Application for Vitamin K2 (Menaquinone-7, MK-7) as a Substance Used for a Nutritive Purpose in Food for Special Medical Purposes

Novozymes Australia Ltd.

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Executive Summary

Novozymes A/S seeks to expand Vitamin K2 (Menaquinone-7, or MK-7) use to the Food for Special Medical Purposes (FSMPs) in Australia and New Zealand. Vitamin K2 (MK-7) is a long-chain menaquinone, which is a form of Vitamin K. Vitamin K2 in this petition is manufactured by Novozymes A/S through production of a purified concentrate that is then diluted to the required potency in sunflower oil.

Vitamin K2 (MK-7) is permitted as a source of Vitamin K for nutritional purposes in the European Union and is Generally Recognized as Safe (GRAS) for use in nutritional beverages in the United States. Additionally, beverage products containing Vitamin K2 are on the market in many additional countries including Vietnam, Malaysia, Philippines and Singapore.

This application includes scientific evidence supporting addition of Vitamin K2 (MK-7) in FSMPs. The physical and chemical characteristics, bioavailability, metabolism, and biological activity of Vitamin K2 are well-established and described in detail. The history of consumption outlines exposure to Vitamin K2 as an inherent nutrient in traditionally consumed foods, as well as its use as a dietary and food supplement. Safety has also been established by a battery of standard *in vitro* and *in vivo* safety studies, as well as by outcomes from multiple human clinical trials. The safety of Vitamin K2 (MK-7) in these products has been assessed positively by the European Food Safety Authority (EFSA), and the United States Food and Drug Administration.

The applicant is making the Application to ensure regulatory certainty noting Section 2.9.5-6(c) of the Code allows for the addition of other permitted substances to FSMPs regardless of form; Vitamin K is permitted to be added to FSMPs by Section 29-7 in the form of Vitamin K1 as phylloquinone (phytonadione).

In summary, this application is seeking for permission the use of Vitamin K2 (MK-7) as an additional permitted form of the currently permitted Vitamin K in FSMPs via an amendment of Schedule 29-20. No amendment to mandatory composition or labelling requirements are being proposed in this application.

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General Requirements (Section 3.1.1)

B. Applicant Details

(a) **Applicant's name/s**

[redacted]

(b) **Company/organisation name**

Novozymes Australia Pty Ltd

(c) **Address (street and postal)**

3/22 Loyalty Road PO Box 4942

2151 NORTH ROCKS NSW, Australia

(d) **Telephone number**

[redacted]

(e) **Email address**

[redacted]

(f) **Nature of applicant's business**

Biotechnology

(g) **Details of other individuals, companies or organisations associated with the application**

Submission prepared by:

[redacted]

C. Purpose of the Application

This application requests an amendment to the Australia New Zealand Food Standards Code (the Code) to explicitly permit the use of Vitamin K2 (also known as Menaquinone-7 or MK-7) as a permitted form of Vitamin K in Foods for Special Medical Purposes (FSMPS).

Vitamin K2 (MK-7) is permitted as a source of Vitamin K in FSMP in the European Union and is Generally Recognized as Safe (GRAS) for use in nutritional beverages in the United States (outlined in Section J.2 of this application).

This application requests an amendment to the Australia New Zealand Food Standards Code (the Code) to permit the use of Vitamin K2 (also known as Menaquinone-7 or MK-7) as a permitted form of Vitamin K in Foods for Special Medical Purposes (FSMPs), in addition to the existing permitted form of Vitamin K1 (also known as phylloquinone/phytonadione) based on Section 29—7 to ensure regulatory certainty. Vitamin K2 (MK-7) is a specific form of Vitamin K with a unique chemical structure that differentiates it from both Vitamin K1 and other forms of Vitamin K2. It is intended to be added to FSMPs sold in powder and liquid formats.

Vitamin K2 is intended to be used as a source of Vitamin K in FSMPs that partially or totally replace the daily diet; products that are recommended to be used under medical supervision. It is therefore possible that Vitamin K2 will be added to FSMPs that are a sole source of nutrition. Section 2.9.5—7 includes compositional requirements for a FSMP that is represented as being suitable for use as a sole source of nutrition. In relation to Vitamin K, this application has no impact on the required limit as specified in section S29—21.

In summary, the purpose of this application is to amend Schedule 29 with inclusion Vitamin K2 (MK-7) as a permitted form of Vitamin K in FSMPs.

D. Justification for the Application

Vitamin K2 (MK-7) is safe for human consumption and provides a safe and nutritionally efficient alternative source of Vitamin K to the currently permitted form listed in the Code. The current Code does not specify the use of Vitamin K2 as a permitted form of Vitamin K in FSMPs. Therefore, as outlined above, the Applicants seeks amendment to the Code to permit addition of Vitamin K2 into FSMP.

D.1 Costs and Benefits of the Application

Consumers of FSMPs containing Vitamin K2 will benefit from it as a permitted form of Vitamin K. Vitamin K2 is a well-accepted form of Vitamin K supported by clinical data that has been proven to be safe for human consumption.

Manufacturers of FSMPs that supply the Australian and New Zealand market can benefit from the permission of using an alternative form of Vitamin K that has been well researched by numerous studies, including human clinical trials.

There should be minimal impact on government from the approval Vitamin K2 as a permitted form of Vitamin K in FSMPs. FSMP is a category of foods that are intended to be used under medical supervision. Vitamin K2 has been demonstrated to be safe and an effective form of Vitamin K. Hence, there is minimal reason for enforcement agencies to be concerned about the presence of Vitamin K2 in FSMP.

D.1 Impact on International Trade

The application is not likely to have an impact on international trade.

E. Information to Support the Application

E.1 Data requirements

Vitamin K2-7 or menaquinone-7, a naturally occurring form of vitamin K2, is easily absorbed and readily bioavailable compared to other forms. Supplementation of the diet with K2-7 has health beneficial effects in various diseases including osteoporosis, cardiovascular diseases, cancer, diabetes and related complications, and neurodegenerative diseases. Clinical studies have unequivocally demonstrated the utility of vitamin K2-7 supplementation in ameliorating peripheral neuropathy, reducing bone fracture risk and improving cardiovascular health. Literature searches were conducted to determine if there is any evidence of role of vitamin K2-7 in various health conditions by using keywords Vitamin K2 and Menaquinone. The database selected to perform these searches was Medline as primary database, followed by EMBASE, TOXLINE and Cochrane Library. The searches were limited to articles from past 33 years (1991 – 2024) to ascertain the validity of the information. The result of the searches provided numerous articles from these databases as there has been a growing interest in understanding the role of Vitamin K2-7 in health and diseases in recent times. Therefore, the articles which are considered the most relevant to this application are provided and summarized in Section 3.3.3-C.1 and C.2.

F. Assessment Procedure

The application is requesting approval for Vitamin K2 (MK-7) as an additional form of Vitamin K in FSMP. The narrow focus of the application should see it assessed by FSANZ under the General Procedure.

G. Confidential Commercial Information (CCI)

The application does not contain information that would be considered Confidential Commercial Information (CCI).

H. Other Confidential Information

The application does not contain other information that would be considered confidential (but non-CCI).

I. Exclusive Capturable Commercial Benefit

This application is not expected to confer an Exclusive Capturable Commercial Benefit.

J. International and Other National Standards

J.1 International Standards

Codex Alimentarius has established a standard (CXS 180-1991) that describes requirements for labelling and claims for Foods for Special Medical Purposes. This standard, nor any other Codex standard, defines compositional requirements for Foods for Special Medical Purposes, nor does any Codex text define a list of substances used for nutritive purposes in Foods for Special Medical Purposes. Therefore, amending the Code to add Vitamin K2 as an additional permitted form of Vitamin K to FSMP would not result in misalignment with Codex standards or country regulations that reference Codex standards.

J.2 Other National Standards or Regulations

United States

Vitamin K2 (Menaquinone-7, MK-7) from Novozymes A/S is Generally Recognized as Safe (GRAS) in the United States for use in nutritional beverages. This GRAS determination was notified to the United States Food and Drug Administration (GRN 887) and received a "No Questions" response on 12 June 2020. The nutritional beverages category that is the subject of this notification covers equivalent products as the Foods for Special Medical Purposes that are the subject of this filing.

European Union

In the European Union, menaquinone (occurring principally as menaquinone-7) is permitted to be used as a source of Vitamin K in both Foods for Special Medical Purposes and Total Diet Replacement Products for Weight Control through Regulation (EU) No 609/2013. This permission is based on the European Food Safety Authority assessment that concluded that the use of Vitamin K2 in foods for the general population including food supplements and in foods for particular nutritional uses (FSMP), other than baby foods and infant formula, was not a safety concern (EFSA. 2008).

K. Statutory declaration

The Statutory Declaration is provided as a separate document together with this submission.

L. Checklist

This application concerns the product intended to be added to FSMPs. Therefore, the relevant documentation according to the Application Handbook from Food Standards Australia New Zealand as of 1 July 2019, are the following sections:

- Section 3.1.1 – General requirements
- Section 3.3.3 – Substances used of a nutritive purpose
- Section 3.6.3 – Special purpose foods, Other foods

Accordingly, the checklist for General requirements as well as the Special purpose foods (Other foods) is included as Appendix 1.

Substances Used for a Nutritive Purpose (Section 3.3.3)

A. Information on the Use of the Nutritive Substance

A.1 Information on the Purpose of the Use of a Nutritive Substance in Food

The information provided in support of this application relates to the use of Vitamin K2 (MK-7) as a permitted additional form of Vitamin K in Food for Special Medical Purposes (FSMP). Vitamin K2 (MK-7) is well accepted as a source of Vitamin K (nutrient/substance used as nutritive purposes).

A.2 General Data Requirements for Supporting Evidence

Vitamin K refers to a group of fat-soluble vitamins considered essential cofactors in humans. Vitamin K is a co-factor for several proteins involved in blood coagulation and bone metabolism as well as renal reabsorption of calcium. There are mainly two biologically active forms of Vitamin K: phylloquinone (Vitamin K1) mainly found in green-leafy vegetables, and menaquinone (Vitamin K2) produced by bacteria and found in animal products and fermented foods. Menaquinone-7 (MK-7) is a specific form of vitamin K2 with a unique chemical structure and metabolism that affects the bioavailability and potential beneficial health outcomes.

Vitamin K2 (MK-7) differs from vitamin K1 in structure, featuring a longer chemical chain length resulting in an exceptionally long half-life, approximately 3-4 days in comparison to 3-4 hours for Vitamin K1. Evidence suggests that the structure of Vitamin K2 leads to more stable serum levels. The structural differences of Vitamin K2 improve bioavailability and help facilitate uptake by extra-hepatic tissues such as bone. By contrast, most Vitamin K1 is retained by the liver and used for synthesis of clotting factor. Please refer to Table 1 for a summary of these differences.

Table 1. Comparison of Vitamin K1 and Vitamin K2 (MK-7)

	Vitamin K2 (MK-7)	Vitamin K1
Major uptake tissue	Extra hepatic (i.e. bone)	Hepatic
Chemical structure	Longer chain	Shorter chain
Bioavailability	Longer half-life (3-4 days)	Shorter half-life (3-4 hours)
Dietary sources	Bacterial origin: animal-based foods and fermented foods	Green, leafy vegetables

B. Technical Information on the Use of the Nutritive Substance

B.1 Information to Enable Identification of the Nutritive Substance

Vitamin K2 (MK-7) is a standardized, fermented, defatted extract prepared by a fermentation and extraction process. Vitamin K2 (MK-7) belongs to "long chain" menaquinones and is part of 14 vitamin K2 [menaquinones (MK)-n; n = 1-14] derivatives. The extract is mixed with food grade material to a desired concentration that is intended to be marketed under the trade name MenaquinGold® (Appendix 2). The chemical structure was described in Figure 1. General descriptive characteristics of Vitamin K2 (MK-7) are summarized in Table 2.

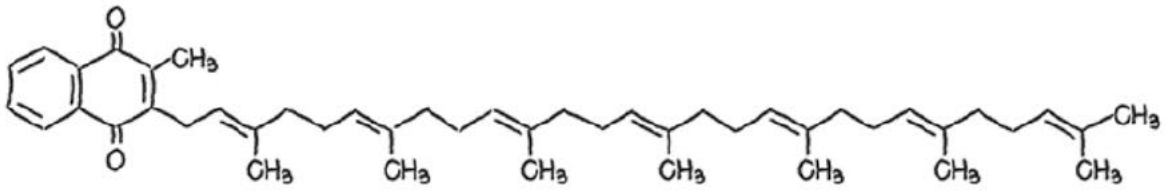


Figure 1: Structure of Vitamin K2 (MK-7)

Table 2. General Description of Vitamin K2 (MK-7)

Parameter	Description
Source	*Bacillus Paralicheniformis* (Appendix 3)
Synonyms	Vitamin MK7; Menaquinone K7; MK7; Vitamin K2
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)-
CAS No.	2124-57-4
Molecular weight	648
Chemical formula	$C_{46}H_{64}O_2$
Recommended International Nonproprietary (rINN)	Vitamin K2-7; MK-7 or Menaquinone -7
Compendial Name	Not applicable
National Approved Names BANM	Vitamin K2-7; MK-7 or Menaquinone -7
Systematic Chemical Name	[E]-2-(3,7,11,15,19,23,27-Heptamethyl-2, 6, 10, 14, 18, 22, 26-Octacosa heptaen yl)-3- methyl-1,4-naphthalienedione
Other names (Proprietary)	MenaquinGold®, Natural Vitamin K2-7
Chemical abstracts service (CAS) registry number (RN)	2124-57-4

B.2 Information on the Chemical and Physical Properties of the Nutritive Substance

The following stability study was used to evaluate the stability of Vitamin K2 (MK-7). A bulk sample of Vitamin K2 (MK-7) oil was packed in 1 kg plastic containers, for use in the following studies (Appendix 4).

Stability Studies at Storage Temperature:

The bulk Vitamin K2 sample was packed in plastic containers and maintained at 30°C ± 2°C / RH 65 % ± 5 %. Samples from three commercial batches of Vitamin K2 (MK-7) were then analyzed at intervals of 1 month, 3 months, 6 months, 12 months, 18 months, 24 months and 30 months.

Accelerated stability studies:

The bulk Vitamin K2 sample was packed in plastic containers and maintained at40°C ± 2°C / RH 75% ± 5%. Samples from three commercial batches of Vitamin K2 (MK-7) were then analyzed at intervals of 1 month, 3 months and 6 months.

The results of both stability studies show that Vitamin K2 (MK-7) is stable for 30 months under both standard storage and accelerated stability conditions with the recovery of Vitamin K2 is 99.10% at the end of 30 months.

Stability study of minimum three commercial batches of Natural Vitamin K2-7 (Menaquinone-7; MK-7) show that in 30 months, assay values are within limit for the material stored at 30^{o}C ± 2^{o}C / RH 65 % ± 5 % (Zone IVa) and 30^{o}C ± 2^{o}C / RH 75 % ± 5 % (Zone IVb).

B.3 Information on the Impurity Profile

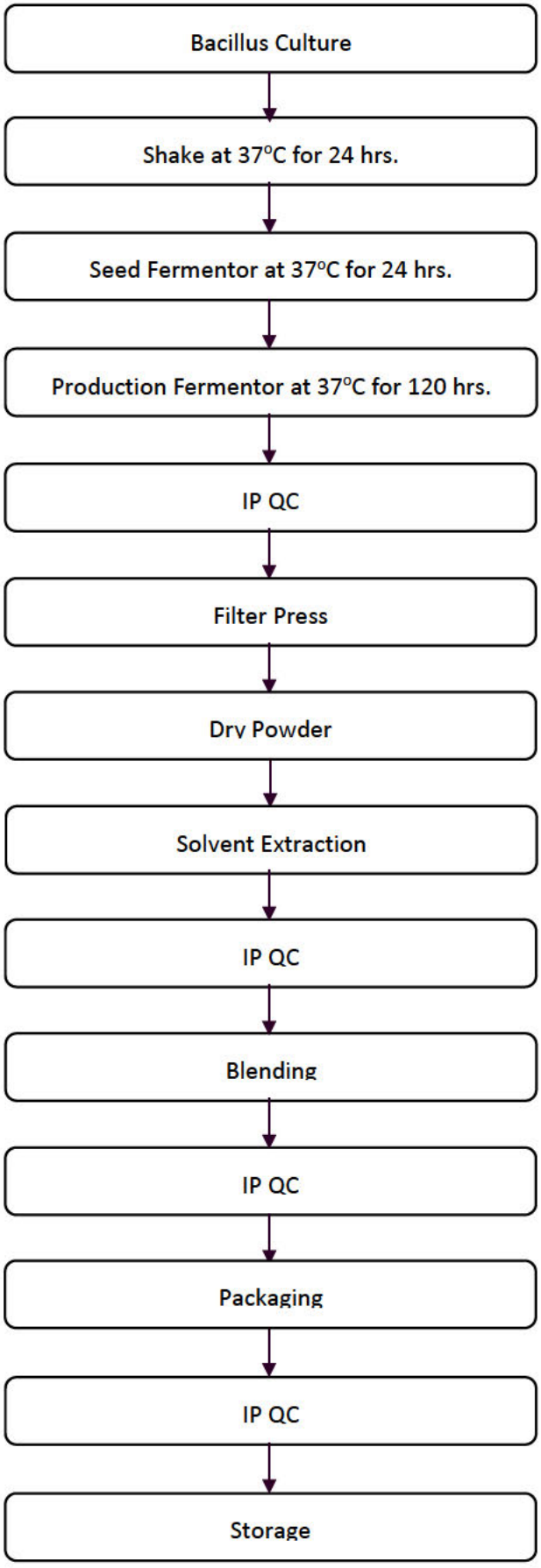
A specification for Vitamin K2 (MK-7) has been established by the United States Pharmacopeia, and this ingredient meets those specifications both in terms of purity and impurity profile. USP monograph document ID: DocId: GUID-F0E343CC-B4C3-40F8-BF5E-3695A790B519_3_en-US. Specifications are complying to USP-NF monograph.

B.4 Manufacturing Process

Vitamin K2 (MK-7) is manufactured via bacterial fermentation using a process outlined in Figure 2. The bacterial strain, *Bacillus paralicheniformis,* used in the production of Vitamin K2, has been well characterized by employing gross morphological characters, biochemical reactions, and by 16S RNA. *Bacillus paralicheniformis* is a non-toxicogenic and non-pathogenic bacterial strain. Gram flour (made from chickpeas) and dextrin are used as substrates for fermentation. Following bacterial fermentation, the fermentation broth (containing Vitamin K2) is spray dried and hexane-extracted. Vitamin K2 is available in both a powder and liquid format. For the powdered Vitamin K2, the concentrated Vitamin K2 oil produced through the process described above is triturated in glycerol monostearate, cooled and milled to the required mesh size for powder form. For the liquid format, the concentrated Vitamin K2 oil produced through the process described above is blended in vegetable oil to a desired potency.

The production procedure and use of food grade materials in that process ensures a consistent Vitamin K2 (MK-7) product. Processing aids, such as the solvent and buffer salts, are all food-grade and comply with specifications described in the current edition of the Food Chemicals Codex.

The flow diagram of manufacturing process of Natural Vitamin K2-7 Oil is shown below.



B.5 Specification for Identity and Purity

The ingredient described in this application complies with the monograph specifications for Vitamin K2 (MK-7) in the United States Pharmacopeia (Table 3). Analytical results from 3 batches demonstrate that Vitamin K2 meets these specifications (Appendix 5).

Table 3. Specification and analysis results for three batches.

Tests	Specifications	Method	Reference Method	Batch# WM/MQG/1000P/		
				2315	2316	2322
Appearance	Off white to pale yellow powder	Visual (STP/QC-03)	In-house & Factory Act, 1948 amended dt.3.1.2012	Off White Powder	Pale Yellow Powder	Off White Powder
Identification A (By TLC)	Under visible light and short-wavelength UV light, the spots from the sample solution should correspond in RF value to those from the Standard solution. After applying the spray reagent, under white light, the spot from the sample solution should correspond in color (dark blue) and RF value to those from the standard solution.	TLC (STP/QC-38)	USPNF<2023>	Confirms	Confirms	Confirms

Identification B (By HPLC)	The retention time of the major peak of the sample solution should correspond to that of the standard solution, as obtained in the test for content of Menaquinone-7.	HPLC (STP/QC-37)	USPNF<2023>	Confirms	Confirms	Confirms
Vitamin K2-7 (dried basis)	90.0% - 120.0% of 1000 µg/g	STP/QC-37	USPNF<2023>	1165	1187	1173
Vitamin K2-6 (dried basis)	NMT 10% of Vitamin K2-7 labelled amount (NMT 100 µg/g)	STP/QC-37	USPNF<2023>	2.0% (20 ppm)	1.9% (19 ppm)	1.4% (14 ppm)
Isomeric Purity	NMT 2% cis-menaquinone-7	STP/QC-39	USPNF<2023>	0.9	1.3	0.6
Loss on drying (at 105^{O}C for 4 Hrs)	5% (Max)	STP/QC-08	IP 2018, Eight Edition & USP 38th Edition, Appendix 731	1.91	2.6	2.81
Elements Impurities:						
Arsenic (As2O3)	0.5 µg/g (Max)	AAS/ICP-MS	AOAC 2015.01 21st Edition	BDL 0.5	BDL 0.5	BDL 0.5
Lead (Pb)	0.5 µg/g (Max)	AAS/ICP-MS	AOAC 2015.01 21st Edition	BDL 0.5	BDL 0.5	BDL 0.5
Cadmium (Cd)	0.5 µg/g (Max)	AAS/ICP-MS	AOAC 2015.01 21st Edition	BDL 0.5	BDL 0.5	BDL 0.5
Mercury (Hg)	0.1 µg/g (Max)	AAS/ICP-MS	AOAC 2015.01 21st Edition	BDL 0.1	BDL 0.1	BDL 0.1
Total Heavy Metals	5 µg/g (Max)	STP/QC-25	IP 2018, Eight Edition	BDL 5	BDL 5	BDL 5
Microbial Enumeration Tests						
a) Total Bacterial Count	Not more than 10^3 cfu/g	USP	USP<2021>	<300	<300	<300
b) Total Yeast and Molds Count	Not more than 10^2 cfu/g	USP	USP<2021>	<100	<100	<100

Test For Special Micro-organisms						
Bile-tolerant Gram-negative bacteria	Absent in 10g	USP	USP<2021>	Absent	Absent	Absent
Escherichia coli	Absent in 10g	USP	USP<2022>	Absent	Absent	Absent
Salmonella spp.	Absent in 10g	USP	USP<2022>	Absent	Absent	Absent
Staphylococcus aureus	Absent in 10g	USP	USP<2022>	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent in 10g	USP	USP<62>	Absent	Absent	Absent

max = maximum; min = minimum; cfu = colony forming units; USP = Untied States Pharmacopeia; AOAC = Association of Official Agricultural Chemists, TLC – Thin-layer chromatography, HPLC – High performance liquid chromatography, AAS – Atomic Absorption spectrophotometry, ICP-MS – Inductive coupled plasma mass spectrometry, BDL – Bellow detection Limit, ppm – Parts per million,

B.6 Analytical Method for Detection

The MP-VKTK method (AOAC International 992.27 and 999.15, modified) is used to do testing of general Vitamin K including Phylloquinone and Phytodione (Vitamin K1), and Menaquinone-7 (MK-7, Vitamin K2). This method is applicable to the quantification of total Vitamin K2 (MK-7) in the Foods for Special Medical Purposes products that are in scope for this submission.

In this method, samples are treated with enzymatic digestion to metabolize all fat in the product. The sample is then extracted with organic solvents and injected on a reverse phase high-performance liquid chromatography (HPLC) system with post-column reduction and fluorescence detection against an external standard curve.

B.7 Information on the Proposed Food Label

Vitamin K2 (MK-7) is intended to be added to FSMP as a permitted form of Vitamin K, and as such would be included in the declaration of the total amount of Vitamin K in the product. This application does not impact on the minimum required level for Vitamin K in FSMP product as stipulated in Section 29—21. In the list of ingredients, it is proposed that Vitamin K2 (MK-7) would be referenced as “Vitamin K2”.

C. Information Related to the Safety of the Nutritive Substance

C.1 Information on the Toxicokinetics and Metabolism of the Nutritive Substance and, if Necessary, its Degradation Products and Major Metabolites

Vitamin K was discovered by the Danish scientist, Henrik Dam, in the 1930s. Dam's discovery was during his quest to understand chicken's cholesterol metabolism by feeding them a diet free of sterols and low in fat (Shampo et al., 1998). This reduced their intake of fat-soluble Vitamin K, resulting in chickens developing large subcutaneous and intramuscular haemorrhages. This initial finding led to isolating, identifying and characterizing the structure of Vitamin K and its importance as an anti-haemorrhagic agent. Of the many metabolic processes related to Vitamin K deficiency, bleeding still remains the potentially most serious generally known consequence. However, the role of Vitamin K's impact on osteoporosis and its inhibitory role in arterial calcification and vascular biology is now recognized in general populations. It is axiomatic that these metabolisms require Vitamin K for γ-carboxylation and that this step is essential to their proper functioning. However, there are many other functions of Vitamin K recently discovered that seem to be independent of its classical co-factor function. Vitamin K's metabolic effects, e.g., ameliorating effect on peripheral neuropathy, cramps, autonomic nervous system, improving perfusion, etc., remain unexplained. Additionally, Vitamin K also acts as a ligand for the receptor SXR, the steroid and xenobiotic sensing nuclear receptor (SXR), which is a transcriptional regulator of the cytochrome P450 gene CYP3A4.

Over the years, the understanding of the Vitamin K family has evolved, with the recognition of two primary forms of Vitamin K, Vitamin K1 (phylloquinone) and Vitamin K2 (menaquinones). Although all forms of Vitamin K have the same function, they differ in bioavailability and bioactivity. Vitamin K2, the main storage form in animals, has several subtypes, which differ in isoprenoid sidechain length. These Vitamin K2 homologues are called menaquinones and are characterized by the number of isoprenoid residues in their side chains. Menaquinones are abbreviated MK-n, where M stands for menaquinone, the K stands for Vitamin K, and the n represents the number of isoprenoid side chain residues. The two prominent menaquinones in human nutrition are MK-4 and MK-7. MK-4 is the most common type of Vitamin K2 in animal products as MK-4 is normally synthesized from all types of Vitamin K1 in certain animal tissues. MK-7 and other long-chain menaquinones are different from MK-4 in that they are not produced by human tissue, but are generated by bacteria in the gut. The available information suggest that a range of Vitamin K2 analogues are present as a mixture in several foods, e.g., in sauerkraut, hard cheese, soft cheese and curd cheese (Schurgers et al., 2000). These foods have a long history of human consumption by humans.

The presence of menaquinones (primarily MK-7) in the diet (Natto and cheeses) supports the safety of this form of Vitamin K. However, because of the role of Vitamin K (K1 and K2) in blood coagulation and potential health benefits, there has been considerable effort to elucidate the mechanism of action of menaquinones, primarily MK-7. There is no known toxicity associated with high doses (dietary or supplemental) of the phylloquinone (Vitamin K1) or menaquinones (Vitamin K2) forms of Vitamin K.

As indicated earlier, all types of Vitamin K have the same function. The main function is to activate proteins that serve important roles in blood clotting, heart health and bone health. However, because of differences in absorption and transport to tissues throughout the body, Vitamin K1 and K2 could have profoundly different effects. Additionally, the available studies also indicate that chain length of Vitamin K2 impacts bioavailability and bioactivity. MK-7 has higher bioavailability than other menaquinone homologs (MK-4) and Vitamin K1. Given this, a summary of the differences in bioavailability of Vitamin K1 and MK-7 and its implications from to safety is provided below.

Bioavailability and Metabolism

It is well recognized that K vitamins are absorbed in the small intestines and enter the circulation via the lymphatic system and are transported in the blood by binding to chylomicrons. Following solubilization of Vitamin K1 (phylloquinone) into mixed micelles composed of bile salts and the products of pancreatic lipolysis, it is absorbed unchanged from the small intestine (Shearer et al., 1974). Phylloquinone in blood appears to be derived exclusively from the diet. However, as regards circulating menaquinones, such as MK-7, it is not clear whether it is derived from the diet, intestinal flora, or a combination of these sources. The translocation of Vitamin K1 and K2, their entry into target tissues, and their excretion are known to be affected by the structural differences in the isoprene side chain between these vitamins. The transport of phylloquinone takes place by triglyceride-rich lipoproteins, whereas long-chain menaquinones are transported mainly by low-density lipoproteins (Kohlmeier et al., 1996).

The predominant Vitamin K in human cortical and trabecular bone has been reported as phylloquinone, and, unlike liver, no menaquinones higher than MK-8 were detected (Usui, Tanimura IL, Nishimura, Kobayashi, Okanoue, & Ozawa et al, 1990). The major circulating form of Vitamin K is primarily phylloquinone. The menaquinones, MK-7, and possibly MK-8 are also found in blood. However, the common hepatic forms such as MKs 9-13 are not detected in the blood (Shearer et al., 1996; Shearer et al., 1992; Suttie et al., 1995 and Hodges et al., 1993).

In three separate studies, Schurgers et al. (2007) compared the bioavailability of Vitamin K1 and Vitamin K2 (MK-7). These investigations revealed maximum serum concentrations of both Vitamin K1

and Vitamin K2 (MK-7) at approximately 4 hours after intake; followed by a steep decline in serum concentrations and then a second phase at 8-96 hours in which Vitamin K1 declined to baseline but Vitamin K2 (MK-7) remained stable for up to 4 days or more. The half-life of MK-7 was estimated as 68 hours for the latter phase of elimination. Using the area under the curve at 24 hours, the ratio of bioavailability of MK-7:Vitamin K1 was 2.5. Using the area under the curve at 96 hours, the ratio of bioavailability of MK-7:Vitamin K1 was 6. Based on these observations, it was concluded that MK-7 has a significantly longer half-life as compared to Vitamin K1 (68 hours vs 1–2 hours).

Both Vitamin K1 and MK-7 had linear dose-response curves at 4 hours post treatment, from 0 to 500 mcg; at 24 hours, there was no effect of Vitamin K1 at up to 200 mcg, but MK-7 at 100 mcg gave an upper limit of normal range for total serum vitamin K (1.5 nM or 1 mcg/L). MK-7 accumulated during the first 2 weeks until it reached a plateau level of approximately 10 nM (6 mcg/L), and Vitamin K1 remained slightly above the placebo values during the entire study period (Schurgers et al., 2007).

In a review article, Shearer et al. (2012) described the absorption, distribution, metabolism, and excretion of Vitamin K2 (MK-7). The findings from this review show that MK-7 is absorbed rapidly and unchanged from the small intestine following incorporation into mixed micelles. In the enterocytes, the mixed micelles are packaged into chylomicrons and secreted by exocytosis from the intestinal villi into the lymphatic capillaries, ultimately reaching the systemic circulation via the larger lymphatic vessels. Circulating MK-7 containing chylomicrons undergo changes in their apoprotein content that facilitate their uptake by receptor-mediated endocytosis in the liver and in bone osteoblasts, involving interactions between surface apoproteins and low-density lipoprotein receptor-related proteins (Shearer et al., 2012).

In several studies, absorption of Vitamin K2 (MK-7) following oral ingestion of Natto and Natto-derived MK-7 by human subjects has been studied. Findings from these studies suggests that MK-7 is absorbed and can be detected in plasma (Sumi et al., 1999; Tsukamoto et al., 2000a; Schurgers et al., 2007; Kaneki et al., 2001). In a study investigating geographic variation in background levels of MK-7, Kaneki et al. (2001) reported that, as compared to women in Britain, the plasma levels of MK-7 were higher in women from Japan (Kaneki et al., 2001). In a single dose study, 8 postmenopausal women consumed 80 g of Natto containing approximately 1100 µg of MK-7. Serum levels of MK-7 were analyzed from blood collected just before eating Natto and on Days 1, 3, 7, and 14 after Natto consumption (Kaneki et al., 2001). This study along with others suggest good bioavailability of MK-7 derived from Natto or with Natto food (Kaneki et al., 2001). These studies reported higher and more stable blood levels of MK-7 as compared to phylloquinone (Kaneki et al., 2001).

In a randomized single-blinded two-way cross-over study, Moller et al. (2016) studied the bioavailability of a synthetic Vitamin K2 (MK-7). In this study, healthy subjects (20-66 years of age) took a single 180 mcg dose of synthetic MK-7 (n=8) or fermentation-derived MK-7 (n=9), and serum MK-7 concentrations were monitored for 72 hours to calculate AUC (0–72 h) and Cmax (Moller et al., 2016). The 90% confidence interval for the ratio of the AUC (0-72 h) values for synthetic and fermentation-derived MK-7 was 83-111, indicating bioequivalence. The 90% confidence interval for the Cmax ratio was 83-131 (Moller et al., 2016).

Knapen et al. (2016) compared the fasting plasma concentrations of MK-7 in healthy men and postmenopausal women (45-65 years) following consumption different foods such as (1) yogurt Kplus [yogurt enriched with MK-7, vitamins D3 and C, magnesium, n-3 poly unsaturated fatty acids (n-3 PUFA) and fish oil], (2) yogurt K [yogurt fortified with MK-7 only], and (3) soft gel capsules containing only MK-7, daily for 42 days (Knapen, et al., 2016). The increase in plasma MK-7 with the yogurt Kplus product was more pronounced than the increase in MK-7 with the capsules. The investigators concluded that dairy matrix and nutrient composition may affect MK-7 delivery and improvement of vitamin K status. Yogurt fortified with MK-7 is a suitable matrix to improve the nutritional status of the fat-soluble vitamins (Knapen et al., 2016).

In a recent bioavailability study conducted by Novozymes A/S (Data on file), the maximal plasma concentration of MK-7 was seen at approximately 4.6 hours after intake, followed by a steep decline. A marked difference was observed during the second phase (between 8 and 24 hours) after mealtime, where MK-7 has remained detectable. The average Cmax and Tmax for MK-7 is 34.45 and 5 respectively. The Area under curve for MK-7 on an average is 262.41. The long half-life time of MK-7, results in more stable serum levels, and accumulation of MK-7 to higher levels (during prolonged intake).

In summary, menaquinones (Vitamin K2) appear to be absorbed unchanged from the gastrointestinal tract. Following absorption, menaquinones are carried in the lymph in mixed micelles composed of bile salts, and subsequently released into circulation. Menaquinones absorbed from circulation are primarily distributed to the liver, in which MK-6 through MK-13 comprise 90% of the total vitamin K composition. Only 10% of the hepatic vitamin K stores consist of phylloquinone (Vitamin K1). Vitamin K1 metabolism primarily takes place in the liver, and involves oxidative degradation of the side-chain resulting in subsequent elimination via the bile or urine. The available studies indicate a 6-10 times better serum/plasma bioavailability of MK-7 compared to Vitamin K1. Given the short half-life, K1 will be eliminated quickly; hence, compared to K1, MK-7 with its relatively longer half-life is likely to build up more stable serum levels.

Vitamin K2 (MK-7) Drug Interaction

Vitamin K Antagonists (VKAs) have a long history of clinical experience as anticoagulant therapies, and there is a known interaction between Vitamin K consumption and these therapies. Individuals receiving VKA therapies, such as warfarin, monitor the effectivity of their treatments through the International Normalized Ratio (INR), which is a measurement of how long it takes blood to clot. The effect of different forms of Vitamin K on warfarin activity, and INR, have been investigated (Schurgers et al., 2007). The findings from a study revealed that a dose of 315 mcg/day of Vitamin K1 and 130 mcg/day of Vitamin K2 (MK-7) changed INR from 2.0 to 1.5 (Schurgers, et al., 2007). The lower dose of Vitamin K2 (MK-7) needed to reduce INR was most likely attributed to its longer half-life and 6-fold higher cofactor activity (Buitenhuis et al., 1990). (Cofactor – A cofactor a non-protein chemical compound or metallic ion that is required for an enzyme's role as a catalyst (a catalyst is a substance that increases the rate of a chemical reaction). Cofactors can be considered "helper molecules" that assist in biochemical transformations.)

Further, the investigators comment that, if expressed as Area Under the Curve (AUC) over 24 hours, the concentration MK-7 is 2.5-fold greater than that of K1. The investigators concluded that “MK-7 supplements containing more than 50 mcg/d may interfere with oral anticoagulant treatment, whereas doses of 50 mcg or less are not likely to affect the INR value in a relevant way”. Despite this, studies suggest that a low dose supplementation of Vitamin K2 is helpful in stabilizing INR (Buitenhuis et al., 1990). Alternatively, the European Food Safety Authority (EFSA 2008) concluded that human studies have demonstrated that consumption of up to 6 mcg/kg body weight in adults (corresponding to 360 mcg for a typical 60 kg adult) and up to 1.5 mcg/kg body weight in children did not affect blood coagulation. (EFSA, 2008).

It should be noted that individuals on VKA therapies are currently provided instructions on limiting or otherwise monitoring Vitamin K intake. This guidance applies not only to food products supplemented with Vitamin K, such as the Foods for Special Medical Purposes in scope for this application, but also for consumption of foods with inherent Vitamin K, such as green vegetables like lettuce, spinach, and broccoli. As Vitamin K2 (MK-7) would be authorized as another source of Vitamin K, this guidance would not need to change and would also apply to use if Vitamin K2 as another form of Vitamin K.

C.2 Information from Studies in Animals or Humans that is Relevant to the Toxicity of the Nutritive Substance and, if Necessary, its Degradation Products and Major Metabolites

The safety assessment of Vitamin K2 (MK-7) is based on the totality of the available evidence, including human clinical observations and animal experimental studies. The presence of menaquinones, primarily Vitamin K2 (MK-7), in the diet (Natto and cheeses) and the lack of known toxicity associated with dietary exposure to Vitamin K1 or Vitamin K2 supports the safety of Vitamin K2. In addition, relevant safety studies on menaquinones, including MK-7 and other structurally closely related substances, are presented in the following sections. Findings from animal studies for acute, sub-chronic, reproductive and developmental toxicity, and genotoxicity and in vitro studies for mutagenicity and carcinogenicity showed no significant risks associated with exposure to menaquinones, including MK-7.

Acute Toxicity

Pucaj et al. (2011) investigated acute toxic effects (limit dose) of Vitamin K2 (MK-7) in a study conducted according to OECD guidelines. In this study, female NMRI mice (n=5; 8 week old; body weight 25-30 g) nulliparous and non-pregnant were given a single oral dose (gavage) of 2000 mg MK-7/kg bw. No adverse clinical observations as evaluated by changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern (including body weight growth) were noted during the 14-day observation period. The absence of adverse effects or death suggest that the minimum lethal dose of Vitamin K2-7 is greater than 2000 mg/kg bw (Pucaj et al., 2011).

In another study, Ravishankar et al. (2015) investigated the effects of oral administration of Vitamin K2 (MK-7) in Wistar rats. In this study, Vitamin K2 (MK-7) was orally administered at 2000 mg/kg bw to 3 male and 3 female rats. Solubility-related issues prevented doses higher than 2000 mg/kg bw from being administered. Following treatment, rats were observed for the next 14 consecutive days for signs of toxicity. None of the animals showed any adverse clinical signs during the observation period. Similar to the above study, the results of this study suggest that LD50 of Vitamin K2 (MK-7) is >2000 mg/kg bw (Ravishankar et al., 2015).

Short-Term Toxicity

In an acute toxicity study, Ravishankar et al. (2015) investigated the effects of Vitamin K2 (MK-7) in male and female rats (Ravishankar et al., 2015). In this study, Vitamin K2 (MK-7) was orally

administered to rats at dose levels of 0.1, 0.5 or 1 mg/kg body weight per day for 14 days. The animals were monitored for general behaviour, toxic signs and symptoms, and mortality during the experimental period. No physical or behavioural changes, no mortality, no differences in body weight gain, no changes in food consumption, and no clinical signs of toxicity were recorded after 14 days in all dose groups, although 2 of 8 animals had mild irritability. The results of 14-day oral administration of Vitamin K2-7 in an acute toxicity study showed no adverse effects of the test compound on either sex of the rats at any dose (Ravishankar et al., 2015).

Long-Term Toxicity and Carcinogenicity

Pucaj et al. (2011) investigated the potential toxicity of Vitamin K2 (MK-7) in rats following sub-chronic exposure. In this OECD study conducted under GLP, Sprague Dawley rats (10/sex/group) were administered Vitamin K2 (MK-7) via gavage at dose levels of 0 (vehicle control, corn oil), 2.5, 5, and 10 mg/kg bw/day for 90 consecutive days. All generated data, including clinical observations, ophthalmology, clinical pathology, gross necropsy, and histopathology, revealed no compound-related toxicity in rats. Any statistically significant findings in clinical pathology parameters and/or organ weights noted were considered to be within normal biological variation. Based on the findings from this study, the no observed adverse effect level (NOAEL) of Vitamin K2 (MK-7), when administered orally to rats for 90 days, was considered to be equal to 10 mg/kg bw/day, the highest dose tested.

In another sub-chronic toxicity study, Ravishankar et al (2015) investigated the effects of Vitamin K2 (MK-7) in male and female rats. In this study, Vitamin K2 (MK-7) was administered to rats at dose levels of 0.1, 0.5 or 1 mg/kg bw/day for 90 days. The body weight and organ weight and macroscopic appearance of thymus, heart, liver, spleen, kidney, testis, prostate, seminal vesicle, and uterus were within the normal range among study groups. In this study, there were several outcomes where differences were observed between treatment groups. Female rats in the 0.5 and 1 mg/kg bw/day dosing groups showed a significant decrease in liver weight, male rats in the 0.5 and 1 mg/kg bw/day dosing groups showed an elevation in serum uric acid, female rats in the 0.5 and 1 mg/kg bw/day dosing groups and an increased number and size of follicles and changes in the myometrium, and male rats in the 0.5 and 1 mg/kg bw/day dosing group exhibited increased spermatogenesis. These changes were not considered adverse, and a lack of observation of these effects in the other study (Pucaj et al., 2011) support a NOAEL from this study of 1 mg/kg bw/day.

Together, these studies support a NOAEL for Vitamin K2 (MK-7) of 10 mg/kg bw/day, based on the highest dose studied.

Genotoxicity

In an in vitro mutagenicity assay, the potential effects of Vitamin K2 (MK-7) to induce reverse mutation in Salmonella typhimurium strains TA1535, TA97a, TA98, TA100, and TA102 in the presence and absence of metabolic activation system (S9) (Ravishankar et al., 2015). Based upon the preliminary solubility/precipitation and cytotoxicity tests, the strains were exposed to Vitamin K2 (MK-7) in triplicate cultures at the doses of 20, 60, 200, 600, and 2000 mcg/plate, both with and without metabolic activation system (S9). Dimethyl sulfoxide was used as a vehicle. The exposed bacteria were plated onto minimal glucose agar medium supplemented with L-histidine. The plates were incubated at 37°C for 48-72 hours after which the histidine revertant colonies were counted and their frequency was compared with that in the vehicle control group. Concurrent negative control group and positive control groups were also included. Results of this test indicated that the frequencies of histidine revertant colonies at all concentrations of MK-7 in strains TA1535, TA97a, TA98, TA100, and TA102, with and without the presence of a metabolic activation system, were comparable to those observed in the vehicle control group. Positive controls demonstrated sensitivity of the assay with and without metabolic activation. The results of this study suggest that Vitamin K2-7 is not mutagenic in S. typhimurium strains (Ravishankar et al., 2015). In another study, rats were divided into six groups (10/group/sex), of which four groups received Vitamin K2 (MK-7) and two control groups received the vehicle (distilled water containing Tween-20- 2 drops/20 ml) Ravishankar at el., 2015. Of the two vehicle treated control groups, one served as control for the micronucleus test (MNT) and the other was used for the comet assay. In the test article treated groups, two groups received Vitamin K2 (MK-7) at a dose level of 100 mcg/kg bw/day, while the remaining two received Vitamin K2 (MK-7) at 1000 mcg/kg bw/day. All animals were treated daily for 28 consecutive days. On Day 29, all control animals received cyclophosphamide (i.p.) at a dose level of 40 mg/kg bw. The control and test groups maintained for assessment in the comet assay received Colchicine at 4 mg/kg (i.p.) 24 hours after cyclophosphamide administration. Blood was collected from all groups for the comet assay and animals were euthanized on the 30th day of the study. The parameters evaluated included clinical observations, feed consumption (daily), body weight (weekly), chromosomal aberration, micronucleus test, and comet assay. The results of the study suggest that treatment with Vitamin K2 (MK-7) at 100 and 1000 mcg/kg bw/day for 28 days did not produce any clinical signs of toxicity in rats. There were no significant differences in any of the parameters in treatment groups at 100 and 1000 mcg/kg bw at all intervals studied. As compared to the Vitamin K2 (MK-7) treated group, the group receiving cyclophosphamide revealed signs of clinical toxicity and genotoxicity (Ravishankar et al., 2015).

TABLE DESCRIBING ALL OF THE ANIMAL STUDIES

Table 4. Summary of Acute and Subchronic Toxicity Studies with Vitamin K2-7

Reference	Study design	Observations	Results
Pucaj et al. (2011)	Acute oral toxicity test. Vitamin K2-7 suspended in sunflower oil was administered to mice by single oral gavage to achieve a dose of 2000 mg/kg body weight	Mice were weighed at days 0, 7, and 14 (termination). Animals were monitored twice daily on the day of dosing and once daily thereafter. Observations included changes in skin, fur, eyes, mucous membranes, and respiratory, circulatory, autonomic, and central nervous systems. Animals were also observed for changes in motor activity and behavior pattern.	At limit dose level of 2000 mg/kg, Vitamin K2-7 did not induce any signs of toxicity in any of the treated mice following dosing or during the 14-d observation period. Body weight gain of treated mice was not adversely affected. Median LD_{50} was > 2000 mg/kg body weight
Ravishankar et al. (2015)	Acute oral toxicity study in rats. Vitamin K2-7 administered orally by gavage at 0.5, 1.0, 10, or 20 mg/kg bw; once daily for 14 days.	Rats were monitored for general behavior, toxic signs and symptoms, or mortality during the experimental period. At end of study, mice were killed and examined for gross necropsy performed in vital organs	No effect of Vitamin K2-7 on food and water consumption, no physical or behavioral changes, and no mortality observed in any group after 14 day. In the 1 mg/kg group, 2 of 8 animals had mild irritability. No statistically significant difference in body weight gain observed in any group. No adverse effects observed in either sex in any group. All rats survived, with no symptoms of distress or toxic effects. LD_{50} was > 2000 mg/kg body weight
Pucaj et al. (2011)	Subchronic oral study. Rats were	Rats were observed for clinical signs and mortality	No deaths occurred, and no compound-related toxicity was

	given Vitamin K2-7 for 90 d at doses of 0, 2.5, 5.0, and 10 mg/kg body weight per day	twice daily throughout the study and for reaction to treatment such as changes in skin, fur, eyes and mucous membranes. Rats were also monitored for changes in respiratory, circulatory, autonomic, and central nervous systems, for changes in somato-motor activity and behavior patterns, and for any other signs of ill health. Terminal body weights were recorded on day 91-92 for main study animals. During the recovery, animal weights were determined. Hematologic and clinical chemistry data of rats were obtained and compared with baseline data.	indicated by clinical observations or by ophthalmology, clinical pathology, gross necropsy, or histopathology. Any statistical significant differences in clinical pathology parameters and/or organ weights noted were considered to be within normal biological variability. Median LD_{50} = 2000 mg/kg and NOAEL = 10 mg/kg body weight per day
Ravishankar et al. (2015)	Subchronic oral toxicity in rats. Vitamin K2-7 administered orally by gavage at 0, 0.1, 0.5, and 1.0 mg/kg bw/day for 90 days. Vitamin K2-7 prepared fresh daily in propylene glycol and	Rats were monitored for changes in behavior, mortality, body weight, and food consumption. Blood was collected on days 15, 45, and 91 (at sacrifice) to determine fasting blood sugar; levels of serum urea, creatinine, uric acid, total cholesterol, triglycerides, total protein, and serum calcium; albumin-to-globulin ratio, liver enzymes SGOT	Normal weight gain pattern in all groups; slight increased weight gain in rats receiving Vitamin K2-7 but increase was not statistically significant. Male and female rats showed significant weight increase at 90 d in all treatment groups compared with controls. Average weights of organs in male and female rats were not significantly different from those in controls (liver, thymus, kidney, spleen, testis, seminal vesicles,

	administered as 1 mL/100 g body weight between 8 am and 9 am	and SGPT, and alkaline phosphatase activity. Other hematologic parameters measured on those days included total WBC count, total lymphocyte count, total monocyte count, total granulocyte count, lymphocyte percentage, monocyte percentage, granulocyte percentage, RBC count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, RBC distribution width, and clotting time. Urine specific gravity and pH were measured at days 15, 45, and 91 (at sacrifice). After 90 days, rats were killed and autopsied, and histological studies of brain, pituitary, thymus, lymph node, heart, lungs, spleen, seminal vesicles, uterus, skin, trachea, liver, stomach, jejunum, kidney, testis, prostate, and ovary were performed	prostate, and uterus). However, female rats in the 0.5 mg/kg group had a statistically significant decrease in heart weight compared with controls. Liver enzymes (SGPT, SGOT and alkaline phosphatase) and, similarly, serum glucose, total protein, creatinine, and blood urea levels showed no significant changes in any group. Uric acid levels were not changed in females, but in males there was a significant decrease at day 45 in the 0.1mg/kg group. Conversely, levels in both sexes were increased significantly decreased at day 90 in both sexes; hemoglobin levels were generally the same except in males on day 45 in the 0.1 mg/kg group (increased), on day 45 in the 1.0 mg/kg group (decreased), and in control females (decreased). Mean corpuscular hemoglobin concentration, corpuscular volume, and RBC distribution width values and clotting time were not affected. Urinalysis showed no significant changes in specific gravity or pH. Histopathological study showed no remarkable changes in organs of control or treated animals

			except in females, in which proliferation of uterine epithelium was seen at all levels in 1-2 rats, while cytoarchitecture was normal in all other rats. Significant levels at $p<0.05$

Abbreviations: LD_{50} = lethal dose 50; NOAEL = no observed adverse effect level; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvate transaminase; RBC = red blood cell; WBC = white blood cell.

Human Studies

Vitamin K2 (MK-7), and other menaquinones, have been investigated in multiple human clinical studies. In an article on the safety of Vitamin K2 (MK-7), the available evidence on the safety of Vitamin K2 (MK-7) as dietary supplement ingredient was reviewed (Marles et al., 2017). In this review, published clinical trials that made no mention of whether adverse events occurred or of any other aspects of safety were excluded. The following section provides an overview of relevant human studies where safety was considered.

In a double-blind, randomized, placebo-controlled trial with study in 55 healthy pre-pubertal children, van Summeren et al. (2009) investigated the effects of 45 mcg of Vitamin K2 (MK-7)/day for eight weeks on different biomarkers and coagulation-related parameters, including serum levels of MK-7 (van Summeren et al., 2009). The details of study participants were as follows: placebo group consisted of 27 male children- age 6-10 years, average height 133.8 cm, weight 30.4 kg, and BMI 16.8; the MK-7 receiving group consisted of 28 male children- age 6-10 years, average height 132.2 cm, weight 29.2 kg, and BMI 16.6. Bone markers and coagulation parameters remained constant over time in both the placebo and treatment group. The results of this study suggest that oral administration of 45 mcg MK-7/day to healthy, pre-pubertal children for eight weeks increased serum levels of MK-7 and osteocalcin carboxylation without affecting blood coagulation. Periodically the subjects were checked for the occurrence of adverse events of treatment and none were reported.

In a randomized controlled trial, McFarlin et al. (2017) investigated the effects of dietary supplementation of Vitamin K2 (MK-7) on cardiovascular responses to a graded cycle ergometer test. In this study, aerobically trained young (average age 21 years) males and female athletes (n=26) were randomly assigned either to a control group that received a rice flour placebo or to an intervention group that received MK-7. For weeks 1 to 4, participants received 320 mcg MK-7/day; for weeks 5 to 8, they received 160 mcg MK-7/day. MK-7 supplementation was associated with a 12% increase in

maximal cardiac output, with a trend toward an increase in heart-rate AUC. No significant changes occurred in stroke volume. As regards safety, the investigators stated, “At no time during the study did any participant report an adverse effect to taking either the supplement or the placebo.” (McFarlin et al., 2017).

Moller et al. (2016) compared the biological effects of placebo, fermentation-derived Vitamin K2 (MK-7) (90 mcg) and 3 doses of synthetic MK-7 (45, 90 and 180 mcg) in a randomized double-blinded parallel study. In this study, healthy adult subjects (n=43; 20-60 years of age) took one of the supplements daily for 43 days, and the fraction of carboxylated osteocalcin (OC) was compared between day 1 and day 43 as a marker for Vitamin K activity. The serum concentrations of carboxylated OC (cOC) and unOC were increased and reduced, respectively, after daily intake of 180 mcg of synthetic MK-7 for 43 days, indicating increased Vitamin K activity. In this study, 27 subjects reported a total of 40 adverse events; 32 of these were judged unlikely to be related to the study supplement. In two cases, the adverse events were judged possibly to be related to the study supplement: dry mouth from day 4 to the end of the study (180 mcg synthetic MK-7 group) and diarrhea (fermentation-derived MK-7 group). Another case of diarrhea in the fermentation-derived MK-7 group was judged probably to be due to the study supplement. The investigators concluded that the findings provide evidence that the tested synthetic form of MK-7 is bioequivalent to fermentation-derived MK-7, exhibits vitamin K activity and is well tolerated in healthy subjects (Moller et al., 2016).

In a double-blind, placebo-controlled trial, Knapen et al. (2015a) investigated effects of 180 mcg MK-7/day supplementation on arterial stiffness. In this study, healthy postmenopausal women (n=244) received either placebo (n=124) or MK-7 (n=120) for three years. At baseline, desphospho-uncarboxylated matrix Gla-protein (dp-ucMGP) was associated with intima-media thickness (IMT), Diameter, carotid-femoral Pulse Wave Velocity (cfPWV) and with the mean z-scores of acute phase markers (APMscore) and of markers for endothelial dysfunction (EDFscore). After three years of MK-7 supplementation, cfPWV and the Stiffness Index-β significantly decreased in the total group, whereas distension, compliance, distensibility, Young's Modulus, and the local carotid PWV (cPWV) improved in women having a baseline Stiffness Index β above the median of 10.8. MK-7 decreased dp-ucMGP by 50% compared to placebo, but did not influence the markers for acute phase and endothelial dysfunction. The investigators concluded that long-term use of MK-7 supplements improves arterial stiffness in healthy postmenopausal women, especially in women having a high arterial stiffness. The investigators stated that no side-effects have been reported for the long-term use of MK-7 (Knapen et al., 2015a).

In another study, Knapen et al. (2015b) investigated the effects of a Vitamin K2 (MK-7)-fortified yogurt drink (28 mcg MK-7/yogurt drink) on Vitamin K status and markers of vascular health. The yogurt drink

was also fortified with n-3 PUFA, Vitamin D, Vitamin C, Ca and Magnesium to support vascular and/or general health. In this study, 32 healthy men and 28 postmenopausal women with a mean age of 56 ± 5 years received either basic or fortified yogurt drink twice per day for 12 weeks. MK-7 was efficiently absorbed from the fortified yogurt drink. Levels of circulating MK-7 were significantly increased from 0.28 to 1.94 ng/ml. Accordingly, intake of the fortified yogurt drink improved Vitamin K status, as measured by significant decreases in uncarboxylated osteocalcin and dp-ucMGP. No effects were seen on markers of inflammation, endothelial dysfunction and lipid metabolism. No adverse effects were reported (Knapen et al., 2015b).

In a three year study, Knapen et al. (2013) investigated the effects of low-dose Vitamin K2 (MK-7) on bone health. In this study, healthy postmenopausal women (n=244) received placebo or MK-7 (180 mcg/day) capsules for three years. In addition to bone mineral density (BMD) and bone mineral content (BMC), circulating ucOC and cOC were measured (the ucOC/cOC ratio served as marker of Vitamin K status) at baseline and after 1, 2, and 3 years of treatment. MK-7 intake significantly improved Vitamin K status and decreased the age-related decline in BMC and BMD at the lumbar spine and femoral neck, but not at the total hip. Bone strength was also favourably affected by MK-7. MK-7 significantly decreased the loss in vertebral height of the lower thoracic region at the mid-site of the vertebrae. At the end of the study, twelve women in the placebo group and nine women in the MK-7 group had withdrawn from the study. The overall drop-out rate was 8.6%. Few complaints were reported during the study. The complaints in the placebo group were: hair loss and/or brittle nails (n=2), hot flashes (n=1), knee pain (n=1), numb sensation in arms and legs, washed-out (n=1), and weight gain (n=2); and in the MK-7 group: bone pain (n=1), hot flashes (n=1), rash around eyes and ears (n=1), smelly capsules (n=1), and weight gain (n=1). Five women withdrew due to these complaints; four women in the placebo group and one in the MK-7 group. Compliance was measured by capsule counts at the end of every half-year period; the mean compliance for both treatment groups was 97%. The results of this study suggest that MK-7 is well tolerated (Knapen et al., 2013).

In a randomized, double-blind, placebo-controlled trial, Dalmeijer et al. (2012) investigated the effects of Vitamin K2 (MK-7) supplementation on carboxylation of matrix Gla-protein (MGP). In this study, 60 subjects (age 40-65 years) received supplementation of 180, 360 mcg/day of MK-7 or placebo for 12 weeks. At the end of 12 weeks, a significant and dose-dependent decrease in desphospho-uncarboxylated MGP (Dp-ucMGP) was noted groups treated with 180 µg and 360 µg MK-7 (31% and 46%, respectively), while dp-ucMGP levels remained unchanged after placebo treatment. The osteocalcin ratio also decreased significantly after 12-week supplementation with 180 mcg (60%) and 360 mcg (74%) MK-7, while levels remained unchanged after placebo treatment. These results indicate improved vitamin K levels and good compliance to the study treatment. Changes over time of dp-

cMGP and t-ucMGP levels did not differ between treatment arms. Other cardiovascular risk factors did not differ between treatments arms. No adverse effects were reported (Dalmeijer et al., 2012).

Theuwissen et al. (2013) carried out a dose-escalation study to measure the antidotal potency of lower doses (10, 20 and 45 mcg/day) of Vitamin K2 (MK-7) supplements in healthy volunteers stabilized on acenocoumarol, a VKA therapy. In addition to conventional INR measurements, response on thrombin generation and the γ-carboxylation status of specific Gla-proteins with coagulation and noncoagulation functions were monitored. In this study, 18 healthy men and women (age 18-45 years) were anticoagulated for four weeks with acenocoumarol; of these 15 subjects attained a target INR of 2.0. In the six successive weeks, subjects were supplemented with increasing doses of MK-7 (10, 20, 45 mcg/day) while continuing acenocoumarol treatment at established individual doses. Apart from the INR, acenocoumarol treatment significantly increased under-carboxylated forms of prothrombin (ucFII), osteocalcin (ucOC) and matrix Gla-protein (dp-ucMGP), and decreased endogenous thrombin generation (ETP). A daily intake of 45 mcg MK-7 significantly decreased the group mean values of both the INR and ucFII by about 40%. Daily intakes of 10 and 20 mcg MK-7 were independently judged by two hematologists to cause a clinically relevant lowering of the INR in at least 40% and 60% of subjects respectively, and to significantly increase ETP by ~20 and ~30%, respectively. Circulating ucOC and dp-ucMGP were not affected by MK-7 intake. The investigators concluded that MK-7 supplementation at doses as low as 10 mcg (lower than commonly recommended dose of 45 mcg) significantly influenced anticoagulation sensitivity in some individuals. Hence, the investigators recommended avoiding use of MK-7 supplements in patients on VKA therapy (Theuwissen et al., 2013).

Theuwissen et al. (2012) investigated the dose-response effects of extra intake of Vitamin K2 (MK-7) on the carboxylation of extra-hepatic vitamin K-dependent proteins in a double-blind, randomized, controlled trial. In this study, a total of 42 healthy adult men and women (age 18 to 45 years) were randomized into seven groups to receive: placebo capsules or MK-7 capsules at a dose of 10, 20, 45, 90, 180 or 360 mcg/day for 3 months. Circulating ucOC, OC and desphospho-uncarboxylated MGP (ucMGP) were measured. As the study was conducted with few participants, in order to increase the statistical power, the researchers collapsed the treatment groups into three dosage groups: placebo, low-dose supplementation (doses below RDA), and high-dose supplementation (doses around RDA). The results of this study showed that MK-7 supplementation at relatively low doses in the order of the RDA increased the carboxylation of circulating OC and MGP. No adverse effects on thrombin generation (blood clotting) were observed (Theuwissen et al., 2012).

In a double-blind, randomized, placebo-controlled trial, Emaus et al. (2010) investigated the effects of Vitamin K2 (MK-7) supplementation on bone mineral density in healthy postmenopausal Norwegian women. In this study, 344 healthy women (ages- 50 to 60 years, 1-5 years after menopause) were

recruited and randomly assigned into two groups, one receiving 360 mcg MK-7 in the form of Natto-derived MK-7 capsules (treatment group- age: 54.7±2.5; weight: 67.5±9.0) and the other with placebo (age: 54.2±2.5 weight: 67.5±9.8) capsules containing olive oil. The subjects were treated daily for 12 months. In the treatment and placebo group, 131 and 133 subjects completed the study, respectively. At baseline and 12 months after supplementation, BMD was measured at total hip, femoral neck, lumbar spine and total body together with serum levels of bone-specific alkaline phosphatase, Crosslaps, total osteocalcin, cOC and ucOC. No statistical differences in bone loss rates between the groups at the total hip or any other measurement site were noted at the end of 12 months. Serum levels of cOC increased and ucOC decreased in the treatment versus the placebo group. No treatment related significant adverse effects of MK-7 were noted. The results of this study suggest that daily ingestion of 360 mcg MK-7/day for one year is safe (Emaus et al., 2012).

Additional Human Studies

Novozymes A/S have conducted additional human clinical trials with Vitamin K2 (MK-7) in subjects at nutritional risk and/or specific medical conditions.

Study 1

A preliminary open labeled observational study conducted showed that daily oral dose of 100 mcg of Vitamin K2 (MK-7) for 3 months was associated with a reduction in the frequency, intensity, and duration of idiopathic muscle cramps (Mehta et al, 2010). This study was conducted on 21 patients aged 18 to 81 of both sexes. Administration of Vitamin K2 (MK-7) was found to be well tolerated and there were no reports of adverse events.

Study 2

A study was conducted on 30 patients to evaluate the effects and tolerability of administration of Vitamin K2 (MK-7) for two months in patients with Peripheral Neuropathy (PN) due to vitamin B12 deficiency and/or diabetes mellitus (Kulkarni et al., 2013). Vitamin K2 capsules of 100 mcg each were given to these 30 patients twice a day for two months. Administration of Vitamin K2 (MK-7) was found to be well tolerated and there were no adverse events were reported during the period of therapy.

Study 3

An open labeled study of Vitamin K2 (MK-7) was conducted in 100 patients with Peripheral Neuropathy (PN) suffering from either Vitamin B12 deficiency and/or diabetes mellitus (Mehta et al., 2018). Subjects were administered 200 mcg of Vitamin K2 orally for 8 weeks and the patients were followed for an additional 4 weeks. Administration of Vitamin K2 (MK-7) was found to be well tolerated and there were no adverse events were reported during the period of therapy.

Study 4

A double-blind, placebo-controlled trial was conducted in 60 patients presenting with Peripheral Neuropathy (PN) suffering from either vitamin B12 deficiency and/or diabetes mellitus (Vladimir et al, 2021). The subjects were administered either 200 mcg of Vitamin K2 (MK-7) or placebo control for 8 weeks, and then followed for an additional 4 weeks. Administration of Vitamin K2 (MK-7) was found to be well tolerated and there were no adverse events were reported during the period of therapy.

Study 6

A double-blind, placebo-controlled was conducted in patients with Peripheral Neuropathy (PN) suffering from either Vitamin B12 deficiency and/or diabetes mellitus with diabetes mellitus (Mehta et al., 2021). In this study, 20 patients were administered either 200 mcg of Vitamin K2 (MK-7) capsules

or a placebo control orally for 8 weeks and the patients were followed up to 12 weeks. Circulating levels of Vitamin K2 were also measured in this study, and results showed that administration of Vitamin K2 resulted in increased circulating levels of Vitamin K2 by the 4^{th} and 8^{th} week.

Study 7

In an Observational study (case series) in 17 Multiple Myeloma patients (age; 18-65 years) having drug-induced (caused by chemotherapy) Peripheral Neuropathy, showed significant relief in the symptoms of peripheral neuropathy after daily administration of Vitamin K2-7 capsules (Bhave et al, 2019).

Recently published Human Studies

In a Randomized Controlled Trial by Nahid (Karamzad et al 2020), it was shown that Vitamin K2-7 supplementation seems to be effective in the improvement of glycemic indices, but not the lipid profile of patients with type 2 diabetes mellitus. In another study by Nahid Karamzad et al (2022), it was observed that Vitamin K2-7 supplementation can be effective in improving PIVKAII levels, of patients with type 2 diabetes mellitus. Habitual natto (particularly rich in Vitamin K2-7) intake is associated with a reduced risk of osteoporotic fractures independent of confounding factors, including bone mineral density, in Japanese postmenopausal women (Kojima A et al 2020). In a one year followup randomized trial, oral administration of vitamin K2-7 in patients on haemodialysis patients reduced serum uc-MGP levels (Oikonomaki T et al 2019). MGP (Matrix Gla Proteins) are one of the most potent inhibitors of vascular calcification. In a randomized controlled trial on pediatric patients on regular hemodialysis, it was observed that Vitamin K2-7 and native vitamin D showed a beneficial effect on calcification regulators (Radwa B, 2022). In a randomized controlled trial (Rahimi Sakak F et al 2020) in individuals with type 2 diabetes, daily intake of Vitamin K2-7 360 mcg for 12-weeks reduces fasting plasma glucose (p-adjusted = 0.031) and glycated hemoglobin (p-adjusted = 0.004). in a 3-year randomized, placebo-controlled clinical trial in postmenopausal women with osteopenia, treatment of Vitamin K2-7 375 mcg daily as an add-on to calcium and vitamin D increased carboxylation of osteocalcin. Vitamin K2-7 acts as a cofactor in the carboxylation of osteocalcin (OC) and carboxylated OC promotes mineralization of bone (Rønn SH et al, 2020). In another randomized placebo-controlled trial by Rønn SH et al (2021), in postmenopausal women who received Vitamin K2-7 375 mcg daily or placebo, as an add-on to calcium and vitamin D for 12 months decreased uncarboxylated osteocalcin and increased p-adiponectin but there was no change in insulin sensitivity. In a prospective cohort study in children with acute lymphoblastic leukemia, an early beneficial effect of the combination of Vitamin K2-7 and vitamin D3 on BMD in all patients especially during the period of intensive steroid

therapy in the first month was observed (Solmaz I, 2021). In a randomized, double-blind, placebo-controlled clinical trial, 84 polycystic ovary syndrome (PCOS) patients, beneficial effects of Vitamin K2-7 on insulin resistance, fat mass, skeletal muscle, and serum levels of triglyceride, dihydrotestosterone, and sex hormone binding globulin were observed (Tarkesh F et al, 2020). In addition to this, a significant improvement in the depression status (P = 0.012) in comparison to placebo was also observed (Tarkesh F et al, 2022) in PCOS. In A prospective multicenter, randomized, controlled, crossover pilot trial by Xu D et al (2022), in hemodialysis patients (N=523), Vitamin K2-7 supplementation decreased the frequency, duration, and severity of muscle cramps. In a prospective and nonrandomized concurrent controlled trial, in patients with osteopenia or osteoporosis who underwent transforaminal lumbar interbody fusion (TLIF) or posterior lumbar interbody fusion (PLIF), concurrent Treatment with Vitamin K2-7 and D3, increased lumbar interbody fusion rates, improved clinical symptoms, promoted bone information, and avoided further decline in BMD within six months after TLIF or PLIF Level of Evidence: 3 (Zhang W et al 2022).

TABLE DESCRIBING ALL OF THE HUMAN STUDIES

Table 5. Summary of the Human Studies with Vitamin K2-7

Clinical study design	Demographic characteristics	Dose	Length of treatment	Endpoint(s)	Reference
Published human studies					
a. Single-dose oral bioavailability b. Escalating dose-response c. Randomized crossover d. Nonrandomized drug interaction study	Healthy men and women 25-35 y; in trial 4, subjects were treated with individualized dose of acenocoumarol to reach target INR value of 2.0 within 3 week, then maintained at stabilizing dose of acenocoumarol while treated	a. MK-7 and K_1 at 2000 mcg each b. MK-7 and K_1 at 50, 100, 150, 200, 250, 300, and 500 mcg each c. MK-7 at 143 mcg/d, K_1 at 99 mcg/d	a. Once b. Once; 2 week washout between doses c. 6 week d. 1 week at each dose level	Comparison of absorption and efficacy (osteocalcin carboxylation) of synthetic vitamin K_1 and natto-derived MK-7	Schurgers et al., 2007

	with escalating doses of MK-7 or K_1	d. MK-7 at 97.4 mcg/d with weekly increment of 97.4 mcg and K_1 at 49.6 mcg/d with weekly increment of 49.6 mcg			
Randomized, partly single-blind, partly open-label bioavailability	Healthy men and postmenopausal women, 45-65 y	MK-7 at 71.2 mcg/d (in yogurt) or 58.3 mcg (in capsule)	42 day	Effect of supplemental MK-7 in yogurts or capsules on fasting plasma MK-7 concentrations	Knapen et al., 2016
Randomized, double-blind, placebo-controlled	Healthy children, 6-19 y, healthy adults, 20-80 y, divided into age groups of 10 y increments; selected for supplementation if circulating values of uc-OC or dp-uc-MGP were significantly higher than those of young	Children: MK-7 at 0 or 45 mcg/d Adults: MK-7 at 0 or 90 mcg/d Linseed oil, casein, or gum Arabic used as carrier	Children: 8 week Adults: 7 week	Effect of MK-7 supplementation on serum uc-OC and dp-uc-MGP	Theuwissen et al., 2013

	healthy adults, 20-29 y				
Randomized controlled trial; conducted in two phases of 4 weeks each	Healthy athletes; average age 21 years	Phase I-320 mcg/day; Phase II-160 mcg/day for 4 weeks	Total 8 weeks; Phase I 4 weeks and Phase II 4 weeks	Effects of supplemental MK-7 on heart rate, stroke volume, cardiac output, oxygen consumption, blood lactate, and ventilation	McFarlin et al., 2017
Randomized, double-blind, placebo controlled trial	Postmenopausal women with osteopenia; average age 67 years	375 mcg/day	For 12 months	Effects of supplemental MK-7 on mineral density (BMD), bone microarchitecture and biochemical bone turnover markers	Ronn et al., 2016
Randomized, double-blind, placebo-controlled, parallel	Healthy postmenopausal women, 55-65 y	MK-7 at 180 mcg/d	3 years	Effect on MK-7 on serum uc-OC and c-OC concentrations and efficacy to decrease bone loss	Knapen et al., 2013
Randomized, double-blind, placebo-controlled	Healthy men and healthy postmenopausal women, 40-60 y	MK-7 at 0, 180, and 360 mcg/d	12 week	Effect of MK-7 on circulating dp-uc-MGP and dp-c-MGP and on total uc-MGP, uc-OC, and c-OC	Dalmeijer et al., 2012
Randomized, double-blind, placebo-controlled exploratory pilot	Healthy men and women, 18-45 y	MK-7 at 0, 10, 20, 45, 90, 180, or 360 mcg/d	12 week	Estimation of dose-response effects of MK-7 supplementation on (a) carboxylation of osteocalcin and MGP (b) thrombin	Theuwissen et al., 2012

				generation as an indicator of safety	
Randomized, double-blind, placebo-controlled	Healthy women, 50-60 y; 1-5 y after menopause	MK-7 at 0 and 360 mcg/d	12 months	Effect of MK-7 supplementation on rate of bone loss among healthy postmenopausal women	Emaus et al., 2010
Randomized, double-blind, placebo-controlled	Healthy postmenopausal women, 50-69 y Healthy men and women, 20-69 y	MK-7 at 0, 50, 100 or 200 mcg/d MK-7 at 0 or 100 mcg/d	4 weeks 12 weeks	Dose finding and efficacy of low dose daily MK-7 supplementation to improve osteocalcin γ-carboxylation	Inaba et al., 2015
Randomized, double-blind, placebo-controlled parallel	Healthy postmenopausal women, 55-65 y	MK-7 at 180 mcg/d	3 years	Effect of MK-7 on arterial stiffness in healthy postmenopausal women	Knapen et al., 2015
Randomized, single-blind, dose-finding intervention	Chronic hemodialysis patients in stable medical condition; men and women, ≥ 18 y	MK-7 at 360, 720, or 1080 mcg, 3 times weekly	8 week	Determination of optimum dose of MK-7 for activation of vitamin K-dependent MGP by measuring reduction of inactive dp-uc-MGP	Caluwe et al., 2014
Nonrandomized prospective pilot study	Pediatric thalassemic osteopathy patients, 3-18 y	MK-7 at 50 mcg + calcitriol at 5 mcg/d	12 months	Efficacy of MK-7 and calcitriol combination to reduce thalassemic osteopathy by improving bone mineral density and z	Ozdemir et al., 2013

				score of lumbar spine	
a. Cross-sectional analysis b. Randomized, double-blind, placebo-controlled, dose-response c. Randomized, double-blind, placebo-controlled	a. Healthy postmenopausal women, 55-65 y b. Healthy postmenopausal women and healthy men, 25-45 y c. Healthy postmenopausal women, 55-75 y	a. Untreated b. MK-7 at 0, 10, 20, 45, 90, 180, or 360 mcg/d c. MK-4 at 0 or 45 mg/d	a. Not applicable b. 12 week c. 3 years	a. Vitamin K status with circulating adiponectin and body composition b. Minimal effective dose for effect on circulating osteocalcin and adiponectin c. Effect of MK-4 on bone loss, bone geometry, body weight, and body composition	Knapen et al., 2012
Non-randomized, non-blinded, bioavailability	Healthy adults, mean age 37 y (SD = 3)	MK-7 at 0, 45, and 90 mcg/d	2 week for each treatment, separated by 2 week washout period	Bioavailability of MK-7 in olive oil (MK-7 plasma levels) and effect on osteocalcin and its carboxylation status	Brugè et al., 2011
Randomized, double-blind, placebo-controlled, prospective, longitudinal	Transplant patients at risk for osteoporosis; stratified by heart vs. lung transplant, men and women ≤ 50 y vs. > 50 y, and sex	MK-7 at 0 or 180 mcg/d	12 months	Effect of MK-7 on bone mass in the 1st year after lung or heart transplant	Forli et al., 2010
Additional Human Studies – Published by Novozymes					
Open-label	Patients of diabetes mellitus (N=7; male, 3 and female, 4)	Vitamin K2-7 capsules (100 mcg/	8 weeks	Effect of MK-7 on peripheral neuropathy due to diabetes mellitus	Kulkarni VK et al 2013

	and/or megaloblastic anaemia (vitamin B12 deficient: N=23; male, 13 and female, 10) with peripheral neuropathy	capsule twice a day)		and/or megaloblastic anaemia	
Open-label	Patients of type 2 diabetes mellitus (N=53; male, 30 and female, 23) or vitamin B12 deficiency N=47; male, 21 and female, 26) with peripheral neuropathy	Vitamin K2-7 capsules (100 mcg/ capsule twice a day)	8 weeks	Effect of MK-7 on peripheral neuropathy due to diabetes mellitus and/or megaloblastic anaemia	Mehta DS et al 2018
Ran domized double-blind placebo controlled trial	Patients (N=60) with peripheral neuropathy suffering from either vitamin B12 deficiency and/or diabetes mellitus	Vitamin K2-7 capsules (100 mcg/ capsule twice a day)	8 weeks	Effect of MK-7 on peripheral neuropathy due to diabetes mellitus and/or megaloblastic anaemia	Vladimir Badmaev et al 2021
Ran domized double-blind placebo controlled trial	Patients (N=20) with peripheral neuropathy suffering from either vitamin B12 deficiency and/or diabetes mellitus	Vitamin K2-7 capsules (100 mcg/ capsule twice a day)	8 weeks	Effect of MK-7 on peripheral neuropathy due to diabetes mellitus and/or megaloblastic anaemia	Mehta DS et al 2021

Open label	Patients (N=17) with multiple myeloma having drug-induced (caused by chemotherapy) peripheral neuropathy	Two capsules (100 mcg or 350 mcg each) a day of vitamin K2-7	In between two chemotherapy cycles	Effect of MK-7 on drug-induced (caused by chemotherapy) peripheral neuropathy with Multiple myeloma	Bhave AA et al 2019
Open label	Patients with idiopathic muscle cramps (N =21)	Vitamin K2-7 capsules (100 mcg/ capsule twice a day)	3 months	Effect of MK-7 on frequency, intensity, and duration of idiopathic muscle cramps	Mehta et al, 2010

In summary, Vitamin K2 (MK-7) has been extensively investigated in over 25 clinical trials, with over 2000 participants. Several of these trials were double-blind, placebo-controlled that are the most likely to capture any adverse effects in order to support the safety of MK-7 in a diverse population. Human clinical studies in which MK-7 was administered up to 180 mcg/day for 3 years, or up to 360 mcg/day for 12 weeks, or up to 1080 mcg thrice weekly for 8 weeks did not reveal any significant adverse effects compared with placebo. Adverse effects specifically attributed to MK-7 were limited to gastrointestinal upset associated with the product's smell. The available information from multiple clinical trials suggest that MK-7 is unlikely to cause any adverse effects in human subjects.

C.3 Safety Assessment Reports Prepared by International Agencies or Other National Government Agencies, if Available

The safety assessment of Vitamin K2-7 (Menaquinone-7) has been evaluated by European Food Safety Authority.

European Food Safety Authority (EFSA)

The European Food Safety Authority concluded that Vitamin K2 (menaquinone) poses no safety concern when used in foods for the general population (including food supplements) and foods for particular nutritional uses (other than baby foods and infant formula) EFSA J. 2008. In this opinion,

EFSA considered the estimated mean daily intake of Vitamin K2 (menaquinone) ranged from 36 mcg (female adults) to 54 mcg (male teenagers), with high intake levels ranging from 75 mcg/day (children) to 115 mcg/day (male teenagers).

United States Food and Drug Administration

The United States Food and Drug Administration reviewed a notification for use of Vitamin K2 (MK-7) in nutritional beverage products intended for consumers ages 1-13 years old, at levels up to 4 mcg per serving. The US FDA concluded that they have no further questions regarding the GRAS determination for use in this product category, which is the closest correlate to a Foods for Special Medical Purposes category in the United States, and represents the same products as what is covered in this application.

Institute of Medicine (IOM) Report

The available scientific literature on phylloquinone (Vitamin K1) and menaquinones (Vitamin K2) was extensively reviewed by the Institute of Medicine IOM. 2000. As regards menaquinone, the IOM report mentions that the human gastrointestinal tract contains a large amount of bacterially produced menaquinones IOM. 2000. However, their contribution to the maintenance of Vitamin K status has been difficult to assess. The IOM report also suggests that long-chain menaquinones (such as menaquinone-7), can serve as active forms of Vitamin K, but they are not widely distributed foods commonly consumed in the United States. The IOM report concludes that there is no evidence of toxicity associated with the intake of either the phylloquinone or menaquinone forms of Vitamin K.

D. Information on Dietary Intake of the Nutritive Substance

D.1 A Detailed List of the Food Groups or Foods in Which the Use of a Nutritive Substance is Proposed, or Changes to Currently Permitted Foods in Which a Nutritive Substance is Used

MK-7 is intended to be used in FSMPs for children and adults that are designed to manage an individual's medically determined nutritional requirements.

D.2 The Maximum Proposed Level of the Use of the Nutritive Substance for Each Food Group or Food, or the proposed Changes to the Currently Permitted Use Levels

The current FSANZ code (S29-21) does not stipulate a maximum limit for nutritive substances and Vitamin K2 is intended for use as an alternative source of Vitamin K. There is no proposal to change the reference to currently permitted use levels of Vitamin K as specified in section S29—21.

D.3 For Foods or Food Groups Not Currently Listed in the Most Recent Australian or New Zealand National Nutrition Surveys (NNSs), Information on the Likely Level of Consumption

Vitamin K2 (MK-7) is being proposed as an alternative source of Vitamin K, in addition to the current Vitamin K source, phylloquinone (Vitamin K1). As an alternative source of Vitamin K, authorization of Vitamin K2 (MK-7) would not change the level of consumption of total Vitamin K. There are no available studies on the intake of Vitamin K2 in Australia or New Zealand from sources inherent in commonly consumed food. While many studies exist for Asian countries where consumption of fermented soy products represents the most abundant source of inherent Vitamin K2 in food, several studies from Europe have also reported the dietary intake of menaquinones (including MK-7) from dietary sources including cheese (Schurgers et al., 2000) that are more relevant for the populations of Australia and New Zealand.

The main source of menaquinones in the Western countries is cheese (e.g., American cheese, Kraft, Land O'Lakes, Cheddar, Mozzarella, Muenster, Jarslberg, Pecorino Romano, Goat milk cheese and Provolone cheese), pork, fish (e.g., eel, plaice) and buckwheat bread. The evidence available suggests that dairy products are likely the predominant dietary sources of long-chain MKs. Cheese and milk products were estimated to contribute to 54% and 22% of total MK intake, respectively, in a cohort of Dutch women in whom long-chain MKs were estimated to account for 9% of the total vitamin K intake (Marles et al., 2017).

While Natto has been reported to contain >100 times more MK-7 than most cheeses, the contribution to Vitamin K2 intake was still substantial (Katsuyama et al., 2002). Depending on the type of cheese, menaquinones, such as MK-6, MK-7, MK-8 and MK-9 are found in varying ratios, especially in fermented cheeses. Long-chain menaquinones (such as MK-7, MK-8, and MK-9) found in Natto are also present in several other foods (Schurgers et al., 1999; Schurgers et al., 2007). These data indicate that Vitamin K2 (menaquinone) intake can comprise between 10-25% of the total Vitamin K intake (Beulens et al., 2013).

D.4 The Percentage of the Food Group to Which the Use of the Nutritive Substance is Proposed or the Percentage of the Market Likely to Use the Nutritive Substance

Vitamin K2 (MK-7) is proposed to be added to FSMPs, which represent a relatively small category in the context of the market for food products generally. FSMPs face unique compositional permissions compared with other foods as they are designed specifically to assist in the dietary management of a patient's medically determined nutrient requirements. They are intended to be consumed in the context of specialized medical supervision, rather than as a discretionary food. If this application is approved, both Vitamin K1 and Vitamin K2 (MK-7) would be permitted for use in FSMP products as a source of Vitamin K. This will likely result in some percentage of FSMP products using Vitamin K2, but it is unlikely that Vitamin K2 would fully replace Vitamin K1 as a source of Vitamin K in FSMP products.

D.5 Information Relating to the Use of the Nutritive Substance in Other Countries

For centuries, Vitamin K2 (MK-7) intake has occurred through inherent levels in commonly consumed foods, primarily from Natto and other fermented soy products. Natto is a traditional Japanese food made from fermenting soybeans with *Bacillus subtilis*, and concentrations of Vitamin K2 in this food product have been shown to range between 780 to 2100 mcg/100 g (Sumi, H. 1999; Schurgers et al., 1999; Schurgers at al., 2000; Tsukamoto et al., 2000; Tsukamoto et al., 2000a; Kaneki at il., 2001; Katsuyama at el., 2004). Similar traditional soybean foods fermented with Bacillus subtilis has been reported in other countries such as China, Korea, Thailand, Nepal and India (Himalayan regions of West Bengal and Sikkim). In addition to Natto, MK-7 is present in certain cheeses, pork, steak, buckwheat bread and eel, all of which have a long history of consumption.

In an analysis of 58 different food items (Kamao et al., 2007) for the presence of phylloquinone and menaquinones, the major contributors to the total daily Vitamin K intake of young women living in eastern Japan was Vitamin K1 from vegetables and algae, and Vitamin K2 from pulses such as peas,

beans and lentils (including fermented soybean foods). The average daily consumption of Vitamin K for women aged 18-29 years living in eastern Japan was estimated to be 230 mcg/person, with the contributions of K1, MK-4, and MK-7 to total Vitamin K intake being 67.7%, 7.3%, and 24.9%, respectively. Based on this analysis, the daily background average intake of Vitamin K2 (MK-7) in this population was estimated at 57 mcg per person. For Natto eaters, the average daily intake was estimated at 133.2 mcg/person (Kamao at el., 2007). The presence of MK-7 in three preparations of fermented soybean Natto, Hikiwari (chopped) Natto, and black-bean Natto was reported as 939 ± 753, 827 ± 194, and 796 ± 93 mcg/100 g, respectively Kamao at el., 2007.

D.6 For Foods Where Consumption has Changed in Recent Years, Information on Likely Current Food Consumption

Not Applicable

E. Information Related to the Nutritional Impact of a Vitamin or Mineral

E.1 Information to Demonstrate a Need to Permit the Addition of a Vitamin or Mineral to Food

The need for Vitamin K is demonstrated by the RDIs issued by FSANZ and the NRVs issued by NHMRC, both recommending dietary intake of Vitamin K. Vitamin K2 (MK-7) is proposed as an alternative source of Vitamin K for use in Foods for Special Medical Purposes.

E.2 Information to Demonstrate the Permitted Addition of the Vitamin or Mineral has the Potential to Address the Deficit or Deliver a Health Benefit to the Population or a Population Subgroup

Vitamin K2 (MK-7) provides a safe and nutritionally efficient alternative source of Vitamin K. Vitamin K2 has been recognized as a source of Vitamin K by several authoritative bodies, and therefore should be allowed as an alternative source of Vitamin K in Foods for Special Medical Purposes.

F. Information Related to the Nutritional Impact of a Nutritive Substance Other Than Vitamins and Minerals

Not applicable

G. Information Related to Potential Impact on Consumer Understanding and Behavior

G.1 Information to Demonstrate the Level of Consumer Awareness and Understanding of the Nutritive Substances in the Food(s)

Vitamin K2 (MK-7) is intended to be added to Foods for Special Medical Purposes as a permitted form of Vitamin K. Vitamin K2 would appear on labels as another source of Vitamin K, and would not be listed separate on the nutritional facts for the product. As this is solely an alternative form of a Vitamin currently being added to Foods for Special Medical Purposes, and addition of this substance an alternative source would not have a meaningful impact on how information about Vitamin K is communicated to consumers, there would be no impact to consumer awareness or understanding.

G.2 Information on the Actual or Potential Behavior of Consumers in Response to Proposed Food(s)

Addition of Vitamin K2 (MK-7) as an alternative source of Vitamin K would not impact behavior of consumers. The amount of Vitamin K2 would be included as Vitamin K on product labels, and therefore would be a transparent change to consumers.

G.3 Information to Demonstrate That the Consumption of Food(s) Containing the Nutritive Substance Will Not Adversely Affect Any Population Groups (e.g. Particular Age or Cultural Groups)

Vitamin K2 (MK-7) has been demonstrated to be safe in a broad range of *in vitro*, animal, and human clinical studies. As an alternative source of Vitamin K, there would be no adverse effect on any population groups.

Special Purpose Foods – Other Foods (Section 3.6.3)

A. Information Related to General Compositional Requirements

A.1 Information on the Identity and Physical and Physiological Need of the Target Population

Vitamin K2 (MK-7) is well accepted as a source of Vitamin K (nutritive substance) and has been incorporated in several hundred products globally including foods and food supplements.

This Application requests that Vitamin K2 (MK-7) be listed as a permitted form of Vitamin K under Section S29-20 for use in FSMPs. Vitamin K2 (MK-7) will be present in FSMPs in which Vitamin K is required to achieve the stated medical purpose.

A.2 Purpose of the Compositional Change

Vitamin K2 (MK-7) is a safe and nutritionally efficient alternative form of Vitamin K, and will therefore benefit consumers who require FSMPs that have been fortified with Vitamin K. This Application is not for a compositional change, but rather for the addition of a new alternative form of Vitamin K that manufacturers may choose to use in FSMPs where appropriate to achieve the stated medical purpose.

A.3 Information Related to the Safety of the Proposed Compositional Change

No compositional change proposed.

A.4 Information Related to the Nutritional Impact or Performance Impact of the Proposed Compositional Change

No compositional change proposed.

B. Information Related to the Dietary Intake or Dietary Exposure

B.1 Data to Enable the Dietary Exposure of the Target Population to Be Estimated

This Application does not propose any amendment to the mandatory compositional requirements for FSMPs. As such, we would not expect Vitamin K2 exposure of the target population to exceed Vitamin K consumption from current sources (Vitamin K1).

B.2 Data on the Recommended Level of Consumption of the Special Purpose Food for the Target Population

The unique usage case of FSMPs means that the level of consumption of Vitamin K through FSMPs will depend on the formulation that is required for the specific medical purpose that the product is intended to perform. FSMP products will also be consumed in the context of specialized medical supervision.

C. Information Related to Labelling Requirements Under Part 2.9 of the Code

C.1 Information Related to Safety or Nutritional Impact of the Proposed Labelling Change

We do not believe that any labelling change is necessary. The addition of Vitamin K2 (MK-7) as additional permitted form of Vitamin K would be adequately addressed through current labelling requirements for FSMPs.

C.2 Information to Demonstrate That the Proposed Labelling Change Will be Understood and Will Assist Consumers, if Applicable

We do not believe that any labelling change is necessary. The addition of Vitamin K2 (MK-7) as additional permitted form of Vitamin K would be adequately addressed through current labelling requirements for FSMPs.

D. Information Related to Internationally Recognised Codes of Practice and Guidelines

This Application is for the addition of Vitamin K2 (MK-7) as a permitted form of Vitamin K for use in FSMPs.

We note that any use of Vitamin K2 (MK-7) in FSMPs would be required to comply with Standard 2.9.5 and other relevant parts of the Code, which also reflect international standards such as EU and *Codex Alimentarius*. This update to the code would allow better harmonization with other global regulations, such as in the European Union (via (EU) No 609/2013) and United States (via GRAS) that currently permit Vitamin K2 (MK-7) as a form of Vitamin K.

To ensure access to FSMPs that are formulated with the most up to date science and harmonization with other global regulations, the Applicant would ask FSANZ to consider further refinement of Section 2.9.5-6(c) to remove any regulatory uncertainty.

Appendix:

Appendix 1 Checklists

Appendix 2 Specification

Appendix 3 Taxonomical identification certificate

Appendix 4 Stability study data

Appendix 5 Certificate of analysis

Reference:

Bhave AA, Mehta DS, Dound YA, Jadhav SS, Vaidya RA, Vaidya AD. Vitamin K2-7, a Promising Solution to Peripheral Neuropathy aroused due to Multiple Myeloma Treatment. The Indian Practitioner. 2019 Jan 1;72(1):16-20.

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