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(CIN : U24230MH2004PTC145390)
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Phone No: +91 22 6266 9600. **Email:** info@synergialifesciences.com

#887

October 8, 2019

RECEIVED
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OFFICE OF
FOOD ADDITIVE SAFETY

Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Campus Drive
College Park, MD 20740

Subject: GRAS Notification for the intended use of Menaquinone-7 (MK-7) in oral nutritional supplements

Dear Sir/Madam:

In accordance with 21 CFR part 170, subpart E, Synergia Life Sciences Pvt. Ltd., (Synergia), Mumbai, INDIA, hereby submits the enclosed notice of a claim that the food ingredient Vitamin K2-7 (Menaquinone-7), derived from *Bacillus licheniformis* preparation for use in Foods for Special Dietary Use (21 CFR 105.3) in oral nutritional supplements such as PediaSure® for children (1 to 13 years of age) as described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be Generally Recognized As Safe (GRAS), based on scientific procedures.

As required, please find enclosed three copies of the notification. If you have any questions or require additional information, please feel free to contact me by phone at +91 22 62669600 or by email at info@viridisbiopharma.com and anmolashit@hotmail.com.

Sincerely,

Ashit Vora
Director

Enclosure: Three copies of GRAS notification

#887

CONCLUSION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF VITAMIN K2 (MENAQUINONE-7) FOR USE IN ORAL NUTRITIONAL SUPPLEMENTS FOR CHILDREN

Prepared for:
Synergia Life Sciences Pvt. Ltd.,
6/312, Jogani Industrial complex,
V.N. Purav Marg, Chunabhatti,
Mumbai - 400022
INDIA

Panel Members

Leon J. Schurgers, Ph.D.
Robert L. Martin., Ph.D.
John A. Thomas, Ph.D., FATS, FACT
Madhusudan G. Soni, PhD, FACN, FATS

September 27, 2019

CONCLUSION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF VITAMIN K2 (MENAQUINONE-7) FOR USE IN ORAL NUTRITIONAL SUPPLEMENTS FOR CHILDREN

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1. PART I- SIGNED STATEMENTS AND CERTIFICATION

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through §170.285, Synergia Life Sciences Pvt. Ltd., (Synergia) hereby informs the FDA that Vitamin K2-7 (Menaquinone-7), derived from *Bacillus licheniformis*, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Synergia's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described below.

It should be noted that the proposed use of Menaquinone-7 (MK-7) is used along with vitamin K1 (phylloquinone) in Foods for Special Dietary Use (21 CFR 105.3) in oral nutritional supplements such as PediaSure® for children (1 to 13 years of age). As Vitamin K2-7 (Menaquinone-7; MK-7) is used along with vitamin K1, the safety in use of total vitamin K is also considered.

1.1. Basis of Conclusion:

This GRAS conclusion for the use of MK-7 has been reached in accordance with requirements in 21 CFR 170.220.

1.3. Name and address of organization:

Synergia Life Sciences Pvt. Ltd
6/312, Jogani Industrial complex,
V.N. Purav Marg, Chunabhatti,
Mumbai – 400 022
INDIA

1.4. Name of substance:

The name of the substance of this GRAS assessment is Vitamin K2-7 (Menaquinone-7; MenaquinGold®). Additionally, the substance is also known as MK-7; Vitamin K2-7; Vitamin MK-7.

1.5. Intended conditions of use:

Menaquinone-7 (MK-7; MenaquinGold®) is intended to be used as a food ingredient and as a nutrient [21 CFR § 170.3(o)(20)][1] in an oral nutritional supplement product or foods for special dietary uses (such as PediaSure®) that will be targeted to children (non-infants; ages 1-13 years). The intended use levels are 4 mcg *per* serving. The uses of both vitamin K1 and MK-7 are as follows: Age range: 1-13 years old; Serving size: 8 fl oz. (or 237 ml); Vitamin K1 per serving: 16 mcg; Vitamin K2-7 per serving: 4 mcg; Caloric density 1 kcal/ml or 237 kcal/serving; Serving per day (3 scenario): (1) Maximum- Each child receives their daily caloric requirements from consumption of this product alone, (2) Typical- One serving/day, (3) High- 6 servings/day (applicable only to children 4-8 years and 9-13 years).

[1] "Nutrient supplements": Substances which are necessary for the body's nutritional and metabolic processes.

1.6. Statutory Basis for GRAS conclusion:

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and 170.30(b).

1.7. Exemption from Premarket approval requirements:

Synergia has concluded that MK-7 is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on our conclusion that MK-7, meeting the specifications cited herein, and when used as nutrient in selected food products such as foods for special dietary uses for children, is GRAS and is therefore exempt from the premarket approval requirements.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that MK-7, when used as described in this dossier, is GRAS based on scientific procedures.

1.8. Availability of data and information:

The data and information that are the basis for this GRAS conclusion will be made available to FDA upon request by contacting

Dr. Dilip Mehta
Director
Synergia Life Sciences Pvt. Ltd.
6/312, Jogani Industrial Complex
V.N. Purav Marg, Chunnabhatti,
Mumbai 400 022
INDIA

Tel: +9122 24055607-09
Email: info@viridisbiopharma.com

The data and information will be made available to FDA in a form in accordance with that requested under 21 CFR 170.225(c)(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

1.9. Data exempt from Disclosure:

Parts II through VII of this GRAS notification does not contain data or information that is exempt from disclosure under the Freedom of Information Act. There is no privileged or confidential information such as trade secrets and/or commercial or financial information in this document and the information contained in this dossier can be made publicly available.

1.10. Certification:

Synergia certifies that, to the best of its knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information, available and obtainable by Synergia, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the use of MK-7. Synergia accepts responsibility for the GRAS determination that has been made for MK-7 as described in this dossier.

1.11. Name, position/title of responsible person who signs dossier and signature:

Dr. Dilip Mehta
Director
Synergia Life Sciences Pvt. Ltd.
6/312, Jogani Industrial Complex
V.N. Purav Marg, Chunnabhatti,
Mumbai 400 022
INDIA

Signature: 

1.12. FSIS/USDA – Use in Meat and/or Poultry:

Synergia does not intend to add MK-7 to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

2. PART II- IDENTITY AND TECHNICAL INFORMATION

2.1. Description

The subject of this GRAS assessment, Menaquinone-7 (MK-7), is a standardized fermented defatted extract prepared by a fermentation and extraction process. 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®. General descriptive characteristics of MK-7 are summarized in Table 1.

In nature, vitamin K occurs as two forms: vitamin K1 (also known as phylloquinone) and vitamin K2 [designated also as menaquinones (MKs)]. Both vitamins share a common 2-methyl-1,4-naphthoquinone ring, also known as menadione. Phylloquinone contains a phytyl side chain which comprises four prenyl units (Shearer and Newman, 2008). The chemical structures of menaquinones contain an unsaturated aliphatic side chain with a variable number of prenyl units. The number of prenyl units indicates the respective type of menaquinone. Vitamin K2 can be divided into subtypes, namely, short-chain (i.e., menaquinone-4; MK-4) and long-chain (i.e., MK-7, MK-8, and MK-9). The chemical structures of various forms of vitamin K and their metabolites are presented in Figure 1.

Table 1. General Description of Menaquinone-7 (MenaquinGold®)

Parameter	Description *
Source	*Bacillus licheniformis*
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	$C_{46}H_{64}O_2$
Appearance	Pale yellow to yellow oil
Solubility	Insoluble in water
Color	Pale yellow
Odor	Characteristic
Taste	Characteristic
Storage	Store in tightly closed containers in a cool place. Exposure to light may deteriorate K2 activity
Shelf life	30 Months

*Based on information provided Synergia

2.2. Specifications and Identity

Food grade specifications of MK-7 from Synergia are provided in Table 2. Analytical results from five non-consecutive lots (Appendix I) demonstrate that MK-7 meets the standard specifications. All specification methods used are validated methods that have been well established at the production facility. Typical compositional analysis of MK-7 is presented in Table 3.

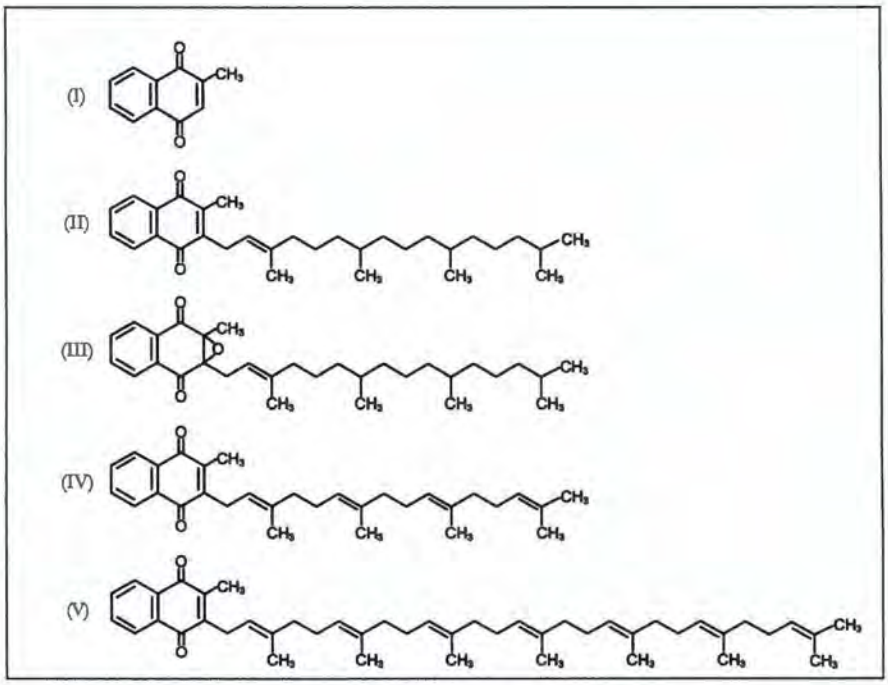

Figure 1. Chemical Structures of Various Isoforms of Vitamin K.

Chemical structures of some K vitamins and metabolites. Nomenclature: chemical name and IUPAC name and abbreviation in brackets:
(I) 2-methyl1,4-naphthoquinone (menadione; K3),
(II) 2-methyl3-phytyl-1,4-naphthoquinone (phylloquinone; K1),
(III) 2-methyl-3-phytyl-1,4-naphthoquinone-2,3-epoxide (phylloquinone epoxide; K1O),
(IV) 2-methyl-3-geranyl-geranyl1,4-naphthoquinone (menaquinone-4; MK-4),
(V) 2-methyl-3-farnesylgeranylgeranyl-1,4-naphthoquinone (menaquinone-7; MK-7),

Table 2. Specifications of Menaquinone-7 (MenaquinGold®; Vitamin K2-7, 1500 µg K2/g)*

Parameter	Characteristics	Methods
Appearance	Pale yellow to yellow oil	IP 2018
Identification	The retention time for principal peak in the chromatogram for the sample corresponds with the standard	USP-40 NF-35
Total vitamin K2-7 as is	1500 µg/g (min)	USP-40 NF-35
Heavy metals		
Arsenic	0.5 µg/g (max)	AOAC 2015.01 21st Edition
Lead	0.5 µg/g (max)	AOAC 2015.01 21st Edition
Cadmium	0.5 µg/g (max)	AOAC 2015.01 21st Edition
Mercury	0.1 µg/g (max)	AOAC 2015.01 21st Edition
Total heavy metals	5.0 µg/g (max)	IP 2018
Microbiological assays		
Total plate count	< 1,000 cfu/g (max)	USP 40
Yeast and Mold	< 100 cfu/g (max)	USP 40
Bile tolerant gram negative bacteria	Absent in 10 g	USP 40
Escherichia coli	Absent in 10 g	USP 40
Salmonella	Absent in 10 g	USP 40
Staphylococcus aureus	Absent in 10 g	USP 40
Pseudomonas aeruginosa	Absent in 10 g	USP 40

*Based on information provided by Synergia; max = maximum; min = minimum; cfu = colony forming units; USP = Untied States Pharmacopeia; AOAC = Association of Official Agricultural Chemists

Table 3. Compositional Analysis of Menaquinone-7 (MenaquinGold®)

Assay	Typical value (per 100 g)
Carbohydrate	<0.1 g
Total Fat	89.29 g
Saturated fat	12.34 g
Protein	<0.1 g
Ash	<0.1 g
Moisture	10.71 g
Calories	803.61 Kcal

*Based on information provided by Synergia

2.3. Manufacturing Process

Menaquinone-7 (MenaquinGold®) is manufactured according to current good manufacturing practice (cGMP) at Synergia Life Sciences Pvt. Ltd., Wada, India. The manufacturing facility is cGMP, NSF cGMP, HACCP, and ISO 22000 certified. The manufacturing process flow diagram is presented in Figure 2. The bacterial strain, *Bacillus licheniformis,* used in the production of menaquinone-7, has been well characterized by employing gross morphological characters, biochemical reactions, and by 16S RNA.

Menaquinone-7 is produced by submerged fermentation using *Bacillus licheniformis,* a non-toxicogenic and non-pathogenic strain, as the production strain. For fermentation gram flour (made from chickpeas) and dextrin as carbon and nitrogen sources are used. The menaquinone-7 in the fermentation broth is then spray dried and the solvent hexane is used for extraction. The concentrated menaquinone-7 oil thus obtained is triturated in glycerol monostearate, cooled and milled to the required mesh size for powder form. Alternately, the concentrated Oil is blended in Vegetable Oil such as Sunflower Oil, or MCT Oil, etc., to a desired potency.

High quality food grade materials are used in the manufacturing process. The production procedure assures a consistent and high quality menaquinone-7 product. The manufacturing process employed in the production of menaquinone-7 ensures that the potential for contamination or the introduction of impurities is low. Each batch manufactured is tested to ensure that the stringent purity criteria are met. No preservatives are used. Processing aids, such as solvent (removed by vacuum evaporation) and buffer salts used in the manufacturing process are all food-grade quality and comply with specifications described in the current Edition of the Food Chemicals Codex. The level of residual solvent (hexane) in the final product was undetectable.

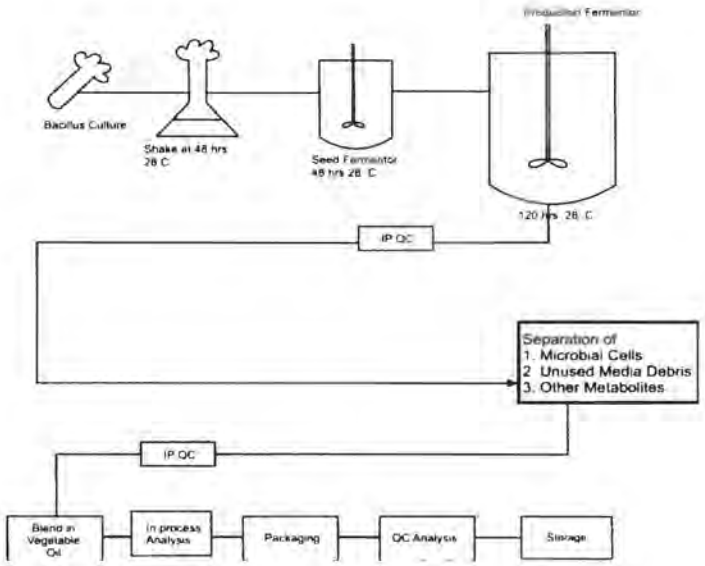

Figure 2. Manufacturing Flow Chart for Vitamin K2 (Menaquinone-7) from *Bacillus licheniformis*

2.4. Biological Activity

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 and Kyle, 1998). This reduced their intake of fat-soluble vitamin K, resulting in chickens developing large subcutaneous and intramuscular hemorrhages. This initial finding led to isolating, identifying and characterizing the structure of vitamin K and its importance as an anti-hemorrhagic 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. As mentioned earlier, the two primary vitamin K forms are vitamin K1 (phylloquinone) and vitamin K2 (menaquinones). Although all K-vitamins 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 and Vermeer, 2000). These foods have a long history of consumption by humans as basic foods.

3. PART III- DIETARY EXPOSURE

3.1. Intended Uses and Food Categories

Synergia intends to use vitamin K2 (MK-7) as a nutrient [21 CFR § 170.3(o)(20)] at levels up to 4 mcg *per* serving. Foods that are intended for infants, such as infant formulas are excluded from the list of intended food uses of the MK-7. As mentioned earlier, MK-7 will be used along with Vitamin K1 in Foods for Special Dietary Use (21 CFR 105.3), such as PediaSure® for children. It should be noted that vitamin K1 is already used in oral nutritional supplement (PediaSure®) that has been marketed for over 30 years as a nutrition drink for children.

The proposed uses of both vitamin K1 and MK-7 are as follows: Age range: 1-13 years old; Serving size: 8 fl oz. (i.e., 237 ml); Vitamin K1 per serving: 16 mcg; MK-7 per serving: 4 mcg; Caloric density: 1 kcal/ml or 237 kcal/serving; Serving per day (3 different scenarios): (1) Maximum- Each child receives their daily caloric requirements from consumption of this product alone, (2) Typical use- One serving/day, (3) High use- 6 servings/day (applicable only to children 4-8 years and 9-13 years). A summary of uses of vitamin K1 and MK-7 is provided in Table 4. Although the total intake of Vitamin K is mentioned in Table 5, as discussed below, and given the differences in pharmacokinetics of MK-1 and MK-2, from a safety assessment point of view the total intake of Vitamin K1 (K1+K2) may not be appropriate.

Table 4. Proposed Uses of Vitamin K1 and MK-7 in Oral Nutritional Supplement

Scenario	Age/Sex Group	Maximum Caloric Requirements[a] (kcal/day)	Servings/day[b]	Vitamin K1[c] (mcg/day)	Vitamin K2[d] (mcg/day)	Total K (mcg/day)
Typical	All ages/sex groups[e]	NA	1	16	4	20
Maximum	12-23 months	1100[f]	5	80	20	100
	2-3 years	1400	6	96	24	120
	4-8 years male	2000	9	144	36	180
	4-8 years female	1800	8	128	32	160
	9-13 years male	2600	11	176	44	220
	9-13 years female	2200	10	160	40	200
High	4-8 years and 9-13 years	NA	6	96	24	120

[a] Based on recommendations in the 2015-2020 DGA (USDA, 2015) unless specified otherwise; [b]Assuming 237 kcal/serving of oral nutritional supplement; [c]16 mcg/serving; [d]4 mcg/serving; [e]As specified in the maximum scenario; [f]Assuming maximum calorie requirement of 1100 kcal/day;

3.2. Methods for Estimates of Background and Proposed Uses

The estimates of daily intake of vitamin K from all sources was performed by Exponent, Inc. (Exponent) (Appendix II). It should be noted that the vitamin K2 intake is

background intake from food and is primarily for MK-4. In an attempt to estimate the cumulative daily intake of vitamin K, Exponent considered the intake of vitamin K1, K2, and related isoforms from background sources and from the Synergia's proposed uses of vitamin K (i.e., K1 and MK-7) in PediaSure® products. The intake estimates of vitamin K was based on food consumption data from foods reported consumed in the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Survey (NHANES) 2011-2014 and provided for children 1-13 years of age. The data and methods used to conduct the intake assessment and results are provided in Appendix II.

As described in the Exponent report, for each food reported in NHANES, the USDA Food and Nutrient Database for Dietary Studies (FNDDS) databases provide information on the amount of energy and approximately 60 nutrients or food constituents per 100 g of each food including vitamin K1 as phylloquinone. Nutrient values for FNDDS are based on the National Nutrient Database for Standard Reference (SR) that is the major source of food composition data in the U.S. and provides the foundation for most food composition databases in the public and private sectors including the FNDDS. The SR database contains data on the level of vitamin K1 and vitamin K2 (limited to MK4 only) in foods included in FNDDS.

Exponent applied the mean nutrient concentrations of vitamin K1 and vitamin K2 as MK4 in foods based on SR28 (USDA, 2016). SR28 is the source of nutrient composition for FNDDS 2013-2014 and contains data on 8,789 food items and up to 150 food components/nutrients.

Additionally, Exponent also identified relevant scientific studies that provided data on the level of vitamin K2 in foods with a preference for those conducted in the U.S. As a result of the literature search and a review of the foods reportedly consumed in the U.S. diet, six studies were included in the assessment (Vermeer et al., 2018; Fu et al., 2017; Fu et al., 2016; Elder et al., 2006; Schurgers and Vermeer, 2000; Sakamoto et al., 1999). Data on the level of other isoforms of vitamin K2, including MK-7, in foods consumed as part of the U.S. diet were based on analytical levels provided in these publications.

3.3. Estimates of Background Intake

Estimates of vitamin K, including vitamin K1, vitamin K2, and vitamin K2 as MK-7, from background sources included reported intakes from all dietary sources and supplements. Estimates of vitamin K1 from background food sources were derived from food consumption data reported in the NHANES 2011 - 2014 in combination with the USDA FNDDS database.

Estimates of vitamin K2 (and MK-7) from background food sources were derived from food consumption data reported in the NHANES 2011-2014 in combination with analytical data from published literature (see Appendix II). For this, Exponent mapped the mean vitamin K2 levels reported in the published literature to foods in the NHANES 2011-2014 through the FNDDS based on the food description and on what was reported to be consumed. In addition, Exponent relied upon the SR28 data on MK-4 levels in foods to supplement the published literature where applicable. It is important to note that the analytical data on the levels of vitamin K2 in background food sources included in the current analysis is limited to what is published and for that reason, the estimated background intake of vitamin K2 and MK-7 from food sources may not reflect all vitamin

K2 that could potentially be present in food. While several of the publications provided total vitamin K2 in the foods included in the analysis (Fu et al., 2017; Fu et al., 2016), some data sources, for example, only focused on MK-4 or MK-7 (Elder et al., 2006).

Intake of vitamin K from dietary supplement use by each respondent was added to the intake of vitamin K from food sources to estimate the total background intake of vitamin K (i.e., vitamin K1, vitamin K2, vitamin K2 as MK-7) per person from both dietary and supplemental sources.

The estimated daily intake of Vitamin K from background (total diet + supplement) for children age 1-13 years, revealed that the highest mean and 90th percentile intake of total vitamin K (K1+K2+MK-7) of 283 and 520 mcg/day, respectively, was noted in 12-23 months age group. In this age group the mean and 90th percentile intake of vitamin K1 was reported as 40.9 and 74.6 mcg/day, while the intake of Vitamin K2 and MK-7 was 242 and 492 mcg/day, and 0.1 and 0.2 mcg/day, respectively. In the report it was noted that the background vitamin K2 and MK-7 were limited to the data available in the published literature and may not reflect all vitamin K2 that could potentially be present in the food (Appendix II).

3.4. Cumulative Intake from Background and Proposed Uses

In order to estimate the cumulative estimated daily intake for vitamin K (including vitamin K1, and vitamin K2 as MK7) from all potential sources, each individual's current background vitamin K intake (food and supplement) was added to his/her potential vitamin K intake from the proposed use of vitamin K in PediaSure®. The details of proposed and cumulative intake of vitamin K under the maximum, typical, and high use scenarios are provided in Appendix II.

Among children 1-13 years of age, the per user mean intake of vitamin K from all sources, including background sources and the maximum proposed use assuming that the child's entire daily caloric requirement is met by consumption of PediaSure®, ranged from 317 to 449 mcg/day among children 2-3 years and males 9-13 years, respectively. The per user 90th percentile intakes of vitamin K from all sources, including background sources and the maximum proposed uses, ranged from 486 to 682 mcg/day among children 2-3 years and males 9-13 years, respectively. The estimated intake of MK-7 from background sources was <1 mcg/day among all subpopulations at both the mean and 90th percentile, while the proposed use in PediaSure® increased MK-7 intakes to >4 mcg/day among all proposed use scenarios.

The cumulative intake of vitamin K, including K1, K2, MK-7, from background (diet + supplement) and from the proposed uses in PediaSure® in the high dose scenario for children (4-13 years) is presented in Table 5.

Table 5. Cumulative Estimated Daily Intake of Vitamin K from background (total diet + supplements) and Proposed uses in PediaSure® by Children 4-13 years: High Use Scenario*

Age/Sex Group	Vitamin Form	N[a]	Estimated Daily Intakes (EDIs) of Vitamin K (mcg/day)		
			Proposed Uses[b]	Cumulative[c] Background + Proposed	
			High	Mean	90th percentile
4-8 years (male)	Vitamin K	818	120	332	507
	Vitamin K1		96	158	195
	Vitamin K2[d]		24	174	323
	MK-7		24	24.2	24.3
4-8 years (female)	Vitamin K	742	120	312	467
	Vitamin K1		96	161	211
	Vitamin K2[d]		24	151	269
	MK-7		24	24.1	24.4
9-13 years (male)	Vitamin K	750	120	349	582
	Vitamin K1		96	177	259
	Vitamin K2[d]		24	172	324
	MK-7		24	24.2	24.4
9-13 years (female)	Vitamin K	756	120	307	466
	Vitamin K1		96	162	218
	Vitamin K2[d]		24	145	274
	MK-7		24	24.2	24.4

[a]N = Unweighted number of survey respondents identified as consumers of vitamin K; 100% user; [b]Applicable only to children 4-13 y; see Table 4; [c]EDIs include background vitamin K from total diet, the reported use of vitamin K-containing dietary supplements, and the maximum proposed use of vitamin K in PediaSure®; [d]Background intakes of vitamin K2 and MK-7 are limited to the data available in the published literature and should not be considered a comprehensive assessment of vitamin K2 intake.

*Source- Appendix II

3.5. Summary of Intake Assessment from Safety Perspective

An attempt has been made to capture all possible outcomes for intake of Vitamin K2 (MK-7) along with vitamin K1 in Foods for Special Dietary Use (such as PediaSure®) based on the different manners these products are typically consumed. Hence, it is important to be specific in describing the consumption scenarios considered in the above analysis:

- Typical consumption: NHANES data and consumer use information demonstrate typical consumption of these products is a single serving per day. These typical consumers would be taking the oral nutritional supplement to complement a general diet. For the purposes of this assessment, this typical consumption is considered mean consumption.
- High consumption: An estimate of high consumption of these products, based on consumer use information, was also developed. These high consumption estimates are only applicable for individuals older than 3 years of age, based on information on product use. This high consumption estimate reflects a situation where these

products are consumed by individuals as a sole source of nutrition, and thus are unlikely to consume other foods. This estimate reflects situations in which individuals, possibly not under direct medical supervision, are consuming these products under a limited duration of time. An example would be an individual that has undergone oral surgery and is unable to consume solid foods, in which these products are consumed as a sole source of nutrition during recovery. For the purposes of this assessment, this high consumption is considered a 90th percentile consumption.

- Maximum consumption: A calculation of the maximum amount of these products that could be consumed as a sole source of nutrition is provided, based on assumptions of the caloric requirements of different age groups. This scenario reflects rare cases in which individuals in long-term care, for example due to chronic illness, are limited to a liquid diet (often through G-tube or J-tube). From a population perspective, this consumption scenario would exceed the 90th percentile intake. Of note, these individuals are also under close medical supervision, in which the medical professionals would be aware of the individuals' full medical condition, including any medications that the individual is being administered. This intake scenario data was generated for the sake of completeness, to account for all potential uses of these products.

Using the typical and high exposure scenarios, the intake of vitamin K2 (both specifically MK-7 and total K2) from these products was estimated, as well as cumulative intake. As shown in Table 6, typical consumption of these products, which would be in addition to vitamin K2 from other dietary sources, would contribute the majority of total MK-7 intake (~95% across all age groups), but only a minor amount of the total dietary vitamin K2 intake (~2% across all age groups).

Table 6. Vitamin K2 intake based on typical consumption (1 serving/day)

Age	Vitamin form	Product contribution mcg/day	Mean background contribution mcg/day	90th percentile background contribution mcg/day	Cumulative intake, mean background mcg/day	Cumulative intake, 90th percentile background mcg/day
12-23 mo	Total K_2	4.0	242	492	246	496
	MK-7	4.0	0.1	0.2	4.1	4.2
2-3 yr	Total K_2	4.0	149	311	153	315
	MK-7	4.0	0.1	0.3	4.1	4.3
4-8 yr, male	Total K_2	4.0	150	299	154	303
	MK-7	4.0	0.2	0.3	4.2	4.3
4-8 yr, female	Total K_2	4.0	127	245	131	249
	MK-7	4.0	0.1	0.4	4.1	4.4
9-13 yr, male	Total K_2	4.0	148	300	152	304
	MK-7	4.0	0.2	0.4	4.2	4.4
9-13 yr, female	Total K_2	4.0	121	250	125	254
	MK-7	4.0	0.2	0.4	4.2	4.4

Table 7 shows the intake of vitamin K2 using high consumption scenario for these products, along with the assumption that consumption of this volume of products does not reduce intake of any of the foods in the background diet. As described in the description of this scenario, an individual consuming six servings per day is likely to be linked with significant reductions or even complete exclusion of other foods. As an additional exercise, Table 8 provides an estimate of intake in the case that background intake is half of the typical mean and 90th percentile estimates. This uses the rationale that individuals consuming six servings per day would not also be consuming a full standard diet. It should be noted that consumption of half of the standard diet is still a likely overestimate of background diet consumption. Under both of these sets of assumptions (Table 7 and 8), consumption of these products supplemented with Vitamin K2 continue to provide nearly all of the daily MK-7 intake. As expected, with this higher rate of product consumption, under these scenarios these products contribute a greater percentage of total Vitamin K2 intake, although MK-7 still only contributes ~25% of total Vitamin K2 intake under maximal exposure scenarios (mean background intake).

Table 7. Vitamin K2 intake based on high consumption (6 servings/day)

Age	Vitamin form	Product contribution mcg/day	Mean background contribution mcg/day	90th percentile background contribution mcg/day	Cumulative intake, mean background mcg/kg	Cumulative intake, 90th percentile background mcg/kg
4-8 yr, male	Total K_2	24.0	150	299	174	323
	MK-7	24.0	0.2	0.3	24.2	24.3
4-8 yr, female	Total K_2	24.0	127	245	151	269
	MK-7	24.0	0.1	0.4	24.1	24.4
9-13 yr, male	Total K_2	24.0	148	300	172	324
	MK-7	24.0	0.2	0.4	24.2	24.4
9-13 yr, female	Total K_2	24.0	121	250	145	274
	MK-7	24.0	0.2	0.4	24.2	24.4

Table 8. Vitamin K2 intake based on high consumption (6 servings/day), with half of estimated background consumption

Age	Vitamin form	Product contribution mcg/day	Mean background contribution mcg/day	90th percentile background contribution mcg/day	Cumulative intake, mean background mcg/kg	Cumulative intake, 90th percentile background mcg/kg
4-8 yr, male	Total K_2	24.0	75	149.5	99	173.5
	MK-7	24.0	0.1	0.15	24.1	24.15
4-8 yr, female	Total K_2	24.0	63.5	122.5	87.5	146.5
	MK-7	24.0	0.05	0.2	24.05	24.2
9-13 yr, male	Total K_2	24.0	74	150	98	174
	MK-7	24.0	0.1	0.2	24.1	24.2
9-13 yr, female	Total K_2	24.0	60.5	125	84.5	149
	MK-7	24.0	0.1	0.2	24.1	24.2

Alternatively, one can express the overall contribution of product consumption to daily total Vitamin K2 intake as a percentage basis (Table 9). In Table 9, the assumption is again used that product consumption (either under the typical or high product consumption

scenario) is in addition to full dietary intake. Using these assumptions, the data show that under typical product consumption, the contribution of these products to total, daily Vitamin K2 intake is very low in the context of both mean dietary intake (< 3.5%) and 90th percentile dietary intake (< 2%), in all age groups. Under the high production consumption scenario (six servings per day), product contribution to total, dietary Vitamin K2 intake is more significant in both the mean dietary intake (~19%) and 90th percentile dietary intake (~9%) scenarios, but still represents a minority of intake.

As mentioned previously, consumption of six servings a day by these individuals (the high product consumption scenario) is highly unlikely to be accompanied by significant intake of Vitamin K2 from other dietary sources. To this end, this data can also be used to compare background dietary Vitamin K2 intake with intake in the high product consumption scenario. Under this sole-source scenario, total vitamin K2 consumption of 24 mcg/day, all in the form of MK-7 (from product consumption), is approximately 19% of mean, total K2 exposure and approximately 9% of 90th percentile, total K2 exposure from the background diet in each population. The data presented in Table 4 provides the information on the incremental addition of total K2 exposure from product consumption to the background total K2 intake. This also represents the percentage of K2 intake from sole-source product consumption, as a percentage of total K2 exposure from dietary sources (without any contribution from product).

Table 9. Percentage increase in total Vitamin K2 intake from product consumption

Age	Mean background		90th percentile background	
	Typical product consumption (% increase from background)	High product consumption (% increase from background)	Typical product consumption (% increase from background)	High product consumption (% increase from background)
12 – 23 mo	1.7%	-	0.8%	-
2 – 3 yr	2.7%	-	1.3%	-
4 – 8 yr, male	2.7%	16.0%	1.3%	8.0%
4 – 8 yr, female	3.1%	18.9%	1.6%	9.8%
9 – 13 yr, male	2.7%	16.2%	1.3%	8.0%
9 – 13 yr, female	3.3%	19.8%	1.6%	9.6%

3.6. Consumption conclusion

When calculating total Vitamin K2 intake from Foods for Special Dietary Use (such as PediaSure®), there are two scenarios that represent greater than 90th percent of consumer exposure:

- Typical consumption:
    - Consumed by individuals as an oral nutritional supplement or supplement between meals.
    - Typical (mean) consumption is one serving per day, likely in combination with other dietary sources of Vitamin K2.
    - In this scenario, intake from Foods for Special Dietary Use contributes nearly all MK-7 (approximately 95% across all age groups), but less than

2% of total dietary Vitamin K2 using both mean and high background dietary K2 exposure scenarios.

- High consumption:
  - Consumed on a short-term basis as a sole source of nutrition,
  - High consumption (90^{th} percentile) is up to six servings per day for individuals three years of age and older.
  - Since the product is being consumed sole-source, intake of Vitamin K2 from other dietary sources would be greatly reduced or eliminated completely.
  - In this scenario, intake from Foods for Special Dietary Use is 24 mcg/day of K2 exposure (all in the MK-7 form), which is < 20% of total K2 intake in these populations from mean background, dietary intake, and < 10% of total K2 from 90^{th} percentile intake.

There are other potential consumption scenarios that involved higher intake, such as long-term, chronic administration of these products as a sole source of nutrition. Those scenarios would only occur when the individual is under close medical supervision. Thus, for safety assessment purposes the maximum exposure to 12 mcg MK-7 and 150 mcg K2 are considered.

4. PART IV- SELF LIMITING LEVELS OF USE

Excessive amounts of Menaquinone-7 (MK-7) are unlikely to be added to food products for economic reasons.

5. PART V- EXPERIENCE BASED ON COMMON USE IN FOODS BEFORE 1958

The statutory basis for the conclusion of GRAS status of Menaquinone-7 (MK-7) in this document is not based on common use in food before 1958. This GRAS assessment is based on scientific procedures. As described below, MK-7 is found in dairy and fermented food products that have been commonly consumed orally prior to 1958. Notwithstanding this, it is reasonable to conclude that, since MK-7 has been used traditionally across the world, it was present in food prior to 1958.

6. PART VI- NARRATIVE

6.1. Traditional and Current Uses

6.1.1. Traditional Uses

As a widely consumed food for centuries in Japan, Natto is considered a rich source of dietary menaquinones. This traditional food has a long history of consumption in Oriental countries (Shurtleff and Aoyagi, 2007). It is reported that the Chinese Salt-Free Soy Nuggets gave origin to Natto food. A description of the process for making different types of nuggets appears in the *Ch'i- min yao-shu*, the earliest encyclopedia of agriculture, written in 535 AD. It is also reported that fermented soybeans were prepared as common food in Japan sometime during the period from the 1st to the 11th century. Since the late 18th century, the Natto preparation has been transformed by the modern science of microbiology. Subsequently, the microbial species responsible for the production of Natto were identified as *Bacillus natto* and *Bacillus mesentericus vulgatus*.

In addition to Natto, MK-7 is present in certain cheeses, pork, steak, buckwheat bread and eel. All of these foods have been consumed for a long time. Natto - the traditional food from Oriental countries (known as Salt-free Soy Nuggets in China; Joenkuk-jang and Damsue-jang in Korea; Thua Nao in Thailand; Kinema in Darjeeling, India and Southwest Nepal; Sereh in Bali, Indonesia) - is a rich source of dietary menaquinones (Shurtleff and Aoyagi, 2007). 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.

6.1.2. Current Approved and other Uses

In the United States, vitamin K, (including MK-7) and other products containing vitamin K, are available as dietary supplements. It is also widely available as a single ingredient dietary supplement in the US; the dosage form is usually 100 mcg/tablet. The Dietary Supplement Label Database[2] lists more than 200 products containing MK-7, the majority of which have a manufacturer's suggested MK-7 dose of 50 mcg/day. These products are regulated under the Dietary Supplement Health and Education Act (DSHEA, 1994). In Japan, Natto has received certification for health claims for both bone health and gut health under the regulation - Food for Specified Health Uses (FOSHU). The European Food Safety Authority (EFSA, 2008) has examined the safety of vitamin K2 (MK-7) and, following consideration of available data on specifications, manufacturing, anticipated intake, bioavailability, metabolism and toxicology, the EFSA Panel concluded that the use of menaquinone-rich edible oil in foods for the general population including food supplements and in foods for particular nutritional uses, other than baby foods and infant formula, at the use levels of 10 mcg/serving was not a safety concern.

Food supplements marketed in the UK may contain up to 45 mcg vitamin K (either as K1 or K2) for general consumption and 200 mcg in supplements intended for women from pre-conception to nursing. In the European Union, the use of MK-7 is permitted as a source of vitamin K for nutritional purposes in foodstuffs. The European Food Safety

[2] Available at: https://www.dsld.nlm.nih.gov/dsld/rptQSearch.jsp?item=MK-7&db=adsld.

Authority (EFSA, 2008) reported that approved uses of MK-7 are estimated to result in a mean daily intake that ranged from 36 mcg (female adults) to 54 mcg (male teenagers), while high intakes ranged from 75 mcg/day (children) to 115 mcg/day (male teenagers).

In a recent article, Marles et al. (2017) summarized the available quantitative data from different sources on levels of MK-7, other menaquinones, and vitamin K1 in foods. These reviewers also noted that the richest dietary sources of long-chain menaquinones are foods fermented by bacteria (not by molds or yeasts), typically represented in Western diets by dairy products such as cheeses (MK-8, MK-9) and in the Japanese diet by Natto (MK-7). Apart from animal livers, meat and fish products generally are low in long-chain menaquinones and are of little importance as dietary sources of vitamin K2. The most commonly consumed menaquinones in the human diet have a side chain with 4, 6, 7, 8, 9, or 10 isoprene residues. 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.

6.1.3. Published Reports on Exposure to MK-7

For centuries, peoples have consumed MK-7 from food, primarily from Natto and to some extent from cheese. The available information indicates that the MK-7 in Natto ranges from 780 to 2100 mcg/100 g (Sumi, 1999; Schurgers et al., 1999; Schurgers, 2000; Tsukamoto et al., 2000, 2000a; Kaneki et al., 2001; Katsuyama et al., 2004). Depending on the method of preparation, Natto may provide between 775 to 1750 mcg of MK-7/100 g of Natto (Tsukamoto et al., 2000). The available studies suggest regular consumption of Natto and in turn MK-7 in Japan. Production of 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 Western countries, including the USA, Natto is marketed. In addition to Natto, MK-7 is also consumed from other dietary sources. MK-7 is also commonly consumed from ordinary food. In the Western diet, common sources of menaquinones include cheese, pork, fish (e.g., eel, plaice) and buckwheat bread.

In an analysis of 58 different food items for the presence of phylloquinone and menaquinones, Kamao et al. (2007) reported that 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 MK-7 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. The contributions of K1, MK-4, and MK-7 to total vitamin K intake were reported as 67.7%, 7.3%, and 24.9%, respectively. Based on this information, the daily background average intake of MK-7 is estimated as 57 µg per person. For Natto eaters, the average daily intake is estimated at 133.2 µg/person. 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 µg/100 g, respectively. These observations are also supported by Isobe et al. (1995) in which MK-7 content in 19 different Natto products was reported to range from 780 to 2100 mcg/100 g. As the average daily Natto serving is 40 - 50 g, this is equivalent to an exposure of about 300 to 800 mcg MK-7/40 g meal.

Several studies from Germany reported the dietary intake of menaquinones (including MK-7) from several sources including cheese (Schurgers et al., 1999; Schurgers and Vermeer, 2000; Geleijnse et al., 2004; Nimptsch et al 2008; Nimptsch et al 2009). As compared to various cheeses that also contains MK-7, Natto has been reported to contain >100 times more MK-7 (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 in fermented cheeses. It has been further observed that long-chained menaquinones, 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). The available information indicate that menaquinone intake comprised 10% of the total vitamin K intake (Geleijnse et al., 2004). In a recent review article, it has been reported that dietary intake of menaquinones accounts for up to 25% of total vitamin K intake and contributes to the biological functions of vitamin K (Beulens et al., 2013).

6.2. Data Pertaining to Safety

6.2.1. Preamble

The presence of menaquinones (primarily MK-7) in the diet (Natto and cheeses) supports the safe use and negates the need for systematic toxicity studies. 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. In several human studies, Natto food, known to contain MK-7, has been investigated for its health benefits.

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 among the K2, that primarily includes MK-4 and MK-7, depending on the chain length, the bioavailability and bioactivity differs. MK-7 has superior bioavailability over other menaquinone homologs (MK-4) and vitamin K1. Given this, it is important to differentiate the potential effects of MK-4 and MK-7 related to safety-in-use. This is particularly important in patients on anti-coagulant therapy. Given this, an attempt has been made to present the differences in bioavailability of MK-4 and MK-7 and its implications from a safety assessment point of view.

The safety assessment of MK-7 for its uses in PediaSure®, is based on the totality of the available evidence, including human clinical observations and animal experimental studies. Efforts have been made to present both the data supporting the safety of menaquinones as well as any data on potential adverse effects. An attempt has been made to interpret the findings from the family of vitamin K substances and its relevance for the current GRAS assessment. The assessment of efficacy studies is limited to a review of the results related to safety and tolerability. Relevant biological and toxicological studies on menaquinones, including MK-7 and other structurally closely related substances, are presented in the following section to provide support for the conclusions reached in this assessment.

6.2.2. 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 (Wildman and Medeiros, 2000). 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, 1996).

Liver stores of vitamin K in humans normally comprise about 90% menaquinones and 10% phylloquinone (Conly and Stein, 1992; Ichihashi et al., 1992). 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 (Shearer, 1988; Usui, 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, 1988; Shearer et al., 1996; Hodges, 1993; Suttie, 1995; Shearer, 1992).

In three separate studies, Schurgers et al. (2007) compared the bioavailability of K1 and MK-7. The details of protocol, including doses used in these studies are provided in Table 10. These investigations revealed maximum serum concentrations of both K1 and 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 K1 declined to baseline but 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:K1 was 2.5. Using the area under the curve at 96 hours, the ratio of bioavailability of MK-7:K1 was 6. Based on these observations, Schurgers et al. (2007) concluded that MK-7 has a significantly longer half-life as compared to K1 (68 hours vs 1–2 hours). Both 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 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 K1 remained slightly above the placebo values during the entire study period. Within 3 days, both K vitamins had induced a statistically significant increase in osteocalcin carboxylation, but only with MK-7 did the ratio of circulating carboxylated osteocalcin to uncarboxylated osteocalcin continue to increase during the entire study period, suggesting that, if taken on a daily basis, MK-7 at 25 mcg/day is more efficacious than vitamin K1 at 100 mcg/day.

Schurgers et al. (2007) also reported that, on a molar basis, MK-7 is 3 to 4 times more potent at interfering with the action of oral anticoagulant drugs, and by weight the effect of MK-7 is approximately 2.5 times that of vitamin K1. These investigators concluded that MK-7 doses of more than 50 mcg/day may interfere with anticoagulant

treatment in a clinically relevant way. Based on these findings of the potency of MK-7 compared with that of vitamin K1 and extrapolation of findings from a previous study in which supplements containing no more than 100 mcg/day of vitamin K1 were not likely to result in clinically relevant adverse effects of oral anticoagulant therapy in healthy individuals. In a review article on the safety of MK-7, Marles et al. (2017) reported that the studies conducted by these investigators were not designed or powered to assess safety.

In a review article, Shearer et al. (2012) described the absorption, distribution, metabolism, and excretion of 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.

In several studies, absorption of 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, 1999: Kaneki et al., 2001; Tsukamoto et al., 2000; Schurgers et al., 2007). 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. In a single dose study by (Kaneki et al., 2001), 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. This study along with others suggest good bioavailability of MK-7 derived from Natto or with Natto food (Vermeer, 2003; Schurgers et al., 2007). These studies reported higher and more stable blood levels of MK-7 as compared to phylloquinone.

In a randomized single-blinded two-way cross-over study, Moller et al. (2016) studied the bioavailability of a synthetic 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. 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.

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. 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.

In a recent review article, Halder et al. (2019) highlighted differences between isoforms vitamin K1 and K2 by means of source, function, and extrahepatic activity. These researchers reported that the difference in structure between K1 and K2 is seen in different absorption rates, tissue distribution, and bioavailability. In spite of differences in structure, both K1 and K2 act as cofactor for the enzyme gamma-glutamylcarboxylase, surrounding both hepatic and extrahepatic activity. Only carboxylated proteins are active and promote a health profile like hemostasis. These investigators noted that MK-7 has been shown to be a bioactive compound in regulating osteoporosis, atherosclerosis, cancer and inflammatory diseases without risk of negative side effects or overdosing.

In a study in healthy women, Sato et al. (2012) compared the bioavailability of MK-4 and MK-7. In this study, 10 female volunteers (age: 20–21 years) were randomized into two groups (n = 5) and treated with a single dose of MK-4 (420 mcg; 945 nmol) or MK-7 (420 mcg; 647 nmol) within 10 minutes after ingesting a breakfast containing 13-17 g of fat. Serum MK-4 and MK-7 levels were determined at different time points up to 72 hours. MK-7 was well absorbed and reached maximal serum level at 6 hours after intake and was detected up to 48 hours after intake. MK-4 was not detectable in the serum in any of the subjects at any time point. Consecutive administration of MK-4 (60 mcg; 135 nmol) or MK-7 (60 mcg; 92 nmol) for 7 days demonstrated that MK-4 supplementation did not increase serum MK-4 levels. However, consecutive administration of MK-7 increased serum MK-7 levels significantly in all subjects. The investigators concluded that MK-4 present in food did not contribute to the vitamin K status as measured by serum vitamin K levels, whereas MK-7 significantly increased serum MK-7 levels.

The comparison of published bioavailability studies indicates a 6-10 times better serum/plasma bioavailability of MK-7 compared to MK-4 or to that of phylloquinone (Schurgers et al., 2007, Schurgers et al. 2002). Ingestion of MK-4 at a dose level of 45 mg/day in osteoporotic patients (n=120; placebo=121) resulted in blood levels of 65 ng/ml (0.15 nM (Shiraki et al. 2000). In the published bioavailability studies, MK-4 is consumed daily as a therapeutic agent at very high doses (3x15 mg/day) - a dose which is 1000-fold higher than presently used for MK-7 as a food supplement in Europe.

In summary, menaquinones 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 metabolism primarily takes place in the liver, and involves oxidative degradation of the side-chain resulting in subsequent elimination via the bile or urine. Knapen et al. (2007) suggested that short half-life of MK-4 will result in fluctuating serum levels. The available studies indicate a 6-10 times better serum/plasma bioavailability of MK-7 compared to MK-4 or to that of 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. Thus, bioavailability among the various forms of menaquinones appears to be related in part to the length of the side chain, as menaquinones with long side chains (e.g., MK-7, MK-8, and MK-9) are better absorbed from food compared with MK-4, which has a short side chain.

6.2.3. Human Studies

6.2.3.1. Human Clinical Studies and Observations

Given the recent increased interest, MK-7 has been extensively investigated in multiple human clinical studies for its efficacy and safety. A summary of some of the relevant clinical trials, including their design, doses used and reported adverse effects noted following menaquinones, including MK-7, ingestion are presented in Table 10. In a recent comprehensive review article on the safety of MK-7, Marles et al. (2017) extensively reviewed the available evidence on safety of MK-7 as an ingredient of dietary supplements. In the review by Marles et al. (2017), published clinical trials that made no mention of whether adverse events occurred or of any other aspects of safety were excluded. In the following section, relevant recent safety related studies are described.

In a recent study, Zwakenberg et al. (2019) assessed if MK-7 supplementation, compared to placebo, decreases vascular calcification in people with type 2 diabetes and known CVD. In this double-blind, randomized, placebo-controlled trial, men and women with type 2 diabetes and CVD were randomly assigned to 360 µg/day MK-7 or placebo for 6 months. Thirty-five patients were assigned to the MK-7 group (33 completed follow-up) and 33 to the placebo group (27 completed follow-up). After the 6-mo intervention, target-to-background ratios tended to increase in the MK-7 group compared with placebo, although this was not significant. Log-transformed computed tomography calcification mass did not increase in the intervention group compared with placebo. MK-7 supplementation significantly reduced dp-ucMGP compared with placebo. No adverse events were reported.

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 MK-7/day for eight weeks on different biomarkers and coagulation-related parameters, including serum levels of MK-7. 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 (van Summeren et al., 2009).

In a recent 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."

In a double blind, placebo controlled trial in 148 postmenopausal women (average age 67 years), Ronn et al. (2016) studied the effects of MK-7 in prevention of age-related deterioration of trabecular bone microarchitecture at the tibia. In this study, the dose of MK-7 used was 375 mcg/day for one year and the effect on under-carboxylated osteocalcin (ucOC), bone mass and quality were investigated. After three months of the administration of MK-7, ucOC was found to be decreased in the MK-7 group as compared with placebo group. High resolution peripheral quantitative computed tomography (HRpQCT) after 12 months demonstrated that tibial trabecular number and trabecular spacing was decreased in placebo group and unchanged in treatment group. It was also observed that trabecular thickness was unchanged in the MK-7 group and increased in the placebo group. There were no differences between the groups regarding adverse events or serious adverse events ($p>0.05$ for both, data not shown).

Moller et al. (2016) compared the biological effects of placebo, fermentation-derived 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.

In a long-term double-blind, placebo-controlled trial, Knapen et al. (2015a) investigated effects of MK-7 (180 mcg MenaQ7/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 year 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.

In another study, Knapen et al. (2015b) investigated the effects of a 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 Mg 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.

In a three year study, Knapen et al. (2013) investigated the effects of low-dose 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 favorably 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%. Only a few women reported complaints 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.

In a more recent report, based on an analysis from 214 postmenopausal women (55-65 years of age), Knapen et al. (2018) reported the effects of high MK-7 (180 mcg/day) or placebo for 3 years on body fat and weight. These investigators reported that MK-7 intake may support reducing body weight, abdominal and visceral fat, notably in subjects showing a strong increase in cOC. A causal relationship between the changes in cOC and body fat or distribution cannot be concluded from these data. The study appears to be an extension of others studies by these investigators. The investigators did not mention about adverse effects in this publication.

In a randomized, double-blind, placebo-controlled trial, Dalmeijer et al. (2012) investigated the effects of 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.

Table 10. Summary of Clinical Trials and Adverse Event Reports from Clinical Trials with MK-7

Clinical study design	No. of subjects	Demographic characteristics	Dose	Length of treatment	Endpoint(s)	Adverse events	Reference
Randomized controlled trial; conducted in two phases of 4 weeks each	N=26 (18 women); 13/group	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	No adverse events reported by subjects	McFarlin et al., 2017
Randomized, double-blind, placebo controlled trial	N= 148 (71 each group)	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	No differences between the groups regarding adverse events or serious adverse events	Ronn et al., 2016
Randomized, partly single-blind, partly open-label bioavailability	N=43 men, 64 women	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	In yogurt-treated groups, 7 cases of satiated feeling, heartburn, stomach ache, abdominal cramps, diarrhea, and nausea attributed to increased yogurt intake	Knapen et al., 2016
Randomized, double-blind, placebo-controlled	N=60 women N=120 men and women	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	No adverse effects associated with study products were observed	Inaba et al., 2015
Randomized, double-blind, placebo-controlled parallel	N=120 treated women, N=124 nontreated women	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	No effect on fasting glucose, acute-phase markers (hs-CRP, IL-6, TNF-α) or markers of endothelial dysfunction (VCAM, E-selectin, and AGE)	Knapen et al., 2015
Randomized,	N=200	Chronic	MK-7 at	8 week	Determination of	Gastrointestinal upset due	Caluwe et al.,

single-blind, dose-finding intervention		hemodialysis patients in stable medical condition; men and women, ≥ 18 y	360, 720, or 1080 mcg, 3 times weekly		optimum dose of MK-7 for activation of vitamin K-dependent MGP by measuring reduction of inactive dp-uc-MGP	to smell of MK-7 tablets in 9 subjects who withdrew	2014
Randomized, double-blind, placebo-controlled, parallel	N=120 treated women, N=124 nontreated women	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	Dropout rate of 8.6%. 12 dropouts in placebo group (hair loss, brittle nails, hot flashes, knee pain, numbness in limbs, fatigue, weight gain); 9 dropouts in MK-7 (bone pain, hot flashes, rash around eyes and ears, smelly capsules, weight gain)	Knapen et al., 2013
Nonrandomized prospective pilot study	N=12 girls; N = 8 boys	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 score of lumbar spine	No noncompliance or side effects observed	Ozdemir et al., 2013
Randomized, double-blind, placebo-controlled	N=42 children N=68 adults	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 healthy adults, 20-29 y	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	1 dropout due to unrelated reasons (broken leg)- no adverse effects noted	Theuwissen et al., 2013

Randomized, double-blind, placebo-controlled exploratory pilot	N=20 men, 22 women	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 generation as an indicator of safety	No dropouts, no adverse effects on thrombin generation observed	Theuwissen et al., 2012
Randomized, double-blind, placebo-controlled	N=60	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	1 dropout after enrollment but prior to treatment; no adverse events, no changes over time of prothrombin time (P = 0.92). Other CVD risk factors such as blood lipid profile or blood pressure did not differ between treatments	Dalmeijer et al., 2012
a. Cross-sectional analysis b. Randomized, double-blind, placebo-controlled, dose-response c. Randomized, double-blind, placebo-controlled	a. N=244 untreated women b. N=22 women, N=20 men, randomized into 7 groups of 6 individuals c. N=124 untreated women, N=133 treated women	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	c. 2 dropped out of placebo group for weight gain; no other adverse effects reported	Knapen et al., 2012
Non-randomized, non-blinded, bioavailability	N=4 males; N=8 females	Healthy adults, mean age 37 y (SD = 3)	MK-7 at 0, 45, and 90 mcg/d	2 week for each treatment, separated	Bioavailability of MK-7 in olive oil (MK-7 plasma levels) and effect on osteocalcin	No dropouts or adverse events reported	Brugè et al., 2011

				by 2 week washout period	and its carboxylation status		
Randomized, double-blind, placebo-controlled	N=334	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	5 participants in each group sustained a fracture; 2 in treatment groups had increased nocturnal hot flushes and abdominal pain, 1 in treatment had increased palpitations that ceased at study end; in placebo group, 4 reports of muscular pain and general unwell feeling, 2 reports of itching	Emaus et al., 2010
Randomized, double-blind, placebo-controlled, prospective, longitudinal	N=35 lung transplant patients, N=59 heart transplant patients	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	10 heart patients did not complete 12 mo follow-up; 1 in MK-7 treatment arm died from causes not connected to study. Other adverse effects not related to treatment and not different between treatment and placebo groups	Forli et al., 2010
a. Single-dose oral bioavailability b. Escalating dose-response c. Randomized crossover d. Nonrandomized drug interaction study	a. N=15 b. N=10 c. N=18 d. N=12	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	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 d. MK-7 at 97.4 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	No adverse reactions in trials a, b, and c; in the interaction trial, doses of K_1 at 315 mcg/d and of MK-7 at 130 mcg/d caused significant decrease in INR from 2.0 to 1.5 (i.e., MK-7 was much more potent)	Schurgers et al., 2007

		while treated with escalating doses of MK-7 or K_1	with weekly increment of 97.4 mcg and K_1 at 49.6 mcg/d with weekly increment of 49.6 mcg				

AGE: advanced glycation end products; c-OC: carboxylated osteocalcin; CVD: cardiovascular disease; dc-uc-MGP: desphospho-uncarboxylated matrix Gla protein; hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin 6; INR: international normalized ratio; MGP: matrix Gla protein; SD: standard deviation; TNF-α: tumor necrosis factor α; uc-OC: uncarboxylated osteocalcin; VCAM: vascular cell adhesion molecule
Adapted from Marles et al. (2017) with inclusion of new studies

In an attempt to understand the dosage range of MK-7 that can be administered without clinically affecting vitamin K antagonists (VKA) stability, 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 MK-7 supplements in healthy volunteers stabilized on acenocoumarol. 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). The findings from this study are further discussed in Section 6.3.3.2. Dietary Vitamin K and Anticoagulation by VKAs.

Theuwissen et al. (2012) investigated the dose-response effects of extra intake of 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.

In a double-blind, randomized, placebo-controlled trial, Emaus et al. (2010) investigated the effects of 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.

In an open label, observational trial, Mehta et al. (2010) recruited 19 subjects (13 female and 6 males; 18-81 years old) with muscle cramps. Depending on the severity of the frequency of cramps, the subjects were divided in two groups (group A: n=9, cramps every day; group B: n=10, 2-3 cramps/week). The subjects received 100 mcg MK-7/day (subject of present GRAS) for three months. At baseline and at the end of treatment period, blood was collected and analyzed for hematology and clinical chemistry parameters that included complete blood cell count, blood biochemistry, TSH, liver function tests, kidney function tests as well as bleeding time and clotting time. Additionally, clinical tolerability and adverse events were monitored. No treatment-related changes in the blood parameters were reported. MK-7 was well tolerated clinically and no subjective or objective adverse effects were reported.

In summary, MK-7 has been extensively investigated for its role in the prevention of osteoporosis and maintenance of cardiovascular health. In the published literature, over 25 clinical trials, in over 2000 participants, investigating the effects of MK-7 have appeared. Several of these trials were double-blind, placebo-controlled that are least likely to result in bias, will capture the adverse effects, and provide an opportunity to assess safety and 'tolerability' of MK-7 in a diverse population. The findings from long-term high dose trials in which treatment with MK-7 at levels 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. MK-7 supplementation at doses as low as 10 mcg significantly influenced anticoagulation sensitivity in some individuals. The available information from multiple clinical trials suggest that MK-7 is unlikely to cause any adverse effects at the proposed use levels in healthy subjects. The findings related to effects of MK-7 and anti-coagulation are further described below.

6.2.4. Animal Toxicity and Genotoxicity Studies

The available published acute and subchronic animal toxicity studies with MK-7 are summarized in Table 11.

6.2.4.1. Acute Toxicity Studies

Pucaj et al. (2011) investigated acute toxic effects (limit dose) of 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 MK-7 is greater than 2000 mg/kg bw.

In another acute oral toxicity study, Ravishankar et al. (2014) investigated the effects of MK-7 in Wistar rats. In this limit dose LD_{50} study, MK-7 at a dose level of 2000 mg/kg bw was

administered orally to 3 male and 3 female rats. Because of solubility-related issues, doses higher than 2000 mg/kg bw could not be 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 LD_{50} of MK-7 is >2000 mg/kg bw.

6.2.4.2. Subchronic Toxicity Studies

Pucaj et al. (2011) investigated the potential toxicity of MK-7 in rats following subchronic exposure. In this study conducted as per OECD and FDA guidance, and in compliance with GLP, Sprague Dawley rats (10/sex/group) were administered 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 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. As compared to the 90th percentile intake of MK-7 (12 mcg/day) from the proposed uses, the rat NOAEL is over 800 fold higher.

In another subchronic toxicity study, Ravishankar et al (2015) investigated the effects of MK-7 in male and female rats. In this study, 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. Female rats receiving MK-7 at doses of 0.5 and 1 mg/kg bw/day showed a significant decrease in liver weight. These changes were not considered as adverse, as no changes were noted in histopathology and in male rats. The blood and urine samples were collected on 0, 45, and 91 day. As compared to the baseline and control group, the test compound did not produce biochemical changes. The only biochemical change observed was an elevation in serum uric acid in male rats receiving 0.5 and 1 mg of MK-7. The increase was not noted in female rats and was not considered as adverse effect. No histopathological lesions or changes were noted in any of the organs studied, except for the following changes in reproductive organs. In ovaries of the females, an increased number and size of follicles were found, and also changes in the myometrium were found. In testicles an increased spermatogenesis was observed. These effects did not occur at the dose of 0.1 mg/kg bw/day. The histopathological changes were not considered as adverse. The results of this study suggest a NOAEL of 1 mg/kg bw/day for MK-7, the highest dose studied.

Table 11. Summary of Acute and Subchronic Toxicity Studies with MK-7

Reference	Study design	Observations	Results
Pucaj et al. (2011)	Acute oral toxicity test. MK-7 suspended in sunflower oil was administered to mice by single oral gavage to achieve a dose of	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	At limit dose level of 2000 mg/kg, MK-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

	2000 mg/kg body weight	central nervous systems. Animals were also observed for changes in motor activity and behavior pattern.	
Ravishankar et al. (2015)	Acute oral toxicity study in rats. MK-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 MK-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 given MK-7 for 90 d at doses of 0, 2.5, 5.0, and 10 mg/kg body weight per day	Rats were observed for clinical signs and mortality 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.	No deaths occurred, and no compound-related toxicity was 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. MK-7 administered orally by gavage at 0, 0.1, 0.5, and 1.0 mg/kg bw/day for 90 days. MK-7 prepared fresh daily in propylene glycol and administered as 1 mL/100 g body weight between 8 am and 9 am	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 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,	Normal weight gain pattern in all groups; slight increased weight gain in rats receiving MK-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, 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

		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	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. Adapted from Marles et al. (2017).

6.2.4.3. Genotoxicity Studies

In an *in vitro* mutagenicity assay, Ravishankar et al. (2014) investigated the potential effects of MK-7 to induce reverse mutation in *Salmonella typhimurium* tester strains TA1535, TA97a, TA98, TA100, and TA102 in the presence and absence of metabolic activation system (S9). Based upon the preliminary solubility/precipitation and cytotoxicity tests, the tester strains were exposed to 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 MK-7 is not mutagenic in *S. typhimurium* strains.

Ravishankar et al. (2014) also studied the potential genotoxicity of MK-7 in rats. In this study, rats were divided into six groups (10/group/sex), of which four groups received MK-7 and two control groups received the vehicle (distilled water containing Tween-20- 2 drops/20 ml). Of

the two vehicle treated control groups, one served as control for the micronucleus test (MNT) and the other was used for comet assay. In the test article treated groups, two groups received MK-7 at a dose level of 100 mcg/kg bw/day, while the remaining two received 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 (ip.) 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 (ip.) 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 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 MK-7 treated group, the group receiving cyclophosphamide revealed signs of clinical toxicity and genotoxicity.

In yet another study, Ravishankar et al. (2014) investigated the role of MK-7 in the prevention of cyclophosphamide induced genotoxicity in Wistar rats. In this study, rats were divided into four groups (10/sex/group). Of the four groups, two received MK-7 (100 mcg/day), while two served as control (vehicle). Both the vehicle and MK-7 were treated daily for 15 consecutive days. On day 16, all animals received cyclophosphamide (ip, 40 mg/kg). For the comet assay, the control and test group received Colchicine (ip. 4 mg/kg) at 24 hours after the cyclophosphamide administration. On study day 17, blood was collected and the animals were euthanized. Clinical symptoms, weekly feed consumption, and body weight change were recorded. The blood was processed for chromosomal aberration, micronucleus test, and comet assay. The result of this study indicated that oral administration of MK-7 at 100 mcg/kg bw for 15 days to Wistar rats did not induce any clinical signs of toxicity. No significant differences in the tested parameters between the active and vehicle receiving groups were noted. The results of this study indicate that MK-7 is not toxic. The results also show that MK-7 did not prevent the toxicity of cyclophosphamide in experimental animals.

6.2.5. Studies with MK-4

6.2.5.1. Human Clinical Studies of MK-4

Several human clinical studies describing the effects of MK-4 have appeared in the published literature. In majority of these studies, the effects of MK-4 as a therapeutic agent (marketed in Japan for over 15 years) have been investigated. In these studies, the effect of MK-4 on various parameters related to bone metabolism were investigated. Some of these clinical studies of MK-4 are summarized in Table 12. The doses used in these trials were usually 45 mg/day. The duration of some of the studies ranged from 24 weeks to 2 years. As described earlier, the only difference between MK-4 and MK-7 or other menaquinones is the number of side chain unsaturated isoprenyl groups at the 3-position. Given the structural similarity, the studies of MK-4 are applicable to the safety assessment of MK-7 with some caveat. The dose of MK-4 in human clinical studies is approximately 1000-fold higher compared to the estimated daily intake of MK-7, the subject of present GRAS assessment.

In a recent systematic review and meta-analysis, Su et al. (2019) comprehensively evaluated the role of MK-4 in the management of osteoporosis. After full text screening by these

researchers, 18 randomized controlled trials with 8882 participants were included in the systematic review and meta-analysis. Pooled analyses showed that MK-4 was more effective than placebo in improving lumbar BMD and decreasing ucOC/OC. In addition to efficacy the trials that reported adverse events, adverse drug reactions, gastrointestinal adverse effects, skin and subcutaneous tissue disorders, or prothrombin time were also evaluated. Compared with placebo, MK-4 significantly increased the incidence of adverse events (AEs) (two studies, N = 1949, RR = 1.47, 95% CI 1.07 to 2.02) and adverse drug reactions (four studies, N = 6102, RR = 1.29, 95% CI 1.07 to 1.56). However, no significant difference in serious adverse events, gastrointestinal adverse events or skin, and subcutaneous tissue disorders was detected between MK-4 and placebo. None of the included randomized controlled trials reported the occurrence of coagulation disorders. It should be noted that in these studies, the dose of MK-4 was quite high (45 mg/day). In reviewing the studies reporting the specific adverse events or adverse drug reactions, Su et al. (2019) found that gastrointestinal disorders and skin/subcutaneous tissue disorders were the two most common adverse events, which were not considered serious and could be resolved after taking action. Overall, the limited evidence showed that MK-4's tolerability may be acceptable.

Table 12. Human Clinical Studies with Menaquinone-4 (MK-4)

Subject/ treatment	Doses/Duration	Observations	Reference
18 MDS refractory anemia patient	0 or 45 mg/day for 16 weeks	Given the absence of toxicity associated with MK-4 administration, recommended its use for all MDS-RA patients.	Takami et al., 2002
25 women with liver cirrhosis, 42-72 years of age	45 mg for 2 years	22 of 25 patients assigned to the test group completed study. No adverse effects related to treatment with vitamin K2 were noted	Shiomi et al., 2002
200 female patients with Alzheimer's disease, 100 healthy females as control group	Vitamin K2 (not specified) supplementation when administered concurrently with 600 mg calcium/day and 1000 IU ergocalciferol/day for 2 years	No adverse effects were reported in study participants. Combined treatment of elderly female Alzheimer's disease patients with MK-4, ergocalciferol, and calcium may represent safe and effective measure for increasing bone mass and reducing the risk of fracture.	Sato et al., 2002
5 male, 25 female osteoporotic patients	45 mg, 3 times/day for >3 weeks	MK4 levels increased at 4 weeks after administration and remained elevated for duration of study period. Plasma levels of phylloquinone and MK-7 unchanged. A group of vitamin K-deficient patients identified. Vitamin K2 can be administered safely without inducing hemostatic disturbances to patients not treated with anticoagulants.	Asakura et al., 2001
43 subjects in test group, 40 subjects in control group	0 or 45 mg/day for 12 months	No adverse events were reported. At 6 months into the study period, the control group also was placed on vitamin K2 supplementation. Adverse reactions reported over the course of the study period limited to 2 incidences of mild skin rash, which subsided once treatment was discontinued.	Bunyaratavej et al., 2001

Subject/ treatment	Doses/Duration	Observations	Reference
110 Japanese females (24 to 52 years of age) diagnosed with endometriosis and/or uterine leiomyomas	All administered Leuprolide therapy for their respective estrogen dependent disease either alone (control) or in conjunction with 45 mg vitamin K2, 0.5 µg 1,25-(OH)2-D3, or 45 mg vitamin K2 and 0.5 µg 1,25-(OH)2-D3 for 6 months.	Vitamin K2 partially prevents bone loss associated with estrogen deficiency resulting from leuprolide treatment. No adverse effects were reported in study participants	Somekawa et al., 1999
75 osteoporotic patients	90 mg or placebo for 24 weeks	Heartburn, stomach upsets, and abdominal fullness were noted. Authors noted that symptoms were unlikely to be related to treatment or could not ascertain a relationship between vitamin K2 treatment and the effects. Increases in GOT, GPT, ALP, and GGPT reportedly observed in single subject in vitamin K2 treatment group and were described as probably related to treatment. All variations were reported to return to normal levels following study completion. No significant differences were reported in prothrombin time between test subjects and the placebo control group.	Orimo et al., 1998
Pregnant mothers (number not specified)	Single intravenous dose of 60 mg MK-4 or 20 mg MK-4 orally for a period of 7 days	MK-4 appears to transfer into placental tissue, where it is stored, and subsequently gradually released into fetal blood. Vitamin K2 found to be concentrated in maternal milk, where on day 4 following intravenous administration, levels 90-times greater than those identified in maternal blood for same period of time were reported. No adverse effects reported in mothers or infants.	Iioka et al., 1991
Pregnant mothers (number not specified)	Oral administration of 20 mg vitamin K2/day for 7 to 10 days prior to delivery	Increased levels of vitamin K2 in mothers' plasma, cord plasma, and breast milk. Postnatally, infants observed for period of 5 days, during which no evidence of bleeding reported.	Motohara et al., 1990
Neonates exhibiting thrombotest results below 20% 48 hours following birth (number not specified)	Single oral administration of up to 6 mg of vitamin K2	Significant increase in thrombotest values without any significant changes in bleeding time or levels of plasma bilirubin and hematocrit	Suzuki et al., 1984

Ushiroyama et al. (2002) investigated the effects of the combined use of MK-4 (45 mg/day) and D3 on vertebral bone mineral density in 172 postmenopausal Japanese women. The treatment continued for two years with bone mineral density measured prior to initiation of the

study and after 6, 12, 18, and 24 months of treatment. Tests of blood coagulation function consisted of measurement of activated partial thromboplastin time (APTT) and analysis of concentrations of antithrombin III (AT III), fibrinogen, and plasminogen. The coagulation and fibrinolytic activity were increased, however, were within the normal range, suggesting that balance was maintained in the fibrinolysis-coagulation system. No adverse reactions were observed. In another study by Takahashi et al. (2001), 43 premenopausal and 48 postmenopausal healthy females, 89 osteoporotic patients with vertebral fractures, and 24 patients with hip fracture were treated with MK-4 and/or vitamin D. After 4 weeks of treatment, ucOC decreased significantly in patients on MK-4 and MK-4 + D. On the other hand, vitamin D did not improve ucOC levels. No side effects of the MK-4 treatment were reported during the course of the study.

In a randomized open label trial, 241 osteoporotic subjects were divided into two groups. Group 1 (n=121) received 45 mg/day (treatment) MK-4 via oral administration for two years, while the group 2 (n=120) served as control. The incidence of clinical fractures during the 2 years of study in the control was higher as compared to MK-4 treated group. The percentages of change from the initial value of lumbar bone mineral density at 6, 12, and 24 months was significantly lower for the MK-4-treated group. MK-4 treatment enhanced gamma-carboxylation of the osteocalcin molecule. No adverse effects of the treatment with MK-4 were reported in the study. In another study, the effect of the combined administration of vitamin D3 and MK-4 (45 mg/day) on bone mineral density (BMD) of the lumbar spine was examined in postmenopausal women with osteoporosis (Iwamoto et al., 2000). In this study, 92 osteoporotic women (55-81 years old), who were in menopause for more than five years were treated with vitamin D3 (0.75 μg/day), MK-4 (45 mg/day), vitamin D3 Pure MK-4 and calcium (calcium lactate, 2 g/day). A significant decrease was observed in BMD in the calcium supplemented group and a significant increase in BMD in the D3 and MK-4 groups compared with that in the calcium group and a significant increase in BMD in the D3/ MK-4 group compared with that in the calcium, D3, and MK-4 groups. There were no reports of adverse effects of the treatment in any of the groups.

Miki et al. (2003) investigated effects of MK-4 on serum ucOC level in elderly women with established osteoporosis. In this study, 12 elderly osteoporotic women with vertebral fractures received either MK-4 at a dose of 45 mg/day or calcium with MK-4 for two weeks. The control group received calcium. Administration of MK-4 significantly raised the serum levels of MK-4. No adverse effects from treatment with MK-4 were reported during the course of this study. In yet another study, MK-4 was administered at a dose 45 mg/day to 23 patients with myelodysplastic syndrome (MDS) (Abe et al., 2002). Six patients showed improvement of anemia. No adverse effects of MK-4 administration were observed, and the time required to obtain the hematological improvement was relatively short, on average three months.

Knapen et al. (2007) tested whether high MK-4 intake promotes bone mineral density and bone strength in a randomized clinical intervention trial. In this study, 325 postmenopausal women (age 55 to 75 years at intake, Caucasian race, apparently healthy, non-osteoporotic) received either placebo or 45 mg/day of MK-4 for three years. The clinical end points evaluated at baseline and after 1, 2 and 3 years included bone mineral density (DXA-BMD), bone mineral content (BMC) and bone strength indices of the femoral neck. MK-4 did not affect the DXA-BMD, but BMC and the femoral neck width had increased relative to placebo. In the MK-4-treated group hip bone strength remained unchanged during the 3-year intervention period, whereas in the placebo group bone strength decreased significantly. The investigators reported

that even though very high doses of MK-4 were used in the study, adverse side effects were minor and not different from the placebo group.

Kakizaki et al. (2007) investigated the effects of MK-4 on the recurrence of the hepatocellular carcinoma and survival of 60 patients diagnosed to be free of the disease. The patients were randomly assigned to either the MK-4 group (n=30) or the control group (n=30). The subjects in the treatment group received an oral dose of MK-4 at 45 mg/day. The study lasted for three years and the recurrence rates were lower in treatment groups as compared to control. The cumulative recurrence-free rates in the MK-4 group were 92.3% at 12 months, 48.6% at 24 months and 38.8% at 36 months; and those in the control group were 71.7%, 35.9% and 9.9%, respectively (P = 0.045). The cumulative survival rates in the MK-4 group were 100% at 12 months, 95.0% at 24 months, and 77.5% at 36 months; and those in the control group were 95.8%, 90.2%, and 66.4%, respectively. No adverse effects of MK-4 treatment were reported.

In summary, in multiple clinical studies, high doses of MK-4 have been well tolerated. In one of the clinical studies, MK-4 at a dose level of 90 mg/day for 24 weeks did not reveal any significant treatment related adverse effects. In other studies, conducted at dose levels of 45 mg/day, most notably for durations of six months, and for two years in one study, also did not reveal any significant treatment related adverse effects. In Japan, patients with osteoporosis are commonly prescribed to take 45 - 90 mg/day of MK-4 and this regimen has been in place for at least 15 years without reported significant adverse effects. In a comprehensive review and meta-analysis of 18 clinical trials, the investigators reported that MK-4's tolerability as a therapeutic agent may be acceptable. The approved uses of MK-4 for therapeutic purposes are over 10000 to 20000 times higher than the proposed use of MK-7 in the current safety assessment.

6.2.5.2. Animal Toxicity Studies of MK-4

In two separate subchronic toxicity studies, the effects of MK-4 in rats and dogs were investigated. No adverse effects were seen in a 13-week oral (gavage) toxicity study conducted in groups of Sprague-Dawley rats (10/sex/group) at 30 mg/kg bw/day (Doi et al., 1995). In a 3-month study in male and female beagle dogs (number per group not identified), the authors identified 200 mg/kg bw/day as being the no observed adverse effect level (NOAEL) (Goldsmith et al., 1995). Longer term, repeat-dose toxicity studies were conducted with rats and dogs receiving oral MK-4 administrations for a period of 1 year (Hosokawa et al., 1995; Vanatta et at., 1995). In the feeding study, Hosokawa et al. (1995) provided MK-4 to Fisher 344 rats (20/sex/group) at dietary levels of 0 (control and non-treated naive groups), 0.04, 0.2, or 1.0% (providing approximately 0, 20, 100, and 500 mg MK-4/kg bw/day, respectively). In this study, the LOAEL was determined to be 20 mg/kg/day, the lowest dose tested. A NOAEL was not established. In the dog study, the l-year treatment period was followed by a 3-month recovery period (Vanatta et al., 1995). MK-4 was provided orally in capsules to groups of 6 male and 6 female dogs to provide doses of 0 (empty capsule), 20, 200, or 2000 mg/kg bw/day. The NOAEL was considered as 200 mg/kg bw/day.

In developmental toxicity studies in mice and rats, Suzuki et al. (1971) investigated the effect of MK-4 on development of the fetuses and offspring. The study was published in Japanese language; however, the details of the protocol and the results of these investigations were described in the EFSA (2008) safety assessment. In the developmental study, MK-4 was administered orally at doses of 0, 10, 500, or 1000 mg/kg bw/day or intraperitoneally at doses of 0, 5, 50, or 100 mg/kg bw/day to pregnant mice and rats for a period of 6 days from days 7 to 12

and 9 to 14 of gestation, respectively. Irrespective of the route of administration, no differences were noted in the number of total implants, percentage of resorptions, dead or live fetuses, mean body weights, and type and number of anomalies in mice or rat fetuses. Compared to the control, mouse fetuses obtained from the group receiving oral administration of 500 and 1000 mg/kg bw/day showed an increased incidence of non-ossified forelimbs compared to fetuses of control mice. In the group receiving 500 mg/kg bw/day, skeletal anomalies were limited to an increased frequency of variations of the cervical ribs. The post-natal development observations in the test groups (results reported for only the 10 and 1000 mg/kg bw/day dose groups) revealed increased separation of ear auricular and emergence of abdominal hair in the high-dose offspring compared to controls. Compared to controls, in male fetuses from the low-dose (10 mg/kg bw/day) treated group, the descent of testes was reduced. In rat fetuses, a decrease in non-ossified forelimbs was observed in the low-dose group, while increases in undeveloped metatarsals and non-ossified hind limbs were noted in fetuses from the high-dose group. No statistically significant variations were reported in the postnatal development or weaning of rat offspring. The investigators concluded that MK-4 did not cause teratogenic effects, mortality, or inhibition of fetal growth, and it produced no effects on postnatal development or weaning of mice and rats (Suzuki et al., 1971).

Mikami et al. (1981) investigated the effects of MK-4 on fertility, and prenatal and postnatal development in rats. This study, published in Japanese with English tables and figures, was also reviewed by EFSA (2008). In this study, rats of unspecified strain (22 to 24/sex/group) were orally administered 0, 10, 100, or 1000 mg/kg bw/day of MK-4 for a period of 14 days. The findings from this study did not reveal any significant malformations in offspring or any other reproductive or developmental effects. The parameters measured included the number of corpora lutea, number of implantations, implantation ratio (total implantation/total corpora lutea) percentage of resorptions and viable fetuses, and fetal and placental weights. There were no significant compound-related effects observed on the physiological, morphological, or functional development of offspring during the peri- and post-natal periods (Mikami et al., 1981).

In summary, in two long-term (one year) toxicology studies with MK-4, the LOAEL in rats and NOAEL in dogs was considered as 20 mg/kg/day and 200 mg/kg/day, respectively. These no effect levels are several orders of magnitude higher compared to the most exaggerated consumption level predicted for total vitamin K. As regards the developmental toxicity study, the EFSA Panel (2008) reviewed data from this study and stated, "... there were no compound-related effects observed on reproductive and developmental parameters measured."

6.2.6. Vitamin K and Coagulation

The role of vitamin K in blood coagulation has been well recognized. Therefore, it is important to understand the potential interference of increased dietary vitamin K in patients on anticoagulant therapy (i.e., Coumadin derivatives). These vitamin K-antagonists (VKA), such as warfarin, inhibit coagulation through antagonizing the action of vitamin K and can thereby interfere with the coagulation cascade. The uncarboxylated, inactive vitamin K-dependent coagulation proteins synthesized in the presence of warfarin result in reduced blood clot formation. The available information suggest that different vitamin K homologs differ in their mode or extent of action in disturbing VKA induced anticoagulation.

Kudo (1990) reported significantly elevated thrombo-test[3] values following intake of Natto in patients undergoing anticoagulant therapy after valve replacement operations from 10 cases (age 25-45 years; 5-male, 5-female). A good correlation between the amount of Natto consumed and thrombo-test value was noted. Serum vitamin K1, MK-4 and MK-7 levels were measured at 0, 24 and 48 hours. No changes in vitamin K1 and MK-4 levels were noted, while a significant increase in MK-7 was noted at 24 and 48 hours. In this article it is stated, "The author assumed earlier that the antagonism of Natto to warfarin might be due to the large amount of vitamin K present in Natto, because warfarin as an anticoagulant inhibits vitamin K dependent coagulation factors. However, the amount of vitamin K present per unit mass of Natto or soybean from which Natto is produced is less than 10% of the vitamin K present per unit mass of cabbage or spinach. Thus as little as 100 g of Natto taken in is unlikely to cause a direct antagonistic effect of warfarin." The author suggested that the antagonistic effects of Natto to warfarin may be attributable to the massive production of vitamin K in the intestine by *B. subtilis* that is present in Natto. Subsequent studies have shown that MK-7 is particularly active in disturbing the anticoagulation and this has been suggested to be due to long half-life of MK-7. As mentioned earlier, 100 g of Natto contains up to 1000 mcg MK-7 and will certainly disturb the VKA treatment. However, it should be noted that the proposed use levels of MK-7 at 4 mcg/serving is very small or negligible (250-fold lower) and is unlikely to affect the INR. Secondly, regular or daily MK-7 supplementation, that has a long half-life as compared to Vitamin K1 or other short-chain K2, is likely to help stabilize INR.

6.2.6.1. Vitamin K and Anticoagulants

Anticoagulants are the cornerstone therapy for thrombosis prevention and treatment. Patients with atrial fibrillation (AF), prosthetic heart valves, venous thromboembolism (VTE) etc. are commonly advised anticoagulant therapy. The mainstay of such therapy has been Vitamin K Antagonists (VKAs), primarily warfarin that has now been in use for over 60 years and remains the most commonly prescribed oral anticoagulant worldwide. Warfarin is used by over 1% of the UK population, and, in the USA there are over 30 million prescriptions annually (Pirmohamed et al., 2015). However, recent data from Warfarin Drug Uses Statistics[4] 2006-2016 shows a steady decline in warfarin prescription since 2010 (when it peaked to about 35 million) to the most recent available data of 2016 (decreased to about 19 million). A search for changes in use (prescription) of warfarin in children (the target population of the subject of present GRAS) over the years did not reveal any specific data. However, it is apparent from the overall decline in the warfarin prescription also indicate decline in use of warfarin in pediatric population.

Warfarin (VKA), 4-hydroxycoumarins, binds to the vitamin K epoxide reductase (VKOR) enzyme and inhibits recycling of vitamin K (Figure 3). Hence, the carboxylation of coagulation factors is inhibited. This results in species that are inactive and non-carboxylate, also known as PIVKAs (Proteins Induced by Vitamin K Antagonists).

[3] A test for the functional intactness of the prothrombin complex that is used in controlling the amount of anticoagulant used in preventing thrombosis

[4] Available at: https://clincalc.com/DrugStats/Drugs/Warfarin

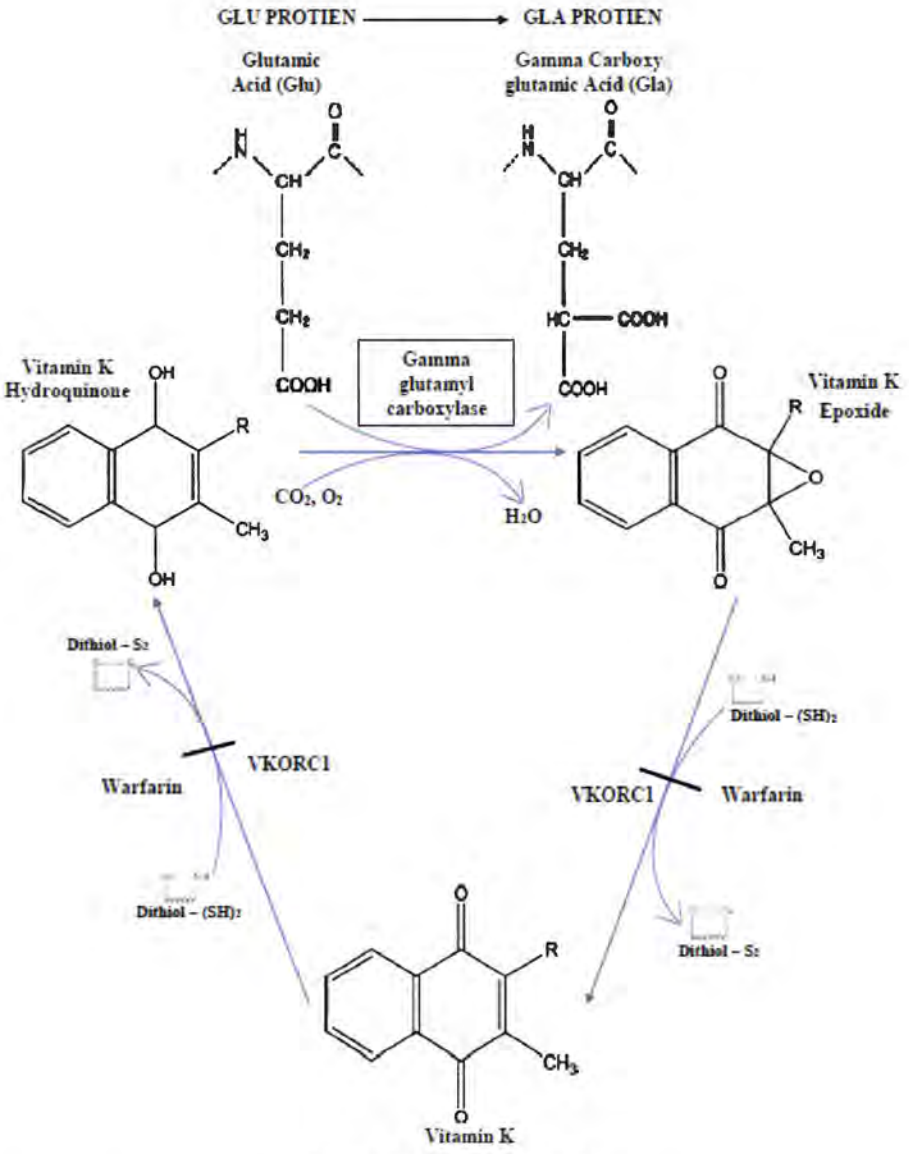

Figure 3. Vitamin K Cycle.

VKORC1: The Vitamin K epoxide Reductase Complex subunit 1 (VKORC1) protein is a key enzyme in the vitamin K cycle. VKORC1 is involved in the vitamin K cycle by reduction of vitamin K epoxide to vitamin K. The availability of reduced vitamin K is of importance for activation vitamin K 2,3-epoxide. The reduction of vitamin K epoxide is then responsible for the carboxylation of glutamic acid residues.

Warfarin is prescribed for individuals in medical conditions that can make blood clot too easily and quickly. Blood clot is formed through coagulation cascade proteins that are formed in the liver and activated by vitamin K. Warfarin works by restricting the vitamin K cycle which otherwise continues to activate coagulation proteins until metabolized. Individuals at risk for developing blood clots take warfarin to prolong the usual time it takes for a clot to form, resulting in an increased INR (International Normalized Ratio)/Prothrombin Time (PT). INR and PT are laboratory test values obtained from measurements of the time it takes blood to clot.

It is common knowledge that dietary vitamin K intake could counteract the anticoagulant effect by VKA (Holbrook et al., 2005; Hirsh et al., 2003). Hence, patients have been advised to reduce dietary vitamin K intake to avoid changes in anticoagulation i.e., International Normalized Ratio (INR) stability. Difficulty to achieve high level of compliance to such restrictions was one of the reasons to develop and introduce non-VKA oral anticoagulants (NOACs), e.g., dabigatran, rivaroxaban, apixaban, endoxaban etc. for the treatment of AF and VTE (Yorkgitis et al., 2015). NOACs pose unique challenge to clinicians in the performance of invasive procedures and during acute hemorrhage. The fact that VKA is still prescribed is a cost issue (much cheaper), tradition (the Dutch thrombosis service for example), and some indications such as no kidney function to clear the drugs (i.e. dialysis) (Ten Cate and Prins, 2017). Hence, VKA continues to be prescribed due to wide clinical experience. However, as indicated earlier there is a steady decline in number of warfarin prescriptions since 2010.

6.2.6.2. Dietary Vitamin K and Anticoagulation by VKAs

Patients starting treatment with VKAs are advised, with great emphasis, on nutritional information and discouraged to consume vitamin-K-rich foods, such as green leafy vegetables. It is essential to understand the putative interaction between dietary vitamin K intake and changes in INR with the VKAs. In a systematic review of interaction between dietary vitamin K intake and anticoagulation by vitamin K antagonists, Violi et al., (2016) evaluated the results of published dietary interventional trials with VKA. The objective of this study was to understand the interaction of dietary vitamin K intake on the anticoagulation by VKA. These investigators found 14,865 potentially relevant studies in their searches, of which 12,819 studies were analyzed in detail, yielding 11 relevant clinical studies. These investigators concluded that:

> "The available evidence does not support current advice to modify dietary habits when starting therapy with VKAs. Restriction of dietary vitamin K intake does not seem to be a valid strategy to improve anticoagulation quality with VKAs. It would be, perhaps, more relevant to maintain stable dietary habit, avoiding wide changes in the intake of vitamin K".

Changes in the daily intake of vitamin K are inevitable. Violi et al. (2016), found the range of daily intake of vitamin K1 variation from 76 to 217 mcg. Analysis of the studies revealed that observable effects on INR were detected only for >150 mcg vitamin K1/day (Schurgers et al., 2004). These investigators point out that the bioavailability factor, difference between the vitamin availability from the plant products to that from the supplemental pure vitamins, should be considered and at the same time significantly suggest that "the putative interaction between food and VKAs should be eliminated from international guidelines".

As described earlier, Schurgers et al. (2007) carried out studies with pure vitamin K during warfarin dosing. The findings from this study revealed that a dose of 315 mcg/day of K1 and 130 mcg/day of MK-7 changed INR from 2.0 to 1.5. Relatively lower value of 130 mcg/day for MK-7 was most likely attributed to much longer half-life and 6-fold higher cofactor activity *in vitro* (Buitenhuis et al., 1990). Further, the investigators comment that, if expressed as AUC over 24 hours (AUC24), the availability MK-7 is 2.5-fold better 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". Further the investigators state: "Hematologists, on the other hand,

need to be aware that relatively low doses of MK-7 may have a larger impact on the stability of oral anticoagulation than K1".

In contrast to their 2007 study (Schurgers et al., 2007), and as described earlier, investigators from the same group (Theuwissen et al., 2013) gave a more cautious advice on the use of MK-7 supplements even at a low dose of 10 mcg/day for those undergoing anticoagulation treatments. The researchers chose 2.0 INR as the target INR and included 18 healthy volunteers in their study. Their reasoning for the choice of 2.0 INR is explained as (A) for the safety of the healthy volunteers and (B) for direct comparison to their previous study. On the other hand, INR of 2.0 to 3.0 is considered safe therapeutic range for the patients undergoing anticoagulation treatment (Baker et al., 2009). A target INR of 2.5 would have been a better choice in determining dose-response relationship.

The 2008 practice guidelines from the American College of Chest Physicians include a recommendation to use long-term oral anticoagulation in patients with atrial fibrillation (AF) and a recent stroke or transient ischemic attack, to a target INR of 2.5 (range 2.0 to 3.0; Grade 1A quality of evidence) (Baker et al., 2009). At the 10 mcg/day dose, variation in INR was 1.91±0.43 after one week of dosing and 1.93±0.35 after two weeks of dosing. Variation of INR of ±0.5 allows to remain within the therapeutic range with a target INR of 2.5. Two hematologists, on the study panel, had to conclude that the emerging data from the study was of clinical relevance. This can be explained by the fact that going below 2.0 is considered outside the therapeutic zone, however, if one were to shift the results to a base of 2.5 INR then the results are within the therapeutic zone. Further, 2016 review and conclusion by Violi et al. (2016), as mentioned earlier, needs to be considered carefully in light of the conclusions they arrived at after reviewing vast amount of data.

6.2.6.3. Risk Related to the Use of VKAs

As described earlier, the use of VKA anticoagulants is associated with impaired function of osteocalcin. Pastoureau et al. (1993) reported that lambs receiving warfarin developed osteopenia within several months. Gage et al. (2006) quantified the association between coumarin anticoagulants and accelerated bone loss and low bone mass. Consequently, long-term use of oral anticoagulants is considered as a risk factor for developing osteoporosis. Matrix Gla-protein (MGP) is an inhibitor of soft tissue calcification by chelating calcium ions which otherwise may deposit in vasa-media and vasa-intima of arterial wall, consequently, hardening the arteries. MGP contains nine glutamate residues five of which can be carboxylated. Only when they are carboxylated they will actively remove calcium from depositing. Hence, uncarboxylated MGP must be regarded as a risk factor for arterial calcification. While warfarin may prevent stroke and pulmonary embolism, it calls for low levels of vitamin K leading to greater risk of osteoporosis, bone fractures, and calcification of arteries.

6.2.6.4. Stability of Anticoagulant Treatment

VKAs need monitoring for efficacy and safety via the international normalized ratio (INR), and the frequency is determined by the stability of the INR within a specific range of 2.0 to 3.0 with a target INR of 2.5 (Baker et al., 2009). van Walraven et al. (2006) evaluated 67 studies involving 50,208 patients with 57,155 patient-years of follow-up. Overall, patients taking vitamin K antagonists for a wide range of indications that included atrial fibrillation, venous thromboembolism, cardiovascular disease other than atrial fibrillation, peripheral vascular disease, valvular heart disease, and other indications were within Time of Therapeutic

Range (TTR) INR range 63.6% of the time. Patients managed in usual care (by community physicians), TTR was 12.2% lower compared with patients managed in anticoagulation clinics. In the study by Baker et al. (2009) that involved meta-analysis of 22,237 warfarin-treated AF patients with 41,199 years of follow-up, the average TTR was found as 55%. Patients in anticoagulation clinics had an average TTR of 63% whereas patients in community practice had an average TTR of 51%. These percentages reveal a gap in protection from ischemic stroke.

6.2.6.5. Low Dose Vitamin K Supplementation to Improve INR Stability

The objective of anticoagulant therapy is to have stable target INR of 2.5 or between 2.0 to 3.0. Practical experience is that half of all the patients who receive warfarin fail to stabilize their target range, with resultant increased risk of thromboembolism and the drugs adverse effect of bleeding (Beyth et al., 2000; Heneghan et al., 20006). Reasons for these risk factors can be concurrent medications, comorbidity and patient compliance besides the intra-individual genetic differences in response to warfarin.

Traditionally, patients taking warfarin have been advised to limit or avoid vitamin K intake from food to settle INR in the therapeutic range. Avoidance of vitamin K depletes vitamin K pool because it is utilized. Vitamin K is a vitamin which means "vital amine: and thus required to receive via the food. Sudden increase of vitamin K intake can significantly decrease the INR with the risk of clot formation. Patients with reduced intake of vitamin K have unstable control of anticoagulation (Scone et al., 2005). In a double-blinded placebo-controlled parallel design study, Sconce et al. (2007) investigated increased stability of anticoagulation control through supplementation of vitamin K. These investigators established benefits of this strategy for stable INR. Supplementation with MK-7, instead of K1, is recommended as it is not preferentially targeted to the liver. MK-7's extrahepatic activity protects against warfarin induced calcification of arteries and osteoporosis. In a letter to Editor, Stafford et al. (2007) support this view and added that the potential benefit is greater in terms of reducing bleeding risk. It is noteworthy to mention that Stafford is an expert on vitamin K metabolism and his group discovered both the carboxylase and VKOR gene and protein (Stafford, 2005).

Based on findings from a multi-center, placebo-controlled, randomized trial, Boonyawat et al. (2016) also reported that low dose oral vitamin K (LDVK) supplementation (150 mcg/day) for a total of six months after a one-month run in period reduced INR excursions suggesting that LDVK did reduce extreme INR variation. LDVK was associated with a decreased number of extreme INR values (<1.5 or >4.5) without increasing risk of adverse events.

Micronutrient deficiencies, through inadequate intakes of vitamins and minerals, result in chronic metabolic disruption, including mitochondrial decay. Ames (2006) in his hypothesis of the triage theory suggests that "micronutrient deficiencies that trigger the triage response would accelerate cancer, aging, and neural decay but would leave critical metabolic functions, such as ATP production, intact". Ames (2010) further state that "A triage perspective reinforces recommendations of some experts that much of the population and warfarin/coumadin patients may not receive sufficient vitamin K for optimal function of VKD proteins that are important to maintain long-term health." This point is critical to consider while controlling INR and balancing vitamin K adequacy. Low dose vitamin K supplementation therapy favors improving the vitamin K status while simultaneously stabilizing INR.

In summary, the intended low dose (4 mcg MK-7) per serving use, addition limited to foods for special dietary uses (PediaSure®) for children should ensure that children currently taking VKA anticoagulants are not negatively affected by the product. Furthermore, the use of warfarin over the past six years for which the data is available is steadily declining. Warfarin is the most frequent anticoagulant used in pediatrics.

6.2.7. MK-7 and Drug Interaction

In the previous section, discussion was related to the biochemical interaction between vitamin K and VKA. In this section, discussion related to metabolic interactions between vitamin K and VKA that may have an impact on INR is presented.

The levels of supplementation of MK-7, or for that matter any other supplementation, be it a vitamin or mineral or herbal or any other variety, must be considered in terms of any on-going therapy and the impact of the resulting drug interaction. In pediatric supplementation of MK-7, just as in adult supplementation of MK-7, one of the main areas of concern is the anticoagulation treatment where the anticoagulant may be interactive with the vitamin. VKA continues to be used as an anticoagulant in pediatric population with the existing vast clinical experience. At a high enough level of vitamin K supplementation, it may affect the INR which needs to be controlled within a specific target range of INR 2.0 to 3.0.

The available literature suggests that a low dose supplementation of vitamin K~~2~~ is helpful in stabilizing INR which is contrary to common belief. At the same time there is a question of how K2 may interfere with VKA. Does vitamin K2 at a low dose supplementation have significant impact on warfarin activity by affecting warfarin concentration in the serum? Further, is there any specific effect on one of the racemic components of warfarin ending up affecting dose to activity relation? The narrow therapeutic index of warfarin poses a unique challenge to the risk of under-dosing, which can result in loss of therapeutic efficacy and potential for thrombosis, with that of overdosing, which may lead to bleeding and potentially fatal hemorrhage. While low dose MK-7 supplementation is helpful in stabilizing INR, the question remains whether it may cause any complications through the cytochrome P450 set of genes.

Cytochrome P450 enzymes metabolize external substances, such as medications that are ingested, and internal substances, such as toxins that are formed within cells. These enzymes also metabolize MK-7 and warfarin. If the same enzyme was involved in metabolizing both the compounds, i.e., MK-7 and warfarin, warfarin concentration may increase if MK-7's interfering metabolism effect sufficiently slows warfarin catabolism. Further, there is also a question whether K2 has any effect on the transcription of the catabolizing enzymes. It can be noted that polymorphism in Cytochrome P450 enzymes plays a role in dosage variability of warfarin and MK-7. This parameter is generally taken into consideration by clinicians while adjusting warfarin dosage.

If a cytochrome P450 enzyme metabolizes a drug slowly, due to K2 affecting the expression of the enzyme, the drug remains active longer; lower dose is needed to get the pharmacological effect. A drug that is quickly metabolized is broken down at an increased level and a higher dose may be needed to be effective. Most of our knowledge is based on vitamin K1 and MK-4 interactions with these enzymes. There is limited knowledge of MK-7 interactions; however, based on the knowledge of vitamin K1 and MK-4 catabolic pathways, the same is viewed as applicable to MK-7. Theuwissen et al. (2013) suggested that the relative

ability of different vitamin K compounds to induce CYPs is an important factor if vitamin K is taken regularly with VKAs. In the following section an attempt has been made to discuss: (1) MK-7 catabolism and (2) Vitamin K as a ligand to SXR.

6.2.7.1. MK-7 Catabolism

CYP4F2 is cytochrome P450's family 4 subfamily F member 2. This gene is part of a cluster of cytochromes P450 genes on chromosome 19. Another member of this family is CYP4F11. These proteins localize to the endoplasmic reticulum. Edson et al. (2013) have shown that CYP4F2 and CYP4F11 are vitamin K1 and K2 ω-hydroxylases and that CYP4F2 also metabolizes vitamin K to the ω-carboxy metabolite. These investigators also state that it is unknown whether MK7, another form of vitamin K2, is a CYP4F2 and CYP4F11 substrate. In the absence of experimental proof, it is assumed that the structurally similar MK-7 will follow the same biochemistry path as with the K1 and MK-4 and thus be metabolized by CYP4F2 and CYP4F11.

Although it is recognized that warfarin is catabolized by CYP4F2, the percentage involved in this catabolism is insignificant, between 1 to 3% (Takeuchi et al., 2009; Chan et al., 2012). Hence, it is concluded that the impact of CYP4F2 in the drug interaction between MK-7 and warfarin is so small that this interaction doesn't contribute significantly.

6.2.7.2. MK-7 as a Ligand of SXR

Vitamin K functions as a ligand for a nuclear receptor, SXR (steroid and xenobiotic receptor), and its murine ortholog, PXR (pregnane X receptor) (Tabb et al., 2003) is a protein that in humans is encoded by the NR1I2 (nuclear Receptor subfamily 1, group I, member 2) gene. It should be noted that MK-7, acting as a ligand for SXR, and thus, controlling transcription and translation of CYP3A4 enzyme is not changing the expression of CYP3A4 materially in a low dose MK-7 supplementation. Importantly, warfarin is a substrate for CYP3A4 and, therefore, any disturbance in the concentration of CYP3A4 by MK-7 could lead to the concentration variation of warfarin. If this variation is of any magnitude it will reflect in the changes of INR value. However, this is very unlikely in the light of the 1-3% catabolism by CYP4F2, indicating that the catabolizing system has great over capacitation.

Further, it is recognized that commercial warfarin is available as a racemic mixture of R and S enantiomers. (R)- and (S)-warfarin differs in the relative plasma concentration, in the antithrombotic potency and in the specific isoenzymes responsible for their metabolism. (S)-warfarin has a three-to-five times greater anticoagulant effect as compared to the (R)-enantiomer and accounts for 60 to 70% of warfarin's overall anticoagulant activity. (S)-warfarin is metabolized almost exclusively by CYP2C9 (Kamiski and Zhanf, 1997; Jones et al., 2010; Piatkov et al., 2010; Kamali and Wynne, 2010). In an earlier study, Choonara et al. (1986) demonstrated the greater potency of (S)-warfarin and suggested that the effect of racemic warfarin resides almost exclusively with the (S)-enantiomer. It is the less active (R)-enantiomer which is metabolized by CYP3A4. Further, CYP3A4 contribution in oxidizing (R)-enantiomer is only to the 10-hydroxywarfarin. The other oxidation products, 4,6,7 and 8 hydroxywarfarin are formed by CYPs that do not have any known interactions with MK-7. However, as precaution the CYP3A4 role in drug interactions of MK-7 and warfarin is reviewed.

Recent studies have demonstrated that the nuclear receptor SXR plays a central role in the transcriptional regulation of xenobiotic detoxifying enzymes such as CYP3A4 (Dussault et al., 2001; Staudinger et al., 2001; Synold et al., 2001; Wilson and Kliewer, 2002). SXR is known to be activated by a diverse array of pharmaceutical agents (Jones et al., 2000; Dussault et al., 2001; Staudinger et al., 2001). SXR functions as a xenobiotic sensor to co-ordinately regulate drug clearance, including participating in the clearance of warfarin working along with other CYPs. Thus, it is of concern if warfarin clearance is significantly changed by K2. In a study conducted by Tabb et al. (2003), it was shown that vitamin K2 binds directly to SXR and activates it to express CYP3A4 in a dose-dependent manner.

In several studies (Tabb et al., 2003; Ichikawa et al., 2006; Azuma et al., 2009) that have investigated vitamin K2 (menaquinones) as a ligand for SXR, these studies mention K2 without identifying the specific side chain of the K2-n series, occasionally mentioning MK-4. Such a mention is not included in the materials section. It appears that these studies may have used MK-4 in their reported results. MK-7 has a similar structure to that of MK-4 with the only difference of having a longer side chain compared to MK-4. Further, MK-7 and MK-4 have the same known biochemistry. Hence, it can be considered that MK-7 will act as a ligand to SXR in a similar way to what publications (Tabb et al., 2003; Ichikawa et al., 2006; Azuma et al., 2009) have referred to as K2.

There are many confounding factors that limit the possibility to pinpoint the exact role in up-regulation of CYP3A4 by menaquinones. Vitamin D3 also contributes to the expression of CYP3A4, though not through SXR. All such CYP3A4 regulatory mechanisms are not considered here. The only available data to assess the effect of vitamin K2 (apparently MK-4) are *in-vitro* results obtained by Tabb et al. (2003) as described below.

A supplementation at use levels of 4 mcg (6.16 nM) per serving of MK-7 is unlikely to influence CYP3A4 to a noteworthy level. Data from the bioavailability study by Schurgers et al. (2007) show that a dose of 0.22 micro-mole MK-7 creates highest steady-state serum concentrations of 10 nM (6 mcg/L). Assuming a linear relationship, a dose of 6.16 nM calculates to 0.28 nM of serum concentration. Tabb et al. (2003) reported that CYP3A4 is expressed two-fold at the K2 concentration of 1000 nm as compared to control. Based on these observations, 0.28 nM of physiological concentration i.e. 4 mcg of MK-7 supplementation will hardly have any effect on the expression of CYP3A4.

In summary, CYP3A4 is involved in the metabolism of approximately 30% of the prescription medication (FDA communication dated 19th July 2018). Any concerns related to the low dose of MK-7 affecting the prescription drug metabolism in the CYP3A4 pathway would be negligible. This is based on discussion vide-supra, where the effect of low dose Vitamin K2, dose of 4 mcg MK-7, is unlikely to have any significant effect on the expression of CYP3A4.

6.2.8. Institute of Medicine Report

The available scientific literature on phylloquinone (vitamin K1) and menaquinones (vitamin K2) has been 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. However, their contribution to the maintenance of vitamin K status has been difficult to assess. The report states that the evidence of vitamin K inadequacy in normal human subjects following dietary restriction of vitamin K

also suggests that menaquinone is not utilized in sufficient amounts to maintain maximal γ-carboxylation of the vitamin K-dependent proteins. Both phylloquinone and the menaquinones have been used to assess the blood status, with phylloquinone as the vitamer[5] usually studied because it is the primary source of dietary vitamin K in western countries.

The IOM report also suggest that the long-chain menaquinones, which are produced in substantial amounts by intestinal microorganisms, can also serve as active forms of vitamin K, but they are not widely distributed in commonly consumed foods. 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. The report considers menaquinone as an active form of vitamin K.

6.2.8. European Food Safety Authority Review

In the European Union, MK-7 is permitted to be used as a source of vitamin K for nutritional purposes in foodstuffs. The European Food Safety Authority (EFSA, 2008) examined the safety of MK-7. After considering the data on specifications, manufacturing, anticipated intake, bioavailability, metabolism and toxicology, the EFSA Panel concluded that the use of menaquinone-rich (primarily MK-7) edible oil in foods for the general population including food supplements and in foods for particular nutritional uses, except baby foods and infant formula, at the use levels of 10 mcg/serving was not of safety concern. The overall estimated mean daily intake of MK-7 ranged from 36 mcg (female adults) to 54 mcg (male teenagers). High intake levels ranged from 75 mcg/day (children) to 115 mcg/day (male teenagers).

6.3. GRAS Panel Review, Summary and Discussion

At the request of Synergia Life Sciences Pvt. Ltd., (Synergia), an independent panel of recognized experts (hereinafter referred to as the Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened to evaluate the Generally Recognized As Safe (GRAS) status of Vitamin K2 (Menaquinone-7; MK-7; MenaquinGold®) a standardized extract for use as a nutrient in foods for special dietary uses (PediaSure®) for children at levels up to 4 mcg *per* serving. A comprehensive search of the scientific literature for safety and toxicity information on MK-7 and other related vitamin K moieties, such as phylloquinone and menaquinones, was conducted through April 2019 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by Synergia and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred on September 27, 2019 and unanimously agreed to the decision described herein.

Synergia ensured that all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety, toxicology, and nutrition. The Expert Panel was selected and convened in accordance with the Food and Drug Administration (FDA)'s guidance for industry on "Best Practices for Convening a GRAS Panel"[6]. Efforts were placed on identifying conflicts of interest or relevant "appearance issues" that could potentially bias the outcome of the deliberations of the Expert Panel and no such conflicts of interest or "appearance issues" were identified. The Expert Panel members received a reasonable honorarium as

[5] A vitamer is any of a number of chemical substances, each of which shows vitamin activity, of a particular vitamin.

[6] Available at: https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm583856.htm

compensation for their time; the honoraria provided to the Expert Panel members were not contingent upon the outcome of their deliberations.

The subject GRAS ingredient, MK-7 is prepared by a fermentation and extraction process, using microbial strain *Bacillus licheniformis*. The production process and the specifications have been fully developed and MK-7 is manufactured according to the current good manufacturing practices. The extract is mixed with appropriate food grade material to a desired concentration that is intended to be marketed under the trade name MenaquinGold®. MK-7 is intended for use in foods for special dietary uses (PediaSure®) for children (non-infants; ages +1 years) at levels up to 4 mcg *per* serving. The proposed use of MK-7 is as follows: Age range: 1-13 years old; Serving size: 8 fl oz. (or 237 ml); MK-7 per serving 4 mcg. The intended use of MK-7 will result in estimated mean and 90th percentile intakes of 4 and 12 mcg/day, respectively.

MK-7 is a form of vitamin K2 biosynthesized by bacteria. It occurs naturally in some meat (<0.5 mcg/ 100 g), dairy (0.1-65 mcg/100 g), and fermented foods (up to 1000 mcg/100g in Natto). Among different foods, Natto, a traditional Japanese food, produced from fermented soybeans and consumed since ancient times, is one of the richest sources of MK-7. The MK-7 levels in Natto have been reported to be approximately 939 mcg/100 g. The history of Natto consumption without reports of any adverse effects in healthy individuals provides support for the safety of MK-7. The available information suggest that overall MK-7 intake contributes probably less than 5 to 10% of the dietary intake of vitamin K, which is primarily as vitamin K1 in Western countries. In Japan, Natto as a food has received certification under FOSHU for health claims for both bone health and gut health. The EFSA has also recognized the use of MK-7 as a novel food for the general population. In the published literature several studies of MK-7, including those of Natto have appeared. In these studies, no adverse effects of MK-7 or Natto consumption were noted in healthy subjects.

The available evidence from multiple studies suggests that all forms of vitamin K (phylloquinone and menaquinone) have a low order of toxicological potential, while the recommended dietary intake is several orders of magnitude lower than doses evaluated in animal and human studies. Different forms of vitamin K2 are absorbed, albeit slowly at normal dietary levels, and they contribute to maintaining function of vitamin K-reliant pathways. In human supplementation studies, higher and more stable plasma levels of vitamin K were reached with supplements containing menaquinone (K2) compared to those containing phylloquinone (K1).

In several animal and *in vitro* toxicity studies adverse effects of menaquinones, including MK-7, have been investigated. Findings from animal studies for acute, subchronic, reproductive and developmental toxicity, and genotoxicity and *in vitro* studies for mutagenicity and carcinogenicity showed no significant risks associated with exposure to menaquinones. Based on the findings from a subchronic toxicity study in rats, NOAEL of MK-7 was considered as 10 mg/kg bw/day, the highest dose tested. This NOAEL is over 800 fold higher as compared to the 90th percentile intake of MK-7 (12 mcg/day) from the proposed uses. In long-term (1 year) toxicity studies with MK-4, LOAEL in rats was established as 20 mg/kg bw/day, while NOAEL in dogs was 200 mg/kg bw/day. As regards developmental toxicity study, the EFSA Panel (2008) after reviewing the data stated, "... there were no compound-related effects observed on reproductive and developmental parameters measured." Although these studies were conducted with MK-4, these findings corroborate the safety of MK-7. The large safety margins indicate that

MK-7 at levels of up to 12 mcg/day in the healthy population is unlikely to pose any risk to health.

Menaquinones appears to be absorbed unchanged from the gastrointestinal tract, released in to circulation and distributed to the liver, comprising 90% (MK-6 to MK-13) of total vitamin K. The available studies suggest that phylloquinone and short-chain menaquinones (MK-4) have short-half-life (1-2 hours) as compared to long-chain menaquinones (MK-7, 68 hours). As compared to MK-4 or phylloquinone, the bioavailability of MK-7 is 6-10 times better (serum/plasma levels) indicating that MK-7 is likely to build up more stable serum levels. This also indicate that in patients on anti-coagulant therapy, the stable levels of MK-7 following regular exposure from the proposed uses may provide advantage.

The findings from multiple published human clinical studies with MK-7 did not reveal any significant adverse effects. In these studies, over 2000 individuals participated and the intake of MK-7 lasted from a few days to 3 years. Among the several clinical studies, in three long-term (two one year and one three year) clinical trials the effects of MK-7 at levels of 180 to 360 mcg/day were investigated. The results of these long-term studies did not reveal any adverse effects of MK-7. Thus, human exposure to MK-7, including from documented clinical studies, confirm that there are no reports of any significant adverse effects. Additionally, in several clinical studies, high doses of MK-4 have been well tolerated. In one of the clinical studies, MK-4 at a dose level of 90 mg/day for 24 weeks did not reveal any adverse effects. These studies with MK-4 corroborate the safety of MK-7 at the proposed use levels.

It is well known that vitamin K interferes with the anticoagulant therapy. Thus, there is a risk for interaction between MK-7 and anticoagulant drugs. Patients on anticoagulation therapy receive advice from their physician and pharmacist specifically warning about both the need to maintain a stable dietary intake of vitamin K (avoid wide fluctuations in vitamin K intake) and the risk of an interaction with vitamin K supplements. The proposed use of MK-7 is at very low levels (4 mcg/serving) and it is used for special dietary uses in a specific product (PediaSure®). MK-7 has a longer half-life than vitamin K1, which may account for better stability of INR values (a clotting measurement) for people on anti-coagulant therapy. The low use levels and specific product should ensure that people currently taking Coumadin anticoagulants are not negatively affected by the proposed uses of PediaSure®. Similarly, any concerns related to the low dose MK-7 affecting the prescription drug metabolism in the CYP3A4 pathway would be negligible as low dose MK-7 is unlikely to have any significant effect on the expression of CYP3A4.

The totality of the available evidence from *in vitro*, animal and human studies, including studies for structurally-related vitamin analogues, and the historical dietary exposure to MK-7 from foods like Natto and cheese, support the safety-in-use of MK-7, at the intended use levels (4 mcg/serving). The clinical evidence of MK-7 safety is supported by:

- As a member of the Vitamin K family, MK-7 has a long history of use for nutritional purposes.
- Humans have been regularly exposed to MK-7 via diet, particularly in Natto and cheese, without reports of adverse effects.
- There is no evidence that consumption of MK-7 either in foods or as a dietary supplement has any cumulative effect.

- Experimental studies, including subchronic toxicity, reproduction and developmental toxicity, and *in vitro* and *in vivo* genotoxicity of MK-7 or MK-4 corroborate the human clinical safety data.
- In multiple human clinical trials, no adverse effects of MK-7 were noted at use levels up to 180 mcg /person/day.
- In multiple human clinical studies, the safety of MK-4, a closely related member has been investigated at doses up to 45 mg/day.

The safety determination of MK-7 (MenaquinGold®) is primarily based on human clinical observations, including its intake from foods, and a variety of animal as well as *in vitro* studies that further corroborate the safety data from human studies. On the basis of scientific procedures[7] and exposure from natural dietary sources, the consumption of MK-7 as an added food ingredient is considered safe at use levels up to 4 mcg/serving resulting in the potential 90th percentile daily intake of 12 mcg/person/day. The intended uses are compatible with current regulations, *i.e.*, MK-7 is used in a specific food (PediaSure®) and is produced according to current good manufacturing practice (cGMP).

[7] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

6.4. GRAS Panel Conclusion

Based on a critical evaluation of the publicly available data summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively concluded that MK-7 (MenaquinGold®), meeting the specifications cited herein, and when used as a nutrient [21 CFR § 170.3(o) (20)] at maximum use levels of up to 4 mcg/serving (when not otherwise precluded by a Standard of Identity) in foods for special dietary uses (PediaSure®) for children, described in this assessment and resulting in the 90th percentile all-user estimated intake of 12 mcg MK-7/person/day is safe.

It is also the opinion of the Expert Panelists that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that MK-7, when used as described, is GRAS based on scientific procedures.

Signatures

Leon J. Schurgers, Ph.D. — 02 October 2019 Date

Robert L. Martin., Ph.D. — Sept. 28, 2019 Date

John A. Thomas, Ph.D., FATS, FACT — 10/1/19 Date

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S.
Advisor to Expert Panel — October 7, 2019 Date

7. PART VII- LIST OF SUPPORTING DATA AND INFORMATION

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8. Appendix I

Certificate of Analysis from Five Nonconsecutive Lots of MK-7

(Attached separately)

9. Appendix II

Estimated Daily Intake of Vitamin K

Proposed for Use in PediaSure® Products

Complete report from Exponent, Inc.

Attached separately- pages 1-23

APPENDIX I - Five CoAs

SYNERGIA LIFE SCIENCES PVT. LTD.

(A Viridis Group Company)(CIN : U24230MH2004PTC145390)
Regd. Off. :6/312, Jogani Industrial Complex, V. N. Purav Marg. Chunabhatti, Mumbai 400 022.
Phone: +91 22 2405 5607-09. Fax: +91 22 2405 5952. Email : info@viridisbiopharma.com
Govt. Recognized One Star Export House

CERTIFICATE OF ANALYSIS

Menaquin Gold
Natural Vitamin K2-7

Natural Vitamin K2-7 Oil 1500 ppm
(Menaquinone-7)

Batch No.: WM/MQG/1500OSF/1907 Shelf Life : 30 Months

Mfg. Date: April, 2019 Expiry Date: September, 2021

- **Product description:** **Vitamin K_2-7 Oil, 1500 µg K_2/g Base Oil : Sunflower Oil**

Parameters	Specifications	Results
Appearance	Pale Yellow to Yellow Oil	Conforms
Identification	The Retention Time for principal peak in the chromatogram for the sample corresponds with the standard	Conforms
Total Vitamin K_2–7 (as is)	1,500 µg/g(min)	1591 µg/g
Arsenic (As_2O_3)	0.5 µg/g (max)	BDL 0.5 µg/g
Lead (Pb)	0.5 µg/g (max)	BDL 0.5 µg/g
Total Heavy metals	5 µg/g (max)	BDL 5 µg/g
Total Plate Count (72 hrs)	10^3 cfu/g (max)	< 300 cfu/gm
Yeast and Molds	10^2 cfu/g (max)	Absent
Bile –tolerant gram-negative bacteria	absent in 10 gm	Absent
Escherichia coli	absent in 10 gm	Absent
Salmonella spp.	absent in 10 gm	Absent
Staphylococcus aureus	absent in 10 gm	Absent
Pseudomonas aeruginosa	absent in 10 gm	Absent

- **Packaging** : 1 kg or 5 kg plastic containers, with inner cap, Nitrogen overlay.
- **Storing and handling :** **Vitamin K_2-7 Oil** is well protected in its original sealed container. Store below 30°C. The product is light sensitive and exposure may deteriorate K_2 activity.

SYNERGIA LIFE SCIENCES PVT. LTD. WADA 421303

The material passes inhouse specification and can be released.

QC Analyst Swapnil Jogmarge | QC Microbiologist Smita Borde | Approved by Dr. Anselm de Souza

Date: 05-Apr-2019

Plant Address: Gut No/S. No.65, H. No. 2-Paiki, At Village: Gatesh Budruk, Talathi: Saja Kone, Taluka: Wada, Dist: Palghar – 421 303, Maharashtra, INDIA.

Rev. 6.0 dt. 01.06.2018

SYNERGIA LIFE SCIENCES PVT. LTD.
(A Viridis Group Company)(CIN : U24230MH2004PTC145390)
Regd. Off. : 6/312, Jogani Industrial Complex, V. N. Purav Marg. Chunabhatti, Mumbai 400 022.
Phone: +91 22 2405 5607-09. Fax: +91 22 2405 5952. Email : info@viridisbiopharma.com
Govt. Recognized One Star Export House

CERTIFICATE OF ANALYSIS

Menaquin Gold
Natural Vitamin K2-7

Natural Vitamin K2-7 Oil 1500 ppm
(Menaquinone-7)

Batch No.: WM/MQG/1500OSF/1901 Shelf Life : 30 Months

Mfg. Date: January, 2019 Expiry Date: June, 2021

- **Product description:** **Vitamin K_2-7 Oil, 1500 µg K_2/g Base Oil : Sunflower Oil**

Parameters	Specifications	Results
Appearance	Pale Yellow to Yellow Oil	Conforms
Identification	The Retention Time for principal peak in the chromatogram for the sample corresponds with the standard	Conforms
Total Vitamin K_2–7 (as is)	1,500 µg/g(min)	1588 µg/g
Arsenic (As_2O_3)	0.5 µg/g (max)	BDL 0.5 µg/g
Lead (Pb)	0.5 µg/g (max)	BDL 0.5 µg/g
Total Heavy metals	5 µg/g (max)	BDL 5 µg/g
Total Plate Count (72 hrs)	10^3 cfu/g (max)	< 300 cfu/gm
Yeast and Molds	10^2 cfu/g (max)	Absent
Bile –tolerant gram-negative bacteria	absent in 10 gm	Absent
Escherichia coli	absent in 10 gm	Absent
Salmonella spp.	absent in 10 gm	Absent
Staphylococcus aureus	absent in 10 gm	Absent
Pseudomonas aeruginosa	absent in 10 gm	Absent

- **Packaging** : 1 kg or 5 kg plastic containers, with inner cap, Nitrogen overlay.
- **Storing and handling :** **Vitamin K_2-7 Oil** is well protected in its original sealed container. Store below 30°C. The product is light sensitive and exposure may deteriorate K_2 activity.

SYNERGIA LIFE SCIENCES PVT. LTD. WADA 421303

The material passes inhouse specification and can be released.

QC Analyst
Swapnil Jogmarge

QC Microbiologist
Smita Borde

Approved by
Dr. Anselm de Souza

Date: 17-Jan-2019

Plant Address: Gut No/S. No.65, H. No. 2-Paiki, At Village: Gatesh Budruk, Talathi: Saja Kone, Taluka: Wada, Dist: Palghar – 421 303, Maharashtra, INDIA.

SYNERGIA LIFE SCIENCES PVT. LTD.
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Phone: +91 22 2405 5607-09. Fax: +91 22 2405 5952. Email : info@viridisbiopharma.com
Govt. Recognized One Star Export House

CERTIFICATE OF ANALYSIS

Menaquin Gold
Natural Vitamin K2-7

Natural Vitamin K2-7 Oil 1500 ppm
(Menaquinone-7)

Batch No.: WM/MQG/1500OSF/1905 Shelf Life : 30 Months

Mfg. Date: February, 2019 Expiry Date: July, 2021

- **Product description:** **Vitamin K_2-7 Oil, 1500 µg K_2/g Base Oil : Sunflower Oil**

Parameters	Specifications	Results
Appearance	Pale Yellow to Yellow Oil	Conforms
Identification	The Retention Time for principal peak in the chromatogram for the sample corresponds with the standard	Conforms
Total Vitamin K_2–7 (as is)	1,500 µg/g(min)	1609 µg/g
Arsenic (As_2O_3)	0.5 µg/g (max)	BDL 0.5 µg/g
Lead (Pb)	0.5 µg/g (max)	BDL 0.5 µg/g
Total Heavy metals	5 µg/g (max)	BDL 5 µg/g
Total Plate Count (72 hrs)	10^3 cfu/g (max)	< 300 cfu/gm
Yeast and Molds	10^2 cfu/g (max)	Absent
Bile –tolerant gram-negative bacteria	absent in 10 gm	Absent
Escherichia coli	absent in 10 gm	Absent
Salmonella spp.	absent in 10 gm	Absent
Staphylococcus aureus	absent in 10 gm	Absent
Pseudomonas aeruginosa	absent in 10 gm	Absent

- **Packaging** : 1 kg or 5 kg plastic containers, with inner cap, Nitrogen overlay.
- **Storing and handling :** **Vitamin K_2-7 Oil** is well protected in its original sealed container. Store below 30°C. The product is light sensitive and exposure may deteriorate K_2 activity.

SYNERGIA LIFE SCIENCES PVT. LTD. WADA 421303

The material passes inhouse specification and can be released.

QC Analyst	QC Microbiologist	Approved by	Date:
Swapnil Jogmarge	Kalyani Pashte	Dr. Anselm de Souza	28-Feb-2019

SYNERGIA LIFE SCIENCES PVT. LTD.
(A Viridis Group Company)(CIN : U24230MH2004PTC145390)
Regd. Off. :6/312, Jogani Industrial Complex, V. N. Purav Marg. Chunabhatti, Mumbai 400 022.
Phone: +91 22 2405 5607-09. Fax: +91 22 2405 5952. Email : info@viridisbiopharma.com
Govt. Recognized One Star Export House

CERTIFICATE OF ANALYSIS

Menaquin Gold
Natural Vitamin K2-7

Natural Vitamin K2-7 Oil 1500 ppm
(Menaquinone-7)

Batch No.: WM/MQG/1500OSF/1906 Shelf Life : 30 Months

Mfg. Date: March, 2019 Expiry Date: August, 2021

- **Product description:** **Vitamin K_2-7 Oil, 1500 µg K_2/g Base Oil : Sunflower Oil**

Parameters	Specifications	Results
Appearance	Pale Yellow to Yellow Oil	Conforms
Identification	The Retention Time for principal peak in the chromatogram for the sample corresponds with the standard	Conforms
Total Vitamin K_2–7 (as is)	1,500 µg/g(min)	1551 µg/g
Arsenic (As_2O_3)	0.5 µg/g (max)	BDL 0.5 µg/g
Lead (Pb)	0.5 µg/g (max)	BDL 0.5 µg/g
Total Heavy metals	5 µg/g (max)	BDL 5 µg/g
Total Plate Count (72 hrs)	10^3 cfu/g (max)	< 300 cfu/gm
Yeast and Molds	10^2 cfu/g (max)	Absent
Bile –tolerant gram-negative bacteria	absent in 10 gm	Absent
Escherichia coli	absent in 10 gm	Absent
Salmonella spp.	absent in 10 gm	Absent
Staphylococcus aureus	absent in 10 gm	Absent
Pseudomonas aeruginosa	absent in 10 gm	Absent

- **Packaging** : 1 kg or 5 kg plastic containers, with inner cap, Nitrogen overlay.
- **Storing and handling :** **Vitamin K_2-7 Oil** is well protected in its original sealed container. Store below 30°C. The product is light sensitive and exposure may deteriorate K_2 activity.

The material passes inhouse specification and can be released.

SYNERGIA LIFE SCIENCES PVT. LTD. WADA 421303

QC Analyst	QC Microbiologist	Approved by
Swapnil Jogmarge	Kalyani Pashte	Dr. Anselm de Souza

Date: 23-May-2019

Plant Address: Gut No/S. No.65, H. No. 2-Paiki, At Village: Gatesh Budruk, Talathi: Saja Kone, Taluka: Wada, Dist: Palghar – 421 303, Maharashtra, INDIA.

Rev. 6.0 dt. 01.06.2018

SYNERGIA LIFE SCIENCES PVT. LTD.
(A Viridis Group Company)(CIN : U24230MH2004PTC145390)
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Phone: +91 22 2405 5607-09. Fax: +91 22 2405 5952. Email : info@viridisbiopharma.com
Govt. Recognized One Star Export House

CERTIFICATE OF ANALYSIS

Menaquin Gold
Natural Vitamin K2-7

Natural Vitamin K2-7 Oil 1500 ppm
(Menaquinone-7)

Batch No.: WM/MQG/1500OSF/1909 Shelf Life : 30 Months

Mfg. Date: May, 2019 Expiry Date: October, 2021

- **Product description:** **Vitamin K_2-7 Oil, 1500 µg K_2/g Base Oil : Sunflower Oil**

Parameters	Specifications	Results
Appearance	Pale Yellow to Yellow Oil	Conforms
Identification	The Retention Time for principal peak in the chromatogram for the sample corresponds with the standard	Conforms
Total Vitamin K_2–7 (as is)	1,500 µg/g(min)	1613 µg/g
Arsenic (As_2O_3)	0.5 µg/g (max)	BDL 0.5 µg/g
Lead (Pb)	0.5 µg/g (max)	BDL 0.5 µg/g
Total Heavy metals	5 µg/g (max)	BDL 5 µg/g
Total Plate Count (72 hrs)	10^3 cfu/g (max)	< 300 cfu/gm
Yeast and Molds	10^2 cfu/g (max)	Absent
Bile –tolerant gram-negative bacteria	absent in 10 gm	Absent
Escherichia coli	absent in 10 gm	Absent
Salmonella spp.	absent in 10 gm	Absent
Staphylococcus aureus	absent in 10 gm	Absent
Pseudomonas aeruginosa	absent in 10 gm	Absent

- **Packaging** : 1 kg or 5 kg plastic containers, with inner cap, Nitrogen overlay.
- **Storing and handling :** **Vitamin K_2-7 Oil** is well protected in its original sealed container. Store below 30°C. The product is light sensitive and exposure may deteriorate K_2 activity.

The material passes inhouse specification and can be released.

QC Analyst	QC Microbiologist	Approved by	Date:
Swapnil Jogmarge	Smita Borde	Dr. Anselm de Souza	

Plant Address: Gut No/S. No.65, H. No. 2-Paiki, At Village: Gatesh Budruk, Talathi: Saja Kone, Taluka: Wada, Dist: Palghar – 421 303, Maharashtra, INDIA.

APPENDIX II

Center for Chemical Regulation and Food Safety

Exponent®

Estimated Daily Intake of Vitamin K Proposed for Use in PediaSure® Products

Exponent

Estimated Daily Intake of Vitamin K Proposed for Use in PediaSure® Products

Prepared for

G. Craig Llewellyn
Principal Toxicologist
Toxicology Regulatory Services
2365 Hunters Way
Charlottesville, VA 22911

Prepared by

Exponent, Inc.
1150 Connecticut Ave, NW
Suite 1100
Washington, DC 20036

February 8, 2019

Contents

List of Tables

List of Acronyms

CEDI	Cumulative EDI
DGA	Dietary Guidelines for Americans
DHHS	U.S. Department of Health and Human Services
DSLD	Dietary Supplement Label Database
EDI	Estimated Daily Intake
FDA	U.S. Food and Drug Administration
FNDDS	Food and Nutrient Database for Dietary Studies
GRAS	Generally Recognized As Safe
µg	Microgram
mL	Milliliter
MK-4	Menaquinone-4
MK-7	Menaquinone-7
mo	Month
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
NIH	National Institutes of Health
SR	Standard Reference
WWEIA	What We Eat In America
U.S.	United States
USDA	U.S. Department of Agriculture
Y	Year

Introduction

At the request of Toxicological Regulatory Services (TRS), and on behalf of their client Abbott Nutrition (Abbott), Exponent, Inc. (Exponent) conducted a dietary intake assessment to estimate the cumulative daily intake of vitamin K (i.e., vitamin K_1, K_2, and related isoforms) from background sources and from Abbott's proposed uses of vitamin K (i.e.,K1 and K_2, in the form of menaquinone-7 (MK-7)) in PediaSure® products. The intake estimates of vitamin K was based on food consumption data from foods reported consumed in the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Survey (NHANES) 2011-2014 and provided for children 1-13 years of age. The data and methods used to conduct the intake assessment and results are summarized in this report.

Proposed Use and Levels

Vitamin K is proposed for use in PediaSure® products marketed for children. TRS provided Exponent with the proposed use levels of both vitamin K_1 and K_2, in the form of menaquinone-7 (MK-7), which are based on the following data and assumptions:

- Age range: Specified for 2-13 years old (medical supervision for children under 2)
- Serving size: 8 fl oz. or 237 mL
- Vitamin K_1 per serving: 16 µg
- Vitamin K_2 (MK-7) per serving: 4 µg
- Caloric density of PediaSure®: 1 kcal/mL or 237 kcal/serving
- Servings per day (3 scenarios):
  - Maximum: Each child receives their daily caloric requirements[1] from consumption of this product alone
  - Typical: 1 serving per day
  - High: 6 servings per day (applicable only to children 4-8 y and 9-13 y)

A summary of the proposed use levels by age group is provided in Table 1.

[1] Maximum caloric requirements of the specific age groups as provided by the Dietary Guidelines for Americans (DGA) 2015-2020 (USDA, 2015)

Table 1. Propose use levels of vitamin K in PediaSure®

Scenario	Age/sex group	Maximum caloric requirements[a] (kcal/day)	Servings/day[b]	Vitamin K_1[c] (µg/day)	Vitamin K_2 (MK-7)[d] (µg /day)	Total vitamin K (µg/day)
Maximum	12-23 months	1100[e]	5	80	20	100
	2-3 y	1400	6	96	24	120
	4-8 y					
	Male	2000	9	144	36	180
	Female	1800	8	128	32	160
	9-13 y					
	Male	2600	11	176	44	220
	Female	2200	10	160	40	200
Typical	All age/sex groups[f]	NA	1	16	4	20
High	4-8 y and 9-13 y only	NA	6	96	24	120

[a] Based on recommendations in the 2015-2020 DGA (USDA, 2015) unless specified otherwise; [b]Assuming 237 kcal/serving of PediaSure®; [c]16 µg/serving; [d]4 µg/serving; [e]Assuming maximum calorie requirement of 1100 kcal/day; [f]As specified in the maximum scenario.

Estimated Daily Intake (EDI)

The estimated daily intake (EDI) of vitamin K among children from background sources (diet and supplements) was determined using two main sources of data: (1) food intake and supplement use data from the National Health and Nutrition Examination Survey (NHANES) (2011-2012 and 2013-2014) in combination with food ingredient weight data (or recipes) from the United States Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies (FNDDS) and (2) nutrient composition data from the USDA National Nutrient Database for Standard Reference (SR) and published literature. The following sections describe the data and method used in this analysis in more detail.

NHANES Data

Intake estimates of vitamin K were based on food consumption records collected in the WWEIA component of NHANES conducted in 2011-2012 and 2013-2014 (NHANES 2011-2014). The NHANES is a continuous survey that uses a complex multistage probability sample designed to be representative of the civilian U.S. population (NCHS 2014, 2016). NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the United States. Statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection and non-response.

NHANES 24-hour Dietary Recall

As part of the examination, trained dietary interviewers collected detailed information on all foods and beverages consumed by respondents in the previous 24 hour time period (midnight to midnight). A second dietary recall was administered by telephone three to ten days after the first dietary interview, but not on the same day of the week as the first interview. The dietary component of the survey is conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). DHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's

dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. A total of 15,179 individuals in the survey period 2011-2014 provided 2 complete days of dietary recalls.

Food and Nutrient Database for Dietary Studies (FNDDS)

For each food reported in NHANES, the USDA Food and Nutrient Database for Dietary Studies (FNDDS) databases provide information on the amount of energy and approximately 60 nutrients or food constituents per 100 g of each food including vitamin K_1 as phylloquinone. Additionally, the FNDDS translates food as reported consumed into its corresponding ingredients (and gram amounts) or recipes. Exponent applied FNDDS version 2013-2014 nutrient composition data and food recipes (corresponding to NHANES 2013-2014) (USDA 2016) to process dietary recall data reported in NHANES 2011-2014 and FNDDS version 2011-2012 recipes (corresponding to NHANES 2011-2012) (USDA 2014) for foods that were only reported consumed in NHANES 2011-2012. Nutrient values for FNDDS are based on the National Nutrient Database for Standard Reference (SR).

National Nutrient Database for Standard Reference (SR)

The SR database is the major source of food composition data in the U.S. and provides the foundation for most food composition databases in the public and private sectors including the FNDDS. The SR database contains data on the level of vitamin K_1 and vitamin K_2 (limited to MK-4 only) in foods included in FNDDS. Exponent applied the mean nutrient concentrations of vitamin K_1 and vitamin K_2 as MK-4 in foods based on SR28 (USDA, 2016). SR28 is the source of nutrient composition for FNDDS 2013-2014 and contains data on 8,789 food items and up to 150 food components/nutrients.

NHANES 24-hour Dietary Supplement Use

Starting in the NHANES 2007-2008 cycle, NHANES collected supplement use data along with food consumption data as part of the 24-hour dietary recall data collection. The data collection for the 24-hour dietary supplement use is administered by the trained dietary interviewers. During the 24-hour recall, NHANES participants who reported taking supplements in the past 30

days in the household questionnaire were asked if they took these supplements in the previous 24 hours, and if so how much they took. All participants in the 24 hour recall were also asked if they took any other supplements, not reported during the 30-day supplement use household interview, and, if so, they were asked to report how much they took. The use of non-prescription antacids containing calcium and/or magnesium is included in this database. NHANES has preprocessed the supplement 24 hour recall data and derived nutrient intakes from supplements for NHANES 2011-2014.

Vitamin K from dietary supplements is reported as phylloquinone (i.e., vitamin K_1) with limited data on vitamin K_2 in supplements. To identify vitamin K_2 from supplements, Exponent reviewed the supplements reported taken that contained either "K_2" in the name and the product's label using the National Institutes of Health (NIH) Dietary Supplement Label Database (DSLD)[2] to determine the concentration and form of vitamin K_2 (as either MK-4 or MK-7).

Published literature

Vitamin K_2 levels in foods in the U.S. diet

As discussed above, the vitamin K levels in foods included in the SR28 are limited to vitamin K_1 and vitamin K_2 in the form of MK-4 only. Data on the level of other isoforms of vitamin K_2 including MK-7 in foods consumed as part of the U.S. diet were based on analytical levels provided in the published scientific literature. Exponent conducted a literature review in December 2018 to identify relevant scientific studies that provided data on the level of vitamin K_2 in foods with a preference for those conducted in the U.S. As a result of the literature search and a review of the foods reported consumed in the U.S. diet, six studies were included in the current assessment (Vermeer et al., 2018; Fu et al., 2017; Fu et al., 2016; Elder et al., 2006; Schurgers and Vermeer, 2001; Sakamoto et al., 1999). A summary of the vitamin K_2 levels included in the current assessment are provided in Appendix A.

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[2] National Institutes of Health (NIH). Dietary Supplement Label Database (DSLD). Accessed 12 December 2018. Available via: https://www.dsld.nlm.nih.gov/dsld/index.jsp.

Analysis

Two-day Average Daily Intake

Using the NHANES 2011-2014 consumption data, Exponent estimated the 2-day average daily intake on a "per capita" and "per user" basis. *Per capita* estimates refer to the consumption based on the entire population of interest whereas *per user* estimates refer to those who reported consuming any of the foods of interest on either of the survey days. In the case of vitamin K, everyone in the population of interest consumed a food containing vitamin K prior to the introduction of vitamin K from the proposed use in PediaSure®. Thus, the *per capita* and *per user* estimates are the same and only the *per user* estimates are presented in this report.

For each subject with a complete 2-day dietary recall, a 2-day average intake estimate was derived by summing the intake(s) of interest on day 1 and day 2 of the survey and dividing that sum by 2. If a survey participant consumed a vitamin K containing-food on only one of the survey days, their intake of interest from that day was divided by two, to obtain their 2-day average intake. The mean and 90th percentile of 2-day average vitamin K intake (from background, proposed new use, and cumulative total from background and proposed new use) were calculated for children 1-13 years (y) of age according to the following subpopulations: 12-23 months (mo), 2-3 y, males and females 4-8 y, and males and females 9-13 y.

Estimates of intake per person were generated using Exponent's Foods Analysis and Residue Evaluation Program (FARE® version 12.29) software. The analysis was limited to individuals who provided two complete and reliable dietary recalls as determined by NCHS. Exponent uses the statistically weighted values from the survey in its analyses. The statistical weights compensate for variable probabilities of selection, adjust for non-response, and provide intake estimates that are representative of the U.S. population.

Background Sources

Estimates of vitamin K including vitamin K_1, vitamin K_2, and vitamin K_2 as MK-7 from background sources included reported intakes from all dietary sources and supplements.

Estimates of vitamin K_1 from background food sources were derived from food consumption data reported in the NHANES 2011-2014 in combination with the USDA FNDDS database.

Estimates of vitamin K_2 (and MK-7) from background food sources were derived from food consumption data reported in the NHANES 2011-2014 in combination with analytical data from published literature (see Appendix A). Exponent mapped the mean vitamin K_2 levels reported in the published literature to foods reported consumed in the NHANES 2011-2014 through the FNDDS based on the food description. In addition, Exponent relied upon the SR28 data on MK-4 levels in foods to supplement the published literature where applicable. It is important to note that the analytical data on the levels of vitamin K_2 in background food sources included in the current analysis is limited to what is published and for that reason, the estimated background intake of vitamin K_2 and MK-7 from food sources may not reflect all vitamin K_2 that could potentially be present in food. While several of the publications provided total vitamin K_2 in the foods included in the analysis (Fu et al., 2017; Fu et al., 2016), some data sources, for example, only focused on MK-4 or MK-7 (Elder et al., 2006).

As described above, the dietary recall portion of the NHANES survey consists of two non-consecutive 24-hr recalls. For each subject with a complete 2-day dietary recall, intake of vitamin K was derived by summing an individual's intake of vitamin K on day 1 and day 2 of the survey and dividing that sum by 2. Intake of vitamin K from dietary supplement use by each respondent was added to the intake of vitamin K from food sources to estimate the total background intake of vitamin K (i.e., vitamin K_1, vitamin K_2, vitamin K_2 as MK-7) per person from both dietary and supplemental sources.

Proposed Use

Intake estimates of vitamin K (including vitamin K_1 and vitamin K_2 as MK-7) from proposed uses in PediaSure® at maximum, typical, and high use levels was calculated for each survey participant (see Table 1 for use levels). It is important to note that the maximum proposed use levels (i.e., maximum servings/day) includes the conservative assumption that every child 1-13 years of age consumes PediaSure® to provide all calories in the diet to meet their daily caloric

requirements. Therefore, additional scenarios were calculated to estimate the EDI assuming a more typical and high use (i.e., servings/day) of PediaSure®.

Cumulative EDI (CEDI)

To estimate the CEDI for vitamin K (including vitamin K_1, and vitamin K_2 as MK-7) from all potential sources, each individual's current background vitamin K intake (food and supplement) was added to his/her potential vitamin K intake from the proposed use of vitamin K in PediaSure®.

Results

The background intake of vitamin K is summarized in Table 2. The proposed and cumulative intake of vitamin K under the maximum, typical, and high use scenarios are presented in Tables 3, 4, and 5, respectively.

Among children 1 -13 years of age, the *per user* mean intake of vitamin K from all sources, including background sources and the maximum proposed use assuming that the child's entire daily caloric requirement is met by consumption of PediaSure®, ranged from 317 to 449 µg/day among children 2-3 y and males 9-13 y, respectively. The *per user* 90th percentile intakes of vitamin K from all sources, including background sources and the maximum proposed uses, ranged from 486 to 682 µg/day among children 2-3 y and males 9-13 y, respectively. Estimated intake of MK-7 from background sources was <1 µg/day among all subpopulations at both the mean and 90th percentile while the proposed use in PediaSure® increased MK-7 intakes to >4 µg/day among all proposed use scenarios.

Table 2. Estimated daily intake of vitamin K from background (total diet + supplements) by children 1-13 y in the U.S. (µg/day)

			Estimated Daily Intakes (EDIs) of Vitamin K (µg/day) Background Sources[b]	
Age/Sex Group	**Vitamin Form**	**N[a]**	**Mean**	**90th Percentile**
12-23 mo	Vitamin K	349	283	520
	Vitamin K_1		40.9	74.6
	Vitamin K_2[c]		242	492
	MK-7		0.1	0.2
2-3 y	Vitamin K	692	197	366
	Vitamin K_1		48.2	88.0
	Vitamin K_2[c]		149	311
	MK-7		0.1	0.3
4-8 y, male	Vitamin K	818	212	387
	Vitamin K_1		61.8	99.2
	Vitamin K_2[c]		150	299
	MK-7		0.2	0.3
4-8 y, female	Vitamin K	742	192	347
	Vitamin K_1		65.1	115
	Vitamin K_2[c]		127	245
	MK-7		0.1	0.4
9-13 y, male	Vitamin K	750	229	462
	Vitamin K_1		81.0	163
	Vitamin K_2[c]		148	300
	MK-7		0.2	0.4
9-13 y, female	Vitamin K	756	187	346
	Vitamin K_1		66.5	122
	Vitamin K_2[c]		121	250
	MK-7		0.2	0.4

Estimates based on 2-day average intakes reported in NHANES 2011-2014.

[a] N = Unweighted number of survey respondents identified as consumers of vitamin K; 100% user; [b]EDIs include background vitamin K from total diet and the reported use of vitamin K-containing dietary supplements; [c]Background intakes of vitamin K_2 and MK-7 are limited to the data available in the published literature and may not reflect all vitamin K_2 that could potentially be present in food.

Table 3. Cumulative estimated daily intake of vitamin K from background (total diet + supplements) and proposed uses in PediaSure® by children 1-13 y in the U.S. (µg/day): Maximum Use Scenario

			Estimated Daily Intakes (EDIs) of Vitamin K (µg/day)		
			Proposed Use[b]	Cumulative[c] (Background + Proposed)	
Age/Sex Group	**Vitamin Form**	**N[a]**	**Maximum**	**Mean**	**90th Percentile**
12-23 mo	Vitamin K	349	100	383	620
	Vitamin K_1		80	121	155
	Vitamin K_2[d]		20	262	512
	MK-7		20	20.1	20.2
2-3 y	Vitamin K	692	120	317	486
	Vitamin K_1		96	144	184
	Vitamin K_2[d]		24	173	335
	MK-7		24	24.1	24.3
4-8 y, male	Vitamin K	818	180	392	567
	Vitamin K_1		144	206	243
	Vitamin K_2[d]		36	186	335
	MK-7		36	36.2	36.3
4-8 y, female	Vitamin K	742	160	352	507
	Vitamin K_1		128	193	243
	Vitamin K_2[d]		32	159	277
	MK-7		32	32.1	32.4
9-13 y, male	Vitamin K	750	220	449	682
	Vitamin K_1		176	257	339
	Vitamin K_2[d]		44	192	344
	MK-7		44	44.2	44.4
9-13 y, female	Vitamin K	756	200	387	546
	Vitamin K_1		160	226	282
	Vitamin K_2[d]		40	161	290
	MK-7		40	40.2	40.4

Estimates based on 2-day average intakes reported in NHANES 2011-2014.

[a] N = Unweighted number of survey respondents identified as consumers of vitamin K; 100% user; [b]See Table 1; [c]EDIs include background vitamin K from total diet, the reported use of vitamin K-containing dietary supplements (see Table 2), and the maximum proposed use of vitamin K in PediaSure®; [d]Background intakes of vitamin K_2 and MK-7 are limited to the data available in the published literature and may not reflect all vitamin K_2 that could potentially be present in food.

Table 4. Cumulative estimated daily intake of vitamin K from background (total diet + supplements) and proposed uses in PediaSure® by children 1-13 y in the U.S. (µg/day): Typical Use Scenario

			Estimated Daily Intakes (EDIs) of Vitamin K (µg/day)		
			Proposed Use[b]	Cumulative[c] (Background + Proposed)	
Age/Sex Group	**Vitamin Form**	**N[a]**	**Typical**	**Mean**	**90th Percentile**
12-23 mo	Vitamin K	349	20	303	540
	Vitamin K_1		16	57	91
	Vitamin K_2[d]		4.0	246	496
	MK-7		4.0	4.1	4.2
2-3 y	Vitamin K	692	20	217	386
	Vitamin K_1		16	64	104
	Vitamin K_2[d]		4.0	153	315
	MK-7		4.0	4.1	4.3
4-8 y, male	Vitamin K	818	20	232	407
	Vitamin K_1		16	78	115
	Vitamin K_2[d]		4.0	154	303
	MK-7		4.0	4.2	4.3
4-8 y, female	Vitamin K	742	20	212	367
	Vitamin K_1		16	81	131
	Vitamin K_2[d]		4.0	131	249
	MK-7		4.0	4.1	4.4
9-13 y, male	Vitamin K	750	20	249	482
	Vitamin K_1		16	97	179
	Vitamin K_2[d]		4.0	152	304
	MK-7		4.0	4.2	4.4
9-13 y, female	Vitamin K	756	20	207	366
	Vitamin K_1		16	82	138
	Vitamin K_2[d]		4.0	125	254
	MK-7		4.0	4.2	4.4

Estimates based on 2-day average intakes reported in NHANES 2011-2014.

[a] N = Unweighted number of survey respondents identified as consumers of vitamin K; 100% user; [b]See Table 1; [c]EDIs include background vitamin K from total diet, the reported use of vitamin K-containing dietary supplements (see Table 2), and the maximum proposed use of vitamin K in PediaSure®; [d]Background intakes of vitamin K_2 and MK-7 are limited to the data available in the published literature and should not be considered a comprehensive assessment of vitamin K_2 intake.

Table 5. Cumulative estimated daily intake of vitamin K from background (total diet + supplements) and proposed uses in PediaSure® by children 4-13 y in the U.S. (µg/day): High Use Scenario

			Estimated Daily Intakes (EDIs) of Vitamin K (µg/day)		
			Proposed Use[b]	Cumulative[c] (Background + Proposed)	
Age/Sex Group	**Vitamin Form**	**N[a]**	**High**	**Mean**	**90th Percentile**
4-8 y, male	Vitamin K	818	120	332	507
	Vitamin K_1		96	158	195
	Vitamin K_2[d]		24.0	174	323
	MK-7		24.0	24.2	24.3
4-8 y, female	Vitamin K	742	120	312	467
	Vitamin K_1		96	161	211
	Vitamin K_2[d]		24.0	151	269
	MK-7		24.0	24.1	24.4
9-13 y, male	Vitamin K	750	120	349	582
	Vitamin K_1		96	177	259
	Vitamin K_2[d]		24.0	172	324
	MK-7		24.0	24.2	24.4
9-13 y, female	Vitamin K	756	120	307	466
	Vitamin K_1		96	162	218
	Vitamin K_2[d]		24.0	145	274
	MK-7		24.0	24.2	24.4

Estimates based on 2-day average intakes reported in NHANES 2011-2014.

[a] N = Unweighted number of survey respondents identified as consumers of vitamin K; 100% user; [b]Applicable only to children 4-13 y; see Table 1; [c]EDIs include background vitamin K from total diet, the reported use of vitamin K-containing dietary supplements (see Table 2), and the maximum proposed use of vitamin K in PediaSure®; [d]Background intakes of vitamin K_2 and MK-7 are limited to the data available in the published literature and should not be considered a comprehensive assessment of vitamin K_2 intake.

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Appendix A. Vitamin K_2 Levels from Published Literature

Food Description from Publication	K_2* (mean)	MK-7 (mean)	Unit	Source
cottage cheese, 4% fat	51.9	0.6	µg/100 g	Fu et al. 2017
cottage cheese, reduced-fat	8.4	0	µg/100 g	Fu et al. 2017
cheddar cheese, full-fat	278.6	0.8	µg/100 g	Fu et al. 2017
cheddar cheese, reduced-fat	46.9	0.7	µg/100 g	Fu et al. 2017
blue cheese	437.1	4	µg/100 g	Fu et al. 2017
fresh cheese (goat, feta, ricotta, cotija, cottage, mozz)	125.3	0.8	µg/100 g	Fu et al. 2017
soft cheese (brie, camembert, crème freche, limburger, mascarpone)	502	4.7	µg/100 g	Fu et al. 2017
semi-soft cheese (moterray jack, havarti, swiss, fontina, gouda, and cream cheese)	285.8	0.6	µg/100 g	Fu et al. 2017
processed cheese (american)	94.1	1.2	µg/100 g	Fu et al. 2017
hard cheese (cheddar, parmesean)	279	0.8	µg/100 g	Fu et al. 2017
mozzerella part skim	100.2	0.5	µg/100 g	Fu et al. 2017
edam (Dutch)	646.6	0	ng/g	Vermeer et al. 2018
gruyere (Swiss)	65.3	0	ng/g	Vermeer et al. 2018
kielbasa, grilled	534.4	0	µg/100 g	Fu et al. 2016
pork sausage, regular fat, pan-fried	383.3	0	µg/100 g	Fu et al. 2016
pork sausage, reduced fat, pan-fried	325.1	0	µg/100 g	Fu et al. 2016
Canadian bacon, cooked	34.7	0	µg/100 g	Fu et al. 2016
broiled ground beef (low fat)	1.7	0	µg/100 g	Elder et al. 2006
broiled ground beef (medium fat)	7.2	0	µg/100 g	Elder et al. 2006
broiled ground beef (high fat)	5.1	0	µg/100 g	Elder et al. 2006
pan-fried beef liver	0.4	0	µg/100 g	Elder et al. 2006
braised beef liver	1.9	0	µg/100 g	Elder et al. 2006
beef, hot hogs, regular fat (uncooked and cooked)	5.7	0	µg/100 g	Elder et al. 2006
ham (with water or natural juices; roasted and pan-broiled)	5.1	0	µg/100 g	Elder et al. 2006
bacon (raw, pan-fried, microwaved, cooked, and baked)	5.6	0	µg/100 g	Elder et al. 2006
meat franks, regular fat (uncooked and cooked)	9.8	0	µg/100 g	Elder et al. 2006
tenderloin	72.2	0	µg/100 g	Fu et al. 2016
pork chops, boneless	68.8	0	µg/100 g	Fu et al. 2016
pork chop with bone	74.8	0	µg/100 g	Fu et al. 2016
St. Louis-style spareribs with bone	164.8	0	µg/100 g	Fu et al. 2016
shoulder blade Boston with bone	127.6	0	µg/100 g	Fu et al. 2016
salmon, raw, Alaska wild (Coho, Sockeye, Chum, and King)	0.3	0	µg/100 g	Elder et al. 2006
regular yogurt, full-fat	23.9	0	µg/100 g	Fu et al. 2017
regular yogurt, fat-free	0	0	µg/100 g	Fu et al. 2017
greek yogurt, full-fat	26.1	0	µg/100 g	Fu et al. 2017

Food Description from Publication	K_2* (mean)	MK-7 (mean)	Unit	Source
greek yogurt, fat-free	0	0	µg/100 g	Fu et al. 2017
cream, heavy	583.4	0	µg/100 g	Fu et al. 2017
cream, light	146.8	0	µg/100 g	Fu et al. 2017
cream, half-and-half	82.7	0	µg/100 g	Fu et al. 2017
milk, 4% (full fat)	56.25	0	µg/100 g	Fu et al. 2017
milk, 2%	22	0	µg/100 g	Fu et al. 2017
milk, 1%	20.5	0	µg/100 g	Fu et al. 2017
milk, fat free	6.25	0	µg/100 g	Fu et al. 2017
buttermilk	2.5	0.1	µg/100 g	Schurgers and Vermeer 2001
eggs, white, fresh, raw	0.4	0	µg/100 g	Elder et al. 2006
eggs, yolk, fresh, raw	15.5	0	µg/100 g	Elder et al. 2006
whole eggs, fried	9	0	µg/100 g	Elder et al. 2006
whole eggs, hard-cooked	7	0	µg/100 g	Elder et al. 2006
hamburger (2-4 oz)	2.3	0	µg/100 g	Elder et al. 2006
hamburger with cheese (2-4 oz)	2.9	0	µg/100 g	Elder et al. 2006
hamburger with sauce (2-4 oz)	1.4	0	µg/100 g	Elder et al. 2006
hamburger with cheese and sauce (> 4 oz)	2.3	0	µg/100 g	Elder et al. 2006
chicken sandwich	2.7	0	µg/100 g	Elder et al. 2006
fish sandwich	0.3	0	µg/100 g	Elder et al. 2006
chicken nuggets	10.6	0	µg/100 g	Elder et al. 2006
chicken tenders	6.3	0	µg/100 g	Elder et al. 2006
burrito with bean	0.6	0	µg/100 g	Elder et al. 2006
burrito with beef	0.9	0	µg/100 g	Elder et al. 2006
taco with beef	1	0	µg/100 g	Elder et al. 2006
taco with chicken	4.5	0	µg/100 g	Elder et al. 2006
pizza, cheese (regular, thin, and thick crust)	1.8	0	µg/100 g	Elder et al. 2006
pizza, pepperoni (regular, thing, and thick crust)	2.1	0	µg/100 g	Elder et al. 2006
pizza, meat and vegetable (regular, thin, and thick crust)	1.9	0	µg/100 g	Elder et al. 2006
shakes, chocolate and vanilla	3.4	0	µg/100 g	Elder et al. 2006
miso (soybean paste)	20	20	ng/g	Sakamoto et al. 1999
natto	10985	9965	ng/g	Vermeer et al. 2018
sauerkraut	55	2.3	ng/g	Vermeer et al. 2018

*Vitamin K_2 levels presented in this table are a sum of the mean level of all K_2 isoforms provided in the listed source.