated forms of prothrombin (ucFII) levels by ~ 40%. 10 and 20 µg/day judged by 2 haematologists to	(Theuwissen et al. 2013)

			cause clinically relevant lowering of the INR in ~ 40% and 60% of subjects respectively, and to increase endogenous thrombin generation (ETP) by ~20 and ~30%, respectively. Authors concluded MK-7 supplements should be avoided in patients receiving VKA therapy	
Randomised, single-blind, dose-finding study	Adult haemodialysis patients (n=200)	MK-7 (360, 720 or 1080 µg) 3 x per week for 8 weeks	Mild gastrointestinal side-effects (nausea, diarrhoea or abdominal discomfort) reported, independent of dose. Most frequent complaint concerned the unpleasant smell of the tablets. Five deaths occurred but not considered treatment-related.	(Caluwe et al. 2014)
Randomised, double-blind, placebo-controlled dose-finding study and trial	1) Healthy post-menopausal women (n=60) 2) Healthy men and women (n=120)	1) MK-7 (0, 50, 100 or 200 µg/day) for 4 weeks 2) MK-7 (0 or 100 µg/day) for 12 weeks	No treatment-related adverse effects observed. Coagulation parameters unaffected by treatment	(Inaba et al. 2015)
Randomised, partly single-blind, partly open-label, bioavailability study	Healthy men and post-menopausal women (n=107)	MK-7 (71.2 µg/day in yogurt or 58.3 µg/day in capsules) for 6 weeks	7 reports of gastrointestinal effects (satiated feeling, heartburn, stomach ache, abdominal cramps, diarrhoea and nausea) in yogurt-treated groups; authors suggested this may be due to increased yogurt consumption (500	(Knapen et al. 2016)

			mL/day)	
Randomised, single-blind, two-way cross-over study and randomised, double-blind, parallel study	1) Healthy adults (n=16) 2) Healthy adults (n=43)	1) MK-7 (0 or 180 µg) as a single dose 2) MK-7 (0, 45, 90 or 180 µg/day) for 6 weeks	1) No treatment-related adverse events; no significant changes in serum CRP, creatinine, ALAT or total cholesterol 2) 3/40 adverse events considered possibly or probably treatment-related: diarrhoea at 90 µg/day (n=2) and dry mouth at 180 µg/day (n=1); no clinically relevant changes in biochemical markers	(Møller et al. 2016)
Randomised, double-blind, placebo-controlled trial	Healthy adult athletes (n=26)	MK-7 (0 or 320 µg/day for 4 weeks, followed by 0 or 160 µg/day for 4 weeks)	No adverse effects reported by study participants	(McFarlin et al. 2017)
Open-label observational study	Adult peripheral neuropathy patients with vitamin B12 deficiency or type 2 diabetes mellitus (n=100)	MK-7 (0 or 200 µg/day) for 8 weeks	MK-7 was well tolerated No treatment-related adverse events Haematology (including prothrombin time), biochemical parameters and organ function tests were in normal limits at baseline, weeks 4 and 8, and 4 weeks post-intervention	(Mehta et al. 2018)
Open-label observational study	Adult multiple myeloma patients with treatment-induced peripheral neuropathy (n=17)	MK-7 (200 or 700 µg/day) for 4 chemotherapy cycles (21 days/cycle)	MK-7 was well tolerated No adverse events reported No adverse effects in haematology (including prothrombin time) and liver or kidney function tests	(Bhave et al. 2019)
Randomised,	Adult peripheral	MK-7 (0 or 200	MK-7 was well	(Mehta et al.

double-blind, placebo-controlled pilot study	neuropathy patients with type 2 diabetes mellitus or vitamin B12 deficiency (n=20)	µg/day) for 8 weeks	tolerated No side-effects or subjective or objective adverse events. No treatment-related effects on haematology (including prothrombin time), clinical chemistry or liver and kidney function tests	2021b)
Randomised, double-blind, placebo-controlled trial	Adult peripheral neuropathy patients with type 2 diabetes mellitus or vitamin B12 deficiency (n=60)	MK-7 (0 or 200 µg/day) for 8 weeks	MK-7 was well tolerated No subjective or objective adverse events No treatment-related effects on haematology (including prothrombin time), clinical chemistry or liver and kidney function tests	(Mehta et al. 2021a)

4.5 Safety assessments by other agencies

The National Health and Medical Research Council (NHMRC) has evaluated nutrient reference values for vitamin K for Australia and New Zealand (NHMRC 2006). The NHMRC concluded that an upper level of intake for vitamin K could not be established as no adverse effects have been associated with vitamin K consumption as food or supplements in humans or animals.

Dietary reference intakes for vitamin K have also been reviewed by the Institute of Medicine (IOM) (IOM 2006). The IOM found no evidence of toxicity associated with intake of either the phylloquinone or the menaquinone forms of vitamin K, and concluded that data were insufficient to set a tolerable upper intake level. It was further noted that no adverse effects have been reported with high intakes of vitamin K from food or supplements in healthy individuals who are not intentionally blocking vitamin K activity with anticoagulation medications.

The European Food Safety Authority (EFSA) has evaluated the safety of vitamin K_2 as a source of vitamin K added for nutritional purposes to food (EFSA 2008). The substance assessed contained vitamin K_2 occurring principally as MK-7 and to a smaller extent as MK-6. EFSA estimated the mean intake of MK-7 resulting from the proposed uses to range from 36 µg/day (female adults) to 54 µg/day (male teenagers). High intake levels ranged from 75 µg/day (children) to 115 µg/day (male teenagers). The highest 97.5th percentile intake on a body weight basis was in children, at 5.4 µg/kg bw/day.

No toxicity studies of MK-7 were available at the time, and EFSA evaluated the studies of

MK-4 that have also been reviewed by FSANZ. EFSA considered the significant decrease in prothrombin time observed in treated males in the 1-year rat study could be an adverse effect and that the lowest doses in this study (20 mg/kg bw/day) represented a lowest observed adverse effect level (LOAEL). The margin of safety calculated from the highest 97.5th percentile intake estimate for children (5.4 µg/kg bw/day) and the LOAEL from the rat study amounts to 3700. It was concluded that there were no safety concerns.

The US Pharmacopeial Convention published a safety evaluation of MK-7 as an ingredient of dietary supplements in 2017 (Marles et al. 2017). The US Pharmacopeial Convention used information on vitamin K_1 intakes in the US as a highly conservative proxy for MK-7 intake, and identified a maximum 95th percentile intake value of 223 µg/day in males aged 51 – 70 years, or 3.7 µg/kg bw/day for a 60 kg individual. Comparison of this value with the NOAEL from the short-term toxicity study of MK-7 by Pucaj et al. (2011) indicated a large margin of exposure (> 500). The review concluded that MK-7, when ingested as a dietary supplement at levels typically recommended, is not likely to be associated with any serious risk to individual or public health.

The US Food and Drug Administration (FDA) has responded that it had 'no questions' regarding the applicant's self-assessment that MK-7 is Generally Recognized as Safe (GRAS) (US FSA 2020).

4.6 Summary of the safety assessment

There is a history of safe human consumption of MK-7 from the diet. Dietary intake levels of menaquinones have not been well studied but it has been estimated that in Western diets approximately 90% of dietary vitamin K is from vitamin K_1 with approximately 10% from menaquinones. MK-7 is also produced endogenously by gastrointestinal bacteria.

MK-7 did not cause acute toxicity in mice or rats. In three 90-day oral toxicity studies in rats, MK-7 was tested at doses up to 1 mg/kg bw/day, 10 mg/kg bw/day or 4500 mg/kg bw/day. No adverse effects were reported in any of these studies. MK-7 was not genotoxic *in vitro* or *in vivo*.

No chronic toxicity/carcinogenicity, developmental or reproductive toxicity studies are available for MK-7. However, studies with the structurally related menaquinone MK-4 were submitted as supporting information.

No adverse effects were observed in a 90-day oral toxicity study of MK-4 in rats at doses up to 30 mg/kg bw/day, the highest dose tested. In a 52-week dietary toxicity study in rats, reductions in prothrombin time were observed in males at all doses tested. However, the study authors reported that the changes were considered to be within the normal range and there was no evidence of clot formation. In addition, no effects on blood coagulation parameters were observed in the toxicity studies with MK-7 or in human clinical studies with MK-7 involving individuals not taking VKA anticoagulant treatments. Changes in haematology parameters, increased spleen weights and histopathological changes indicative of extramedullary haematopoiesis in the spleen were observed in this study at doses ≥ 0.2% (equivalent to 100 mg/kg bw/day). It was unclear if these changes were adaptive or adverse, and the NOAEL was conservatively set at 0.04% MK-4 (equivalent to 20 mg/kg bw/day).

MK-4 was also evaluated in 3-month and 1-year oral toxicity studies in dogs. The NOAEL in the 3-month study was 200 mg/kg bw/day based on changes in haematology parameters at 2000 mg/kg bw/day. However these changes were not reproduced in the 1-year dog study in which the NOAEL was 2000 mg/kg bw/day, the highest dose tested. No adverse effects were observed in developmental and reproductive toxicity studies in mice and/or rats at doses up to 1000 mg/kg bw/day.

MK-7 was well tolerated and not associated with significant adverse events in human clinical studies in which it was administered up to 360 µg/day for 12 months, 180 µg/day for 3 years or 1080 µg/day three times/week for 8 weeks.

In studies with individuals taking VKAs, MK-7 treatment was associated with reduced blood clotting times as would be expected given the known interaction between vitamin K and these anticoagulant therapies. However, patients on anticoagulant therapy receive medical advice about the risk of an interaction with vitamin K supplements, and individuals consuming FSMP are under medical supervision.

No upper levels of intake have been established for vitamin K. As the applicant is not requesting a change in the permitted level of addition, the use of MK-7 is not expected to affect vitamin K intake from FSMP and a dietary intake assessment was not considered necessary. A comparison of the 95th percentile intake estimated by the US Pharmacopeial Convention (3.7 µg/kg bw/day) with the conservative NOAEL of 20 mg/kg bw/day in the 52-week rat toxicity study with MK-4 results in a margin of exposure of approximately 5400, indicating no safety concerns. The MOE is even larger in comparison with the NOAEL of 4500 mg/kg bw/day from the 90-day toxicity study in rats with MK-7, > 1,000,000.

5 Conclusions

FSANZ has assessed an application from Novozymes Australia Pty Ltd. to amend the Code to permit the use of MK-7 as a permitted form of vitamin K in FSMP. Vitamin K functions as a co-factor in the vitamin K cycle for the γ-carboxylation of VKDPs that is required for multiple cellular functions. Several studies have shown that MK-7 performs an equivalent role within the vitamin K cycle to vitamin K_1.

The method of production of MK-7 is via a standard submerged fermentation, using the bacterial strain *Bacillus paralicheniformis*. MK-7 can be added and incorporated in a uniform manner into food products in the same way as other lipid soluble vitamins, including vitamin K_1. It has good stability at both standard and accelerated storage conditions. There is a specification for MK-7 in the Code, which data provided by the applicant demonstrated it is able to comply with.

To determine the bioavailability of MK-7, FSANZ considered studies in humans on the effect of MK-7 supplementation on serum or plasma concentrations of MK-7 and biomarkers of vitamin K status. In these studies, blood MK-7 concentration increased compared to placebo or baseline at doses greater or equal to 45 µg/day in healthy adults and children, with a greater increase reported at similar doses of vitamin K_1. MK-7 supplementation at doses of 90 to 360 µg/day were associated with an improvement in biomarkers of vitamin K status relative to placebo treatments or baseline values, indicating that MK-7 is a bioavailable form of vitamin K. FSANZ concludes that based on the body of evidence in humans, MK-7 would be expected to support normal physiological function at doses of 90 to 360 µg/day.

Due to the different molar mass of MK-7 and vitamin K_1, the Adequate Intake of vitamin K as MK-7 would result in lower intake compared to vitamin K_1. FSANZ did not identify any studies in adults that compared MK-7 and vitamin K_1 bioavailability at doses equivalent to the Adequate Intake (60 µg/day in women, 70 µg/day in men) and therefore cannot conclude whether the lower intake would support essential requirements if it was the only form of vitamin K in the diet.

No evidence was identified to suggest that MK-7 supplementation would inhibit or modify the absorption of other nutrients.

There is a history of safe human consumption of MK-7 from the diet, and MK-7 is also produced endogenously by gastrointestinal bacteria. No adverse effects of MK-7 were identified in toxicity studies in laboratory animals and clinical studies in humans. Toxicity studies with the structurally related compound MK-4 were also considered as supporting evidence. A comparison of estimated dietary intakes of MK-7 to the NOAEL in a chronic toxicity study with MK-4 resulted in a large margin of exposure (< 5400), indicating no safety concerns. There is a potential for interaction between MK-7 and VKA anticoagulant drugs, but patients on anticoagulant therapy receive medical advice about the risk of an interaction with vitamin K supplements, and individuals consuming FSMP are under medical supervision.

The assessment concluded that there are no public health and safety concerns associated with the use of MK-7 as a permitted form of vitamin K in FSMP.

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