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February 25, 2008

02-29-08 A11:08 OUT

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Dr. Robert L. Martin

Dear Dr. Martin:

On behalf of NattoPharma ASA of Lysaker, Norway, we are submitting for FDA review a GRAS notification for Menaquinone-7, which is to serve as a dietary source of vitamin K_2. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
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Enclosure: GRAS Notification – Menaquinone-7 (in triplicate)

000002

I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

Menaquinone-7, meeting the specifications described below for MenaQ7, has been determined to be Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination was made by experts who are qualified by scientific training and experience to make such safety determinations. The GRAS evaluation is based on scientific procedures as described in the following sections, and the evaluation accurately reflects the conditions of the ingredient's intended use in foods.

Signed:

February 25, 2008

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074

Date

B. Name and Address of Notifier

NattoPharma ASA
Lysaker Torg 5
P O Box 397 Lysaker
NO-1326
Lysaker, NORWAY

As the notifier, NattoPharma ASA ("NattoPharma") accepts responsibility for the GRAS determination that has been made for menaquinone-7 as a dietary source of vitamin K_2 as described in the subject notification; consequently, menaquinone-7 meeting the conditions described herein is exempt from pre-market approval requirements for food ingredients.

C. Common Name and Identity of the Notified Substance

Menaquinone-7 or MK-7; also see Sections II.B.1 and II.B.2.

[1] See 62 FR 18938 (17 April 1997).

D. Conditions of Intended Use in Food

Menaquinone-7, in the form of selected MenaQ7 products, is intended to be added to designated foods as a nutrient supplement as defined in 21 CFR 170.3(o)(20). The per serving levels added to dairy products and butter and margarine substitutes range from 6.7 – 10 mg of MenaQ7 to yield a total daily dietary consumption of 50 µg of menaquinone-7 as a source of vitamin K_2.

E. Basis for the GRAS Determination

Pursuant to 21 CFR 170.30, menaquinone-7 as MenaQ7 has been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR 97702-3074.

II. Chemistry and Manufacture of MenaQ7

A. Description of Natto

The US Soybean Export Council (USSEC) defines natto as a fermented soybean product.[2] There are several commonly used foods in various Asian countries that originate from fermented soybeans including soy sauce, miso, sulfu, natto, tempe, taosi, meitauza, and meju. The fermented soybean foods differ in the form of soybean used as substrate---sometimes in combinations with other grains---and the microorganism used in fermentation (Francis, 1999).

Natto is fermented from whole soybeans using *Bacillus subtilis* or *Bacillus subtilis natto.* Its appearance is that of cooked beans bound together with a sticky substance formed in fermentation. It has a musty or ammonia odor and is commonly consumed as a main dish over rice (Francis, 1999). A more detailed description of natto as a product of soybean agriculture appears on the USDA website:

> Soybean production in the Far East in the 1990's, as in ancient times, is primarily for food. Chinese writings more than 2000 years ago refer to soybean as one of the five sacred food grains. In Japan, natto is an important soy food eaten as a companion to rice and first popularized about 1000 AD. Noted for its strong fermented aroma and dark brown color, natto has quite a consumer following in modern Japan and many food companies compete in this lucrative market. Because of the high population in Japan, the country is not self sufficient in soybean production and pays handsomely for specialty beans from the USA. For natto type specialty beans, Japanese buyers pay U.S. farmers up to $6 per bushel above market price. Although U.S. farmers are vitally interested in this lucrative market, growing the right variety for Japan's needs has proven difficult. Most U.S. commodity-type soybeans are unsuited to the natto market.

USDA sponsored research is developing new varieties of soybean that US farmers are growing and exporting for the Asian market.[3] Exports from Delaware[4] as well as North Dakota and Minnesota[5] are reported to be increasing.

Fermented soybean products that are commonplace in the US diet include soy sauce and Worcestershire sauce. Natto is freely available as a food at Asian specialty stores in the US (Francis, 1999).

B. Vitamin K Chemistry, Common or Usual Name, and Background Information

1. **Molecular Structures of Vitamin K.** The collective term, vitamin K, encompasses several lipid-soluble 2-methyl-1,4-naphthoquinone derivatives. Naturally occurring forms of vitamin K include vitamin K_1 and vitamin K_2 (*i.e.*, phylloquinone and the menaquinone series, respectively), which are characterized by lipophilic side chains at the 3-position of the 2-methyl-1,4-naphthoquinone ring structure. The side chain of phylloquinone is unsaturated and fixed length while the side chain of menaquinones are unsaturated and of variable length.

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[2] See Glossary of Terms on USSEC website at http://www.ussoyexports.org/buying_u.s._soy/IP_book/Glossary.pdf.
[3] See http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=188029.
[4] See http://www.desoybeans.org/soynews/soynewsjanuary2007.pdf.
[5] See http://www.ussoyexports.org/news/SoyExportNews/Jun06.htm.

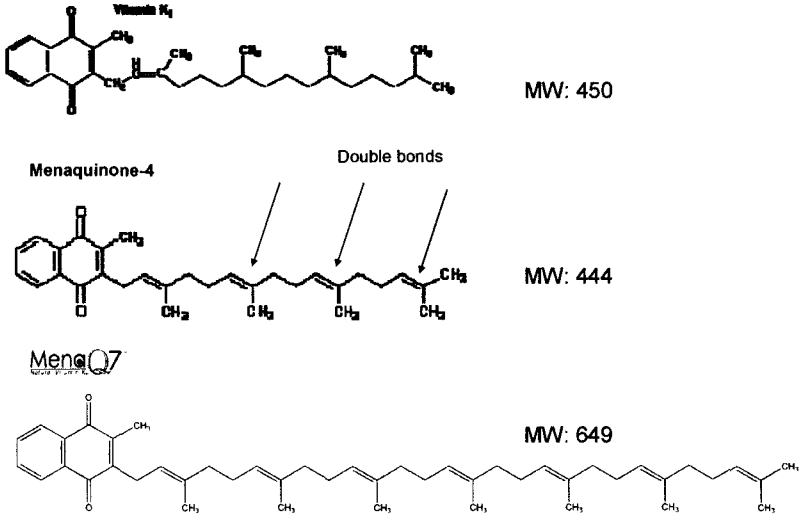
More specifically, vitamin K_2 (2-methyl-3-*all-trans*-polyprenyl-1,4-naphthoquinones), or the menaquinone series, is a group of prenylated naphthoquinone derivatives (Merck, 2001). The number of isoprene residues, where 1 isoprene unit consists of 5 carbons comprising the side chain, is used to characterize the menaquinone homologues. The nomenclature is based on the number of isoprene units, such that the name "menaquinone" or "MK" is followed by the number of units. Although menaquinones with side chains of up to 15 isoprene units have been identified, menaquinones of only 2 to 13 isoprene units have been encountered in human and animal tissues (PDR, 2001).

Demethylmenaquinones, which are menaquinones unconjugated at the carbon-2 position, also have been identified (Conly and Stein, 1992).

The structures of phylloquinone and two common menaquinone derivatives are illustrated in Figure 1.

Figure 1. Molecular Structures and Molecular Weights of Phylloquinone, Menaquinone-4, and Menaquinone-7 (MenaQ7)

Molecular structures K1, MK-4 and MK-7



2. **Common or Usual Name**. The generic common or usual name for vitamin K_2 is menaquinone, and the principle constituents of menaquinone are menaquinone-4 (MK-4) and menaquinone-7 (MK-7). Menaquinone-7 is the common or usual name of the subject material of this notification. The specific substance evaluated is MenaQ7 as marketed by NattoPharma.

Table 1. Nomenclature and Chemical Descriptions of Phylloquinone and Common Menaquinones

Common or Usual Names	Chemical Names and Synonyms
Phylloquinone	1,4-Naphthalenedione, 2-methyl-3-[(2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecenyl]-; phytonadione; vitamin K_1
Menaquinone (generic)	Vitamin K_2
Menaquinone-7 (MK-7)	(*all-E*)--2-(3,7,11,15,19,23,27-heptamethyl-2,6,10,14,18,22,26-octacosaheptaenyl)-3-methyl-1,4-naphthalenedione; Vitamin MK-7; Menaquinone K7; MK-7; Vitamin $K_{2(35)}$
Menaquinone-4 (MK-4)	(*E,E,E*)-2-methyl-3-(3,7,11,15,-tetramethyl-2,6,10,14,-hexadecatetraenyl)-1,4-naphthalenedione; 2-Methyl-3-*trans*-tetraprenyl-1,4-naphthoquinone; Menaquinone K4; MK4; Menatetrenone; Vitamin $K_{2(20)}$

Common or Usual Names	Chemical Abstracts Service (CAS) Number	Molecular Formula	Molecular Weight (grams/mole)
Phylloquinone	84-80-0	$C_{31}H_{46}O_2$	450.68
Menaquinone (generic)	11032-49-8	Varies	Varies
Menaquinone-4 (MK-4)	863-61-6	$C_{31}H_{40}O_2$	444.66
Menaquinone-7 (MK-7)	2124-57-4	$C_{46}H_{64}O_2$	649.00

3. **Vitamin K Background Information.** There are three common forms of synthetic vitamin K: phytonadione or phylloquinone (K_1); menatetrenone (K2/MK-4), and menadione (K_3). Menadione, a synthetic form known as vitamin K_3, is not used in products for humans but is widely used in animal feed and may serve as a vitamin K_2 precursor in animals. Acetomenaphthone (vitamin K_4) is another synthetic form available (Gilman et al., 1990; Sweetman, 2002).

   Vitamin K_1 is regarded as the major dietary source of vitamin K, occurring naturally in green plants, algae, and photosynthetic bacteria (Gilman *et al.*, 1990; Merck, 2001). In contrast to vitamin K_1, dietary contributions of vitamin K_2 on a weight basis is much less (PDR, 2001). Dietary sources of vitamin K_2 include chicken egg yolk, butter, cow liver, certain cheeses, and natto (Schurgers and Vermeer, 2000; PDR, 2001). Vitamin K_2 is of microbiological origin, and its most common occurrence is in fermented foods or animal-derived foods, especially if the animals have been fed vitamin K_3.

   The preponderance of the evidence suggests that bacterially synthesized menaquinones, particularly in the ileum, can and do play a significant role in contributing to vitamin K requirements in humans (Conly and Stein, 1992). In bacteria, vitamin K_2 synthesis is associated with the inner cytoplasmic membrane, where it functions as a redox compound in bacterial respiration (Conly and Stein, 1992). Depending on the specific growth conditions, bacteria contain menaquinones at concentrations in the micromolar range (Conly and Stein, 1992). A variety of microbial

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species have been identified to contain menaquinones in the human intestinal tract including *Escherichia coli* [menaquinone-8 (MK-8)], *Bacteroides fragilis* [menaquinone-10 (MK-10) and menaquinone-11 (MK-11]). MK-7, and to a smaller extent, MK-6 and MK-8 are synthesized by *Vibrio parvula*, a *fusobacterium* species.

C. Chemistry of MenaQ7

1. **Product Description.** MenaQ7 is NattoPharma's brand name for products containing Vitamin K_2. The products are manufactured from ethanol extracts of fermented soy protein and then further processed into unique forms.

2. **Product Composition.** MenaQ7 is available for use in food in two forms. MenaQ7 Oil M 1500 contains Vitamin K_2, in the form of MK-7 with minor amounts of MK-6, in an oil formulation with not less than 0.15% total menaquinone. MenaQ7 Powder P 1000 contains a similar ratio of MK-7 to MK-6 formulated as a powder with not less than 0.1% vitamin K_2.

a. MenaQ7 Oil M 1500

i. **Physical Properties and Specifications.** MenaQ7 Oil M 1500 is a yellow, odorless oil, consisting of not less than 0.15% total vitamin K_2 (as MK-7 and MK-6) and not more than 99.85% sunflower oil as a formulation aid. The chemical specifications are listed in Table 2. All specification methods used are validated methods, and all are included in other official monographs.

Table 2. Chemical Specifications for MenaQ7 Oil M 1500

Specification Parameter	Specification	Method
Total Vitamin K_2 (ppm)	Not less than 1,500	HPLC[a]
Acid Value (mg KOH/g fatty acids)	Not more than 10	AOCS[b] Official Method Te 1a-64
Peroxide Value (mEq H_2O_2/1,000 g sample)	Not more than 3 (at the time of filling)	Acetic Acid-Isooctane Method (AOCS Official Method Cd 8b-90)

[a] HPLC - High Performance Liquid Chromatography. [b] American Oil Chemists' Society.

ii. **Impurities.** NattoPharma has detailed product specifications and has done extensive analyses of its MenaQ7 Oil M 1500. The primary impurities resulting from the manufacturing process of MenaQ7 Oil M 1500 include unsaponifiable matter (0.85%) and phospholipids (0.002%). Low levels of various tocopherols are also found. Typical data are shown in Table 3.

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Table 3. Typical Composition of Oil M 1500 (Other than Manaquinones)

COMPOUNDS	CONTENT (%)	METHOD
Triglycerides	99.148	AOCS Official Method Ca 5b-71
Unsaponifiable Matter	0.850	AOCS Official Method Ca 6a-40
Sterol	0.404	J-Oil Mills Internal Method
Total Tocopherol	0.075	AOCS Official Method Ce 8-89
α-tocopherol	0.048	AOCS Official Method Ce 8-89
β-tocopherol	0.000	AOCS Official Method Ce 8-89
γ-tocopherol	0.022	AOCS Official Method Ce 8-89
δ-tocopherol	0.005	AOCS Official Method Ce 8-89
Phospholipids	0.002	AOCS Official Method Ca 19-86

The manufacturing process employed in the production of MenaQ7 Oil M 1500 ensures that the potential for contamination or introduction of impurities is low, and each batch manufactured is tested to ensure that the stringent purity criteria are met. Specifications include limits on arsenic, cadmium, lead, mercury, aflatoxins and dioxins.

Product specifications for MenaQ7 Oil M 1500 and sample results of heavy metal analysis are given in Appendix A.

iii. **Stability.** Total vitamin K_2 concentrations in unopened MenaQ7 Oil M 1500 product vials were measured by HPLC at room temperature for a period of up to 36 months. The results of the stability testing shown in Figure 2 demonstrate that the total vitamin K_2 content of MenaQ7 Oil M 1500 is little affected by storage times of the final product based on data acquired over at least 3 years. NattoPharma recommends that the commercial formulation, MenaQ7 Oil M 1500, be stored at a temperature of not more than 15°C in a cool, dry, and dark place, and away from high heat, humidity, and sunlight.

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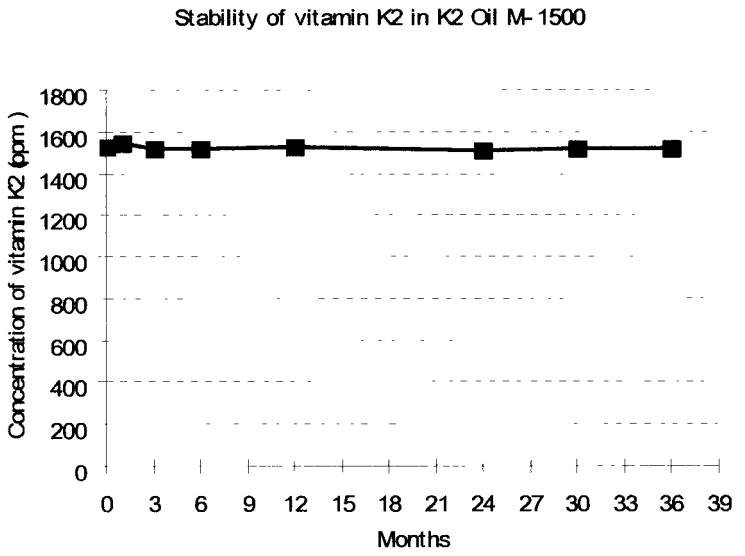
Figure 2. Stability of Vitamin K_2 in MenaQ7 Oil M 1500



b. **MenaQ7 Powder P 1000.** MenaQ7 Powder P 1000 is a powder formulation based on the same stock oil formulation, but it is dispensed in a powder formulation with not less than 0.10% (1,000 ppm) total vitamin K_2 (occurring principally as MK-7 and, to a minor extent, MK-6). The remaining components of the product are listed in Table 4. Product specifications are listed in Appendix A.

Table 4. Non-Menaquinone Components of MenaQ7 Powder P 1000

Compounds	Content (%)	Function	Relevant FDA Clearance
Dextrin	60 - 65%	Delivery vehicle of product	172.892; 184.1277
Sunflower Oil	20 – 25%	Delivery vehicle of MK-7 and MK-6	GRAS
Sodium casein	10%	Powderization material	182.1748
Starch sodium octenylsuccinate	2%	Powderization material	172.892
Glycerol fatty acid ester	1%	Emulsifiers	184.1505
L-Ascorbyl palmitate	0,5%	Antioxidant	182.3149

D. Manufacture of MenaQ7

1. **Raw Materials.** The raw materials used in the production of menaquinone, formulated as MenaQ7 Oil M 1500, include soybean protein isolate, corn starch, water, *B. subtilis natto*, ethanol, and sunflower oil.

   a. **Soybean Protein Concentrate.** Soybean protein concentrate, which is used as the primary starting material in the fermentation of *B. subtilis natto*, is derived from non-genetically modified soybeans that are handled under identity preserved conditions.

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b. **Corn Starch.** Corn starch, which also is used in the fermentation with *B. subtilis natto*, is derived from non-genetically modified corn that is handled under identity preserved conditions. In the US, corn starch is approved for use in food for human consumption as a starting material for maltodextrin (21 CFR 184.1444), corn sugar (21 CFR 184.1857), corn syrup (21 CFR 184.1865), and high fructose corn syrup (21 CFR 184.1866), all of which are approved as direct food substances affirmed as GRAS (21 CFR 184) (FDA, 2004).

c. ***Bacillus Subtilis Natto.*** Natto, which has a history of dietary consumption in Japan dating back at least 1,000 years, is rich in vitamin K_2 as MK-7 (Sumi et al., 1990). It is produced via the natural fermentation of boiled soybeans through the action of bacteria, which has been specifically referred to as *B. subtilis natto* (Sato et al., 1997). Currently, natto is manufactured and sold as a food product in the US market (US Soyfoods Directory, 2003). *B. subtilis natto*, itself, is currently used in Japan as a dietary supplement source of vitamin K_2 (PDR, 2001).

   In the US, carbohydrase (21 CFR 184.1148) and protease (21 CFR 184.1150) from *B. subtilis*, meeting food grade specifications, are affirmed as GRAS for use in foods as enzymes at levels not exceeding current good manufacturing practice (cGMP) (FDA, 2001, 2004). The affirmations are predicated on the use of non-pathogenic and nontoxigenic strains of *B. subtilis* (FDA, 2004).

d. **Ethanol.** Food grade ethanol which meets Food Chemicals Codex specifications is used as an extraction solvent in the production of MenaQ7 Oil M 1500 (Council of the European Communities, 1988).

e. **Sunflower Oil** Sunflower oil is used as the final solubilizing agent and vehicle for MenaQ7 Oil M 1500. The sunflower oil used meets the food grade specifications for non-hydrogenated sunflower oil as published in the Food Chemicals Codex (FCC).

E. Manufacturing Process

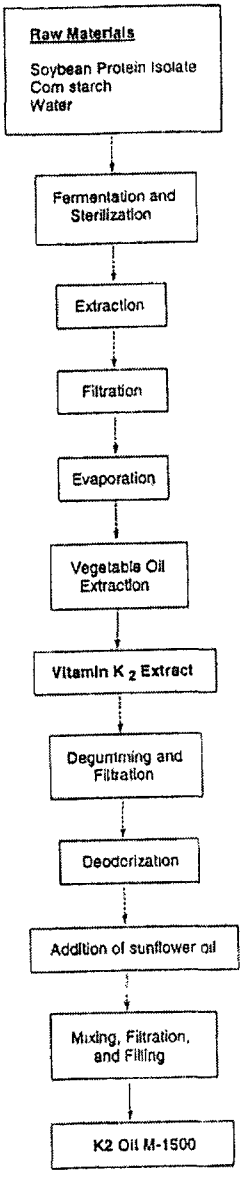
The production process is carried out by Sumitomo Corporation, J-Oil Mills plant, in Japan. The facility is registered with FDA, registration number 13885804934.

The natural vitamin K_2 MK-7 is a long chain menaquinone synthesized by bacteria which utilize MK-7 in their own energy metabolism. The non-pathogen bacteria, *B. subtilis natto,* is the key microbial participant in the ongoing production of the soy-based traditional natto fermentation, as various strains are known for their capacity to produce and secrete large quantities of enzymes.

As noted in Section II.D.1, MK-7 production takes place by using non-GMO soy bean concentrate and non-GMO corn derived starch as substrates for the fermentation. After prolonged fermentation, the mixture is sterilized and extracted with ethanol and filtered before the remains of the ethanol are evaporated. The fat soluble menaquinones are then extracted with sunflower oil and processed through multiple separations steps (stem distillation, filtrations) before a stock solution of MK-7 is obtained.

The various commercial products are then diluted either in sunflower oil or in a powder mixture with various components listed in Table 4.

Figure 3. Manufacturing Diagram for MenaQ7 Oil M 1500



III. Nutritional and Food Aspects of Vitamin K

A. Nutritional Functions of Vitamin K

The fact that vitamin K is essential for blood clotting has been known for over 70 years. The alphabetical designation "K" was derived from the German description "koagulationvitamin," and at the time of its identification, vitamin K's only known role was in the mediation of clot formation. Evidence since then suggests vitamin K plays a crucial role in many other bodily functions involving calcium ion metabolism.

All forms of vitamin K function as cofactors for carboxylation of specific protein-bound glutamate residues which are converted into γ-carboxy glutamate (GLA) (Schurgers et al, 2007a). These GLA residues form calcium binding sites that are essential for activating the proteins to function in the tissues in which they are found (Schurgers et al, 2007a). The first identified GLA-containing proteins were associated with blood coagulation regulation and were recognized as playing an important role in hemostasis through the activation of blood coagulation and anticoagulation factors in the liver. Another GLA-containing protein (vitamin K-dependent) not related to blood clotting has more recently been isolated in bone and tooth dentine. Osteocalcin is a protein synthesized by osteoblasts, and while full understanding of its function is unclear, it appears to play an important role in bone mineralization (EVM, 2003; Wildman and Medeiros, 2000). Accumulating evidence suggests that low vitamin K intake results in low bone mineral density, increased fracture risk and osteoporosis (Horiuchi et al., 2004; Schurgers et al., 2007b; Tsugawa et al., 2006).

Matrix GLA protein (MGP) is another vitamin K-dependent GLA-protein synthesized primarily in cartilage and blood vessel walls. Studies demonstrate that MGP prevents arterial calcification (atherosclerosis) while facilitating normal bone growth and development (National Academy of Sciences, 2001). Moreover, low vitamin K_2 intake is associated with an increased risk of cardiovascular disease and mortality (Geleijnse et al, 2004). Vitamin K supplements were shown to slow down menopausal bone loss and to block age-related arterial stiffening (Schurgers et al., 2007b). Increasing dietary vitamin K intake is potentially beneficial for bone and vascular health (Schurgers et al, 2007a).

GLA proteins have also been isolated in kidney tissue and may be involved in reabsorption of calcium by the kidney tubules and solubilization of calcium salts in urine (EVM, 2003; Wildman and Medeiros, 2000). Other GLA-containing proteins have been found in placenta, pancreas and spleen but have not been thoroughly characterized (EVM, 2003). Vitamin K-dependent proteins are also thought to function in cell signaling and brain lipid metabolism (EVM, 2003).

The review of nutritional requirements for vitamin K by the National Academy of Sciences (2001) largely discussed vitamin K_1. However, a combination of direct and indirect evidence demonstrates that dietary vitamin K_2 is nutritionally important. It is very difficult to induce vitamin K deficiency. It was demonstrated in human volunteers that inducing deficiency required both a vitamin K free diet as well as application of broad spectrum antibiotics (Frick et. al., 1967). The need for antibiotics implicates a role for intestinal bacteria in maintaining vitamin K levels. Hodges et al. (1991) found that elderly patients with low plasma levels of MK-7 and MK-8 were more likely to experience bone fractures. MK-7 plasma values were found to be lower in osteoporotic patients than in non-osteoporotics (Horiuchi et al., 2004). Geleijnse et al. (2004) found lower cardiovascular disease in population groups with higher dietary levels of menaquinone. Dietary MK-7 was

shown to be more effective in maintaining osteocalcin function than dietary phylloquinone (Schurgers, et al., 2007a).

B. Natural Occurrence of Vitamin K in Food

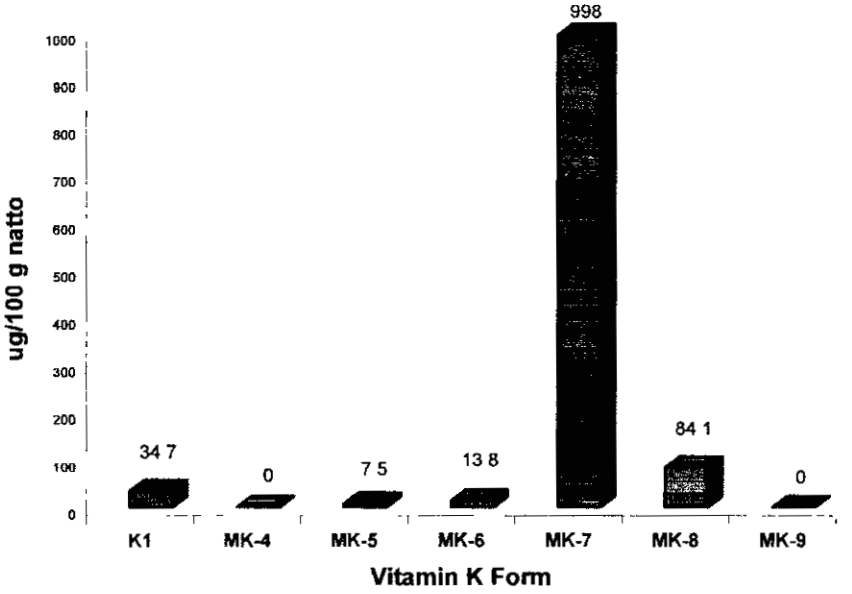
Various forms of vitamin K occur naturally in food. The most prevalent and studied form of vitamin K is K_1 or phylloquinone. Vitamin K_1 is most remarkably present in dark green leafy vegetables such as kale, spinach, collards, broccoli and Brussels sprouts. Except for some plant oils, most foods have far lower phylloquinone content (Booth and Suttie, 1998).

It is apparent that phylloquinone is distributed throughout the Western diet, and the range of concentrations in different food categories varies widely. In general, the relatively high value in vegetables confirms the association of phylloquinones with the photosynthetic tissues of plants, with the highest values being found in green, leafy vegetables. The next best sources are certain vegetable oils. The large difference between vegetable oils presents problems for calculating phylloquinone content of prepared foods containing oil when the type of oil is not known.

Vitamin K_2, the designation for the family of menaquinones---MK-4 through MK-13---is typically synthesized by bacteria, specifically including intestinal bacteria. While menaquinones from intestinal bacteria were once believed to be insignificant contributors in satisfying the body's requirement for vitamin K, accumulating evidence suggests differently---especially the benefits derived from the long-chained menaquinones, MK-7, MK-8 and MK-9---which are also present in fermented foods, notably natto (NattoPharma ASA, 2007; Schurgers et al., 1999; Schurgers et al., 2007a).

Natto is a fermented soy product that has been a staple food of eastern Japan for over 1,000 years. Many westerners find it unpalatable due to its texture and strong flavor. It is, however, the richest dietary source of vitamin K presently known---almost all of which occurs in the form MK-7 (NattoPharma ASA, 2007; Schurgers et al., 2007a.); however, there is some MK-8 and MK-6 present along with some vitamin K_1 (NattoPharma ASA, 2007). The amounts of various forms of menaquinone are given in Figure 4.

Figure 4. Content of Various Forms of Vitamin K in Natto[a, b]



[a] From Schurgers, L J., 2002 [b] Zero means not detectable

The quantitative determination of menaquinones in food is tedious and to date has not been standardized As quality assured analytical assays for the quantitative determination of menaquinone forms become available, the full extent of their presence can be established. Table 5 shows typical levels of phylloquinone, MK-4, and total menaquinones found in food

Table 5. Concentrations of K-Vitamins in Food[a]
Vitamin K Concentration Range µg/100g or µg/100mL

NUTRIENT	K_1	MK-4	MK-n[b]
Meats	0 5 - 5	1 - 30	nd[c]
Fish	0 1 - 1	0 1 - 2	0.1 - 2
Fruits	0.1 - 3	nd	nd
Green vegetables	100 - 750	nd	nd
Natto	20 - 40	nd	900 - 1200
Cereals	0 5 - 3	nd	nd
Cheese	0 5 - 10	0 5 - 10	40 - 80
Other dairy produce	0 5 - 15	0 2 - 15	0 - 35
Eggs	0.5 - 2.5	10 - 25	nd
Margarine/plant oils	50 - 200	nd	nd
Wine and beer	nd	nd	nd

[a] From Schurgers et al , 1999 [b] MK-n consists of all forms of menaquinones other than MK-4 [c] Not detectable

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In the Western diet nutritionally significant amounts of long-chain menaquinones have only been found in animal livers and cheeses (NattoPharma, 2007; Schurgers et al., 1999; Schurgers et al. 2007a; Matschiner, et al., 1967). Yeasts do not synthesize menaquinones and menaquinone-rich foods are those that include a bacterial fermentation stage.

The relative dietary importance of vitamin K_2 is difficult to evaluate because concentrations in foods may depend on the geographic differences in animal husbandry practices and the use of menadione (vitamin K_3) in feedstock (Booth and Suttie, 1998; Matschiner, et al., 1967). MK-4 is synthesized from menadione in animal tissues. There is also evidence that animal tissues and dairy products may contain some MK-4 as a consequence of tissue synthesis from phylloquinone itself (Booth and Suttie, 1998; Matschiner et al., 1967).

C. Current Estimates of Vitamin K in Total Diets

1. **Intake from Food.** It is common for newborn infants in the US to receive an injection of vitamin K to compensate for immature blood coagulation systems at birth. FDA requires vitamin K supplementation of infant formula.[6] FDA also requires that olestra-containing foods be supplemented with vitamin K to counteract the action of olestra to interfere with the absorption of fat-soluble vitamins.[7] It is interesting to note that there are no food additive or GRAS listings for sources of vitamin K. It is suspected that phylloquinone is added in these cases, presumably sanctioned by an independent GRAS determination.

   Studies evaluating the average daily intake of vitamin K within diets of various worldwide population groups are spotty, at best. Booth and Suttie (1998) observed that while medical and nutritional texts state that the average American diet contains between 300 and 500 µg of vitamin K per day, in reality the vitamin K intake of most individuals is closer to the recommended dietary allowance (RDA) of 1 µg/kg body wt/day and that many individuals fail to even meet this lower level on a daily basis. Until recently, menaquinones were thought to contribute little to the body's requirement for vitamin K, so most data measuring vitamin K intake in the standard American diet focus solely on phylloquinone intake concentrations. Table 6 summarizes Booth and Suttie's findings which compared 11 studies that were conducted to measure vitamin K_1 consumption by age group. While the numbers come primarily from American diet studies, one Dutch and one Scottish study are included.

   Compared to the United States, Schurgers et al. (1999) report that the mean dietary intake of vitamin K_1 in the Netherlands is high (80 vs. 250 µg/day). In a study measuring vitamin K_1, MK-4, and MK-n intake in 5435 elderly Dutch, the mean phylloquinone intake was reported as 249 ± 2 µg/day, mean MK-4 intake was 6.8 ± 0.04 µg/day and the mean intake of the higher menaquinones, especially MK-7, was 21.6 ± 0.2 µg/day. Geleijnse et al. (2004) report similar data for Dutch men and women aged 55 years and older with no history of myocardial infarction.

   In another study, Kaneki et al. (2001) report a large geographic difference in serum vitamin K_2 (menaquinone-7, MK-7) levels in postmenopausal women. Serum MK-7

[6] See 21 CFR 107.100.
[7] See 21 CFR 172.867(d).

Table 6. Dietary Phylloquinone Intake Per Day in Healthy Men and Women[a]

Gender	Age in Years	Number of Subjects	Mean Intake (mg)
M + F	> 60	1,216	80 (American Study)
M + F	60 - 80	17	143 (American Study)
M	55 - 75	9	164 (American Study)
F	41 - 71	362	156 (American Study)
F	60 - 79	80	210 (Dutch Study)
M + F	20 - 40	17	111 (American Study)
M + F	22 - 54	65	68 (Scottish Study)
M + F	18 - 55	221	61 (American Study)
M + F	28 - 45	1,490	71 (American Study)
M	20 - 35	10	77 (American Study)
M	20 - 28	9	83 (American Study)

[a] From Booth and Suttie, 1998.

concentrations were 5.26 ± 6.13 ng/mL (mean ± SD) in Japanese women in Tokyo, 1.22 ± 1.85 in Japanese women in Hiroshima, and 0.37 ± 0.20 in British women. Serum concentrations were significantly higher in regions where natto is frequently eaten. Natto, with its large amount of MK-7, is eaten often in eastern Japan (Tokyo) but seldom in western Japan (Hiroshima).

2. **Intake from Dietary Supplements and Other Forms.** Vitamin K is commonly found in multiple vitamins as phylloquinone at levels of 10 - 80 µg per daily dose (Johnson, 2005). Single ingredient dietary supplements of vitamin K_1 are widely available in the US, and the dosage form is usually 100 µg per tablet.

   In Europe as well as in the US, MenaQ7 is sold in dietary supplements in a recommended dose of 45 ug MK-7/day for adults.

   MK-4 is registered as a drug (brand names GLAKAY, EISAI) in Japan and is commonly used in Japan to treat osteoporosis in multiples of 45 mg/day (Plaza & Lamson, 2005).

D. Nutritional Requirement for Vitamin K

The National Academy of Sciences, Institute of Medicine's Food and Nutrition Board (NAS, 2000) acknowledges that, because of the lack of data to estimate the daily average dietary requirement, an Adequate Intake (AI) is estimated based on representative dietary intake data from healthy individuals. The AI for vitamin K_1 for men is set at 120 µg/day and that for women is 90 µg/day. This was apparently set largely based on data on vitamin K_1. Because no adverse effects have been reported for individuals consuming higher amounts of vitamin K, a Tolerable Upper Intake Level (UL) was not established.

European experts in the field of vitamin K suggest that daily intakes between 200 and 500 µg/day, preferably in the form of vitamin K_2, may be required for optimal carboxylation of extrahepatic GLA proteins (Askim, 2001; Vermeer et al., 2003). The Expert Group on Vitamins and Minerals (EVM, 2003) reports that an intake of 0.001 mg/kg body weight/day (60 µg/day) was probably adequate for proper coagulation function.

IV. Intended Food Use and Dietary Estimates

A. Intended Use of MenaQ7 in Food

NattoPharma intends to add the two existing MenaQ7 products to selected processed food categories as a nutrient supplement as defined in 21 CFR 170.3(o)(20). The MenaQ7 products will be added at levels ranging from 6.7 - 10 mg/serving to deliver 10 µg of MK-7 per serving of food. NattoPharma is developing other MenaQ7 products of varying concentrations of MK-7 for formulation flexibility for the food industry.

The foods to which MenaQ7 will be added include dairy products and butter and margarine substitutes. The dairy products targeted include yogurt, cottage cheese, hard cheeses, and milk drinks and substitute products. NattoPharma recognizes that certain foods may have compositional restrictions because of specified food standards; for those foods MenaQ7 can only be added if allowed by the food standards or if incorporated into substitute or alternate foods that are not subject to the food standards limitations.

B. Estimated Dietary Intake for the United States

USDA survey data indicate that the average consumption of total dairy products for Americans is 1.5 servings per day.[8] Based on FDA guidance, a level of twice the average (or 3 servings per day) can be considered an overestimate of the 90th percentile of total dairy products (FDA, 2006).

USDA survey data indicate that the average consumer consumes 4 g/day of table fats, the category which includes butter and margarine products.[9] Given the serving size of 5 g, this equates to 0.8 servings per day for the butter and margarine type products; at twice the consumption of the mean to estimate the 90th percentile consumer, we obtain the estimated consumption of butter and margarine products to be about 1.6 servings per day.

To deliver 10 µg of MK-7 per serving of food, 6.7 mg per serving of MenaQ7 Oil M 1500 (which contains 0.15% MK-7) will be added to the designated food. When adding MenaQ7 Powder P 1000 (which contains 0.10% of MK-7), 10 mg per serving will be added to the designated food.

Thus, a total daily dietary intake of MK-7 using either MenaQ7 Oil M 1500 or MenaQ7 Powder P1000 as described above provides 50 µg of MK-7. Since the MenaQ7 Oil M 1500 is 95+% sunflower oil, 5 total servings of the designated foods would provide about 35 mg/day of sunflower oil to the diet. At 5 servings per day, the consumption of added components of MenaQ7 Powder P 1000 products will be as represented in Table 7.

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[8] USDA, 1999. Table 4a. Pyramid Servings Data for Dairy Products.

[9] USDA, 1997. Table 9.6. Eggs; legumes; nuts and seeds; fats and oils; sugars and sweets: Mean quantities (in grams) consumed per individual, by sex and age, 1 day, 1994-95.

Table 7. Non-Menaquinone Components of MenaQ7 Powder P 1000

COMPOUNDS	CONTENT (%)	MAXIMUM CONSUMPTION BASED ON 5 SERVINGS PER DAY	FRACTION OF DAILY DIET BASED ON 3 KG DIET (PPM)
Dextrin	60 -- 65	35 mg/day	12
Sunflower Oil	20 – 25	12.5 mg/day	4
Sodium casein	10	5 mg/day	2
Starch sodium octenylsuccinate	2	1 mg /day	0.3
Glycerol fatty acid ester	1	0.5 mg/day	0.2
L-Ascorbyl palmitate	0.5	0.25 mg/day	0.1

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V. Safety Data for MenaQ7

Due to the structural similarities of phylloquinone and the various menaquinones, the safety data for all these forms have been considered as part of the safety review of MK-7 and MenaQ7.

A. ADME Studies

The 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). Several forms of vitamin K_2 differ by the length of the side chain at the 3-position of quinone ring as discussed in Section II. MK-4, which is the major form synthesized by intestinal bacteria and found in meat and cheese, has a side chain with four isoprene units. MK-7, which is the major form synthesized in the fermentation process for natto, has seven isoprene units on the side chain. Other forms which exist naturally and are designated as MK-5 through MK-11.

The function, tissue distribution and the metabolic interconversions of the various forms of vitamin K are not entirely understood. NAS (2000) summarized the level of understanding in the year 2000.

> The human gut contains a large amount of bacterially produced menaquinones, but their contribution to the maintenance of vitamin K status has been difficult to assess (Booth and Suttie, 1998). Although the content is extremely variable, human liver contains about 10 times as much vitamin K as a mixture of menaquinones than as phylloquinone (Shearer, 1992; Thijssen and Drittij-Reijnders, 1994; Usui et al., 1990). Absorption of these very lipophilic membrane-associated compounds from the distal bowel has been very difficult to demonstrate (Ichihashi et al., 1992).

Some light has been shed on these issues in recent studies by examining the absorption, distribution and metabolism of excretion of various forms of vitamin K_2.

Several published studies in human volunteers with oral doses of natto showed that MK-7 is absorbed and can be detected in plasma (Sumi, 1999; Tsukamoto et al., 2000; Kaneki et. al., 2001, Schurgers et al., 2007). Plasma levels of MK-7 were found to be higher in women from Japan than women from Britain. The difference was correlated to the use of natto in the diet by the Japanese women (Kaneki et al., 2001). In two studies, good bioavailability of MK-7 was observed form natto capsules and natto food (Vermeer, 2003a; Schurgers et. al., 2007a). Higher and more stable blood levels of vitamin K were seen with MK-7 than phylloquinone in both studies.

Thijssen et al. (2002) demonstrated that MK-4 found in human breast milk is derived from phylloquinone in the maternal diet. The plasma half-life of MK-9 from dietary absorption is longer that that of phylloquinone and MK-4 (Schurgers and Vermeer, 2002). Schurgers et al. (2007a) demonstrated that the half life of MK-7 in plasma was longer than for phylloquinone when similar levels of either form was consumed in the diet.

Degradation of the side chain can occur in mammalian tissues. Based on studies with phylloquinone and MK-4, the liver is likely the principal site of vitamin K_2 metabolism involving oxidative degradation of the side-chain with the resulting subsequent elimination via the bile or urine (IARC, 2000).

B. Clinical Studies on Vitamin K Derivatives

1. **Studies on MK-7.** In a single dose study, 8 postmenopausal women consumed 80 g of natto (Kaneki, et al., 2001). Each dose contained approximately 1100 µg of MK-7. The blood levels of MK-7 were followed for 14 days. Good bioavailability of MK-7 was found. There was no mention of adverse effects in any of the subjects. Three published clinical studies with repeated dose regimens shed light on the safety of natto and MK-7.

   Tsukamoto et al. (2000) studied the influence of intake of fermented soybeans (natto) containing MK-7 (menaquinone 7) on increased serum MK-7 and y-carboxylated osteocalcin in 48 adult volunteers in a short-term study. It is known that the intake of MK-7 may be significant in reducing age-related bone loss. Three groups of 16 adults each (15 males and one female) were given sequentially the natto (50 g) with three different concentrations of MK-7 once a day for 14 days as follows: regular natto with 865 µg MK-7/100 g of natto; reinforced natto containing 1295 µg MK-7/100 g, or 1730 µg MK-7/100 g. Reinforced natto already contains more MK-7 than regular natto. The intake of natto was performed between 6 AM and 8 AM each day, and blood samples were taken between 10 AM and 12 AM on the day prior to the intake or 7, 10 and 14 days after the intake. Serum MK-7 and osteocalcin were measured using analytical methods. The serum MK-7 and y-carboxylated osteocalcin concentrations were raised 7, 10 and 14 days after the start of the intake of the reinforced natto containing 1295 µg or 1730 µg MK-7. The intake of regular natto containing 865 µg MK-7 significantly raised serum MK-7 levels, but not osteocalcin levels. These results suggest that the MK-7 addition to reinforced natto leads to the y-carboxylation of osteocalcin, which plays an important role in bone formation. No adverse effects in subjects were noted by the authors.

   Seventy-three (73) healthy pre-menopausal women were randomly allotted to one of four fermented soybean (natto) consumption regimes (Katsuyama et al., 2004). The women consumed 0 (control), 1 serving/month, 1 serving/week, or 3 servings/week of natto at lunch for a period of 1 year. Each natto serving contained 175 µg of MK-7. Bone turnover markers and a stiffness index, measured by quantitative ultrasound, were measured at baseline, 6 months, and after 1 year. No significant differences were observed in the stiffness index of the control and natto treatment groups. BAP (bone specific alkaline phosphatase---a marker for bone formation) was reported to be significantly higher in women consuming 3 natto servings/week compared to those consuming 1 serving/week after 1 year. The level of undercarboxylated osteocalcin (a marker for inactive osteocalcin) was observed to be significantly lower in the women consuming 3 natto servings/week compared to all other women after 6 months. Furthermore, as compared to the control group, two markers for bone formation---BAP and undercarboxylated osteocalcin—both changed in the direction of improved bone health in the highest treatment group (3 servings/week). An increase in natto intake may have contributed to the promotion of bone formation in pre-menopausal women. No adverse effects were reported in study participants.

   In the most recent study, 18 volunteers received either 180 µg MK-7 form natto capsules or 150 µg of phylloquinone once a day for six weeks (Schurgers, et al., 2007a). The primary purpose of the study was to measure vitamin K blood levels and forms of osteocalcin. No adverse effects were noted during the course of the study.

The results of these studies are summarized in Table 8.

Table 8. Clinical Studies on Natto and Other Sources of MK-7

Study	Treatment Group	Doses /Duration	Results and Remarks
Tsukamoto et al., 2000	3 groups of 16 adults each (15 males and one female	Regular natto with 865µg MK-7/100 g of natto; reinforced natto containing 1295 µg MK-7/100 g, or 1730 µg MK-7/100 g/ 14 days	Results suggest that MK-7 addition to reinforced natto leads to the y-carboxylation of osteocalcin, which plays an important role in bone formation. No adverse effects in subjects were noted.
Katsuyama et al., 2004	73 healthy pre-menopausal women in 4 groups	0 (control), 1 serving/month, 1 serving/week, or 3 servings/week of natto at lunch for a period of 1 year. Each natto serving contained 175 µg of MK-7	Increase in natto intake may have contributed to promotion of bone formation in pre-menopausal women. No adverse effects reported in study participants.
Kaneki et al., 2001	Groups of healthy postmenopausal women from Tokyo (49 participants, aged 50-84), Hiroshima (25 participants, aged 51-66), and London (31 participants, aged 48-84)	Single dose of natto used which contained 18 ng/g of MK-4, 13,998 ng/g MK-7 and 168 ng/g MK-8	Overall, the authors conclude that there is a large geographic difference in vitamin K, specifically MK-7, serum levels, and that that difference may be partially ascribed to natto intake. No adverse effects noted.
Schurgers, et. al., 2007a	18 healthy volunteers	Daily doses of 180 µg MK-7 from natto capsules or 150 µg phylloquinone for 6 weeks	Blood levels of vitamin K more stable in subjects receiving MK-7 rather than phylloquinone. MK-7 reduced concentration of undercarboxylated osteocalcin. No adverse effects noted.

2. Clinical Studies on Other Derivatives of Vitamin K

Several clinical studies were conducted with MK-4. Many of these studies were conducted to evaluate the effect on various parameters related to bone metabolism. A few others studied the effects on various disease states. The doses in these studies were usually multiples of 45 mg per day. The duration of the more significant of these studies ranged from 24 weeks to two years. The results are summarized in Table 9.

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Table 9. Clinical Studies on Other Forms of Vitamin K_2

Study	Treatment Group	Doses / Duration	Results and Remarks
Shiomi *et al.*, 2002	25 women with liver cirrhosis, 42-72 years of age	45 mg/2 year	22 of 25 patients assigned to the test group completed study. No adverse effects related to treatment with vitamin K_2 were noted.
Orimo et al., 1998	75 osteoporotic patients	90 mg or placebo/24 weeks	Adverse effects included heartburn, stomach upsets, and abdominal fullness. Authors either considered symptoms to be unlikely related to treatment or were unable to ascertain a relationship between vitamin K_2 treatment and the effects. Increases in GOT, GPT, ALP and γ-GPT reportedly observed in single subject in vitamin K_2 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.
Asakura et al., 2001	5 male, 25 female osteoporotic patients	45 mg, 3x/day/>3 weeks	Levels of MK-4 increased significantly 4 weeks after administration and remained elevated for duration of study period. Plasma levels of phylloquinone and MK-7 unchanged from pre-treatment levels. No variations observed in any of 10 hemostatic parameters examined. A group of vitamin K-deficient patients identified. Overall, concluded that vitamin K_2 can be administered safely without inducing hemostatic disturbances to patients not treated with anticoagulants.
Bunyaratavej et al., 2001	43 subjects in test group, 40 subjects in control group	0 or 45 mg/day/12 months	Bone mass monitored and levels of circulating undercarboxylated osteocalcin. Throughout study period, women attended monthly clinic, at which point any adverse events were reported. At 6 months into the study period, the calcium control group also was placed on vitamin K_2 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.
Takami et al., 2002	18 MDS-refractory anemia patients	0 or 45 mg/day/16 weeks	Symptoms of MDS-RA were reported to improve with menatetrenone treatment in 5 of 9 patients (56%), while only one (11%) of the group 2 patients displayed improvement in MDS-RA symptoms. In treatment group, absolute neutrophil count (ANC) rose significantly in 3 patients, major response in hemoglobin concentration observed in 2 patients, and major response in platelet count observed in another 2 patients. Overall, ANC of treatment group increased following menatetrenone treatment and decreased slightly in control group. Significant improvement seen in final platelet count, but not in hemoglobin concentration. Given the absence of toxicity associated with menatetrenone administration, recommended its use for all MDS-RA patients.
Sumi et al., 1999	6 males	2 to 100 g of natto (26.4 µg menanquinone-7 per 2 g natto) or 1 g of a *natto bacilli* powder containing 23.0 µg menaquinone-7/g powder	Neither blood coagulation nor fibrinolytic systems as determined on the basis of thromboerastography (TEG) patterns, prothrombin time (PTT), and APTT were affected.
Iioka et al., 1991, 1992	Pregnant mothers (number not specified)	Single intravenous dose of 60 mg menatetrenone or 20 mg menatetrenone orally for a period of 7 days	Authors noted that menatetrenone appears to transfer into placental tissue, where it is stored, and subsequently gradually released into fetal blood. Vitamin K_2 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.

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Motohara et al., 1990	Pregnant mothers (number not specified)	Oral administration of 20 mg vitamin K_2/day for 7 to 10 days prior to delivery	Significantly increased levels of vitamin K_2 in mothers' plasma, cord plasma, and breast milk following oral administration. Postnatally, infants observed for period of 5 days, during which no evidence of bleeding reported.
Suzuki et al., 1984	Neonates exhibiting thrombotest results below 20% 48 hours following birth (number not specified)	Single oral administration of up to 6 mg of vitamin K_2 *via* 1 mL of syrup	Vitamin K_2 significantly increased thrombotest values without significantly affecting bleeding time or levels of plasma bilirubin and hematocrit.
Sato et al., 2005	200 female patients with Alzheimer's disease, 100 healthy females as control group	Vitamin K_2 (dose not specified) supplementation when administered concurrently with 600 mg calcium/day and 1000 IU ergocalciferol/day/ 2 years	BMD of control group significantly higher than that of untreated and treated groups at baseline. In treated group, 86% fewer nonvertebral fractures and 87% fewer hip fractures observed compared to untreated group. 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. No adverse effects were reported in study participants.
Somekawa et al., 1999	110 Japanese females (24 to 52 years of age) diagnosed with endometriosis and/or uterine leiomyomas	All were administered leuprolide therapy[a] for their respective oestrogen-dependent disease either alone (control) or in conjunction with 45 mg vitamin K_2, 0.5 µg 1,25-$(OH)_2$-D_3, or 45 mg vitamin K_2 and 0.5 µg 1,25-$(OH)_2$-D_3/ 6 months	Percent decrease from baseline of lumbar BMD of women receiving vitamin K_2, alone or in conjunction with 1,25-$(OH)_2$-D_3, were significantly lower than percent decrease reported for control group. Significantly larger increase from baseline in serum bone formation markers observed in groups administered vitamin K_2 compared to control group, while no significant differences observed in serum bone resorption markers. Administration of vitamin K_2 reported to partially prevent bone loss associated with oestrogen deficiency resulting from leuprolide treatment. No adverse effects were reported in study participants.

C. Animal and Other Toxicology Studies of Vitamin K Derivatives

1. **Acute Studies**. Two acute oral studies in mice are reported for menaquinone. No deaths or side effects were reported following administration of a single oral dose of 540 mg vitamin K_2/kg body weight to mice (no other details were provided; Takehara, 1981). In an older study, a LD_{50} value of 500 mg /kg was reported for menanquinone in mice (Molitor and Robinson, 1940).

2. **Subchronic and Chronic Studies**. Two subchronic studies have been conducted on MK-4. No adverse effects were seen in a 13-week oral toxicity study conducted in groups of Sprague-Dawley rats (10/sex/group) at 30 mg/kg body weight/day administered by gavage (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 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 menatetrenone administrations for a period of 1 year (Hosokawa et al., 1995; Vanatta et al., 1995). Hosokawa et al. (1995) provided menatetrenone to Fisher 344 rats (20/sex/group) at dietary concentrations of 0 (control and non-

treated naïve groups), 0.04, 0.2, or 1.0% (providing approximately 0, 20, 100, and 500 mg menatetrenone/kg body weight/day, respectively). Based on the results obtained under the conditions of this study, the dietary concentration of 0.04% (approximately 20 mg/kg body weight/day) was determined as the NOAEL in rats.

In the dog study, the 1-year treatment period was followed by a 3-month recovery period (Vanatta *et al.*, 1995). Menatetrenone was provided orally in capsules to groups of 6 male and 6 female dogs to provide doses of 0 (empty capsule), 20, 200, or 2,000 mg/kg body weight/day. The authors concluded that 200 mg/kg body weight/day was the NOAEL in this study.

3. **Reproductive and Developmental Studies.** Menatetrenone was administered orally at doses of 0 (control), 10, 500, or 1,000 mg/kg body weight/day or intraperitoneally at doses of 0 (control or solvent), 5, 50, or 100 mg/kg body weight/day to pregnant mice and rats for a period of 6 days from days 7 to 12 and 9 to 14 of gestation, respectively (Suzuki et al., 1971). No differences were observed 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 irrespective of the route of administration. Following oral administration, mouse fetuses obtained from the mid- and high-dose groups (500 and 1,000 mg/kg body weight/day, respectively) exhibited an increased incidence of non-ossified forelimbs compared to fetuses of control mice. Skeletal anomalies were limited to an increased frequency of variations of the cervical ribs in the mid-dose fetuses. Compared to controls, variations in post-natal development in the test groups (results reported for only the 10 and 1,000 mg/kg body weight/day dose groups) included increased separation of ear auricular and emergence of abdominal hair in high-dose offspring. Descent of testes was reduced in low-dose (10 mg/kg body weight/day) male fetuses compared to untreated controls. 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 observed in fetuses from the high-dose group. No statistically significant variations were reported in the postnatal development or weaning of rat offspring.

   No significant malformations in offspring or any other reproductive or developmental effects were seen a study with rats of unspecified strain (22 to 24/sex/group) which were orally administered 0, 10, 100, or 1,000 mg/kg body weight/day of MK-4 for a period of 14 days (Mikami *et al.*, 1981). There also were no significant compound-related effects observed on the physiological, morphological, or functional development of offspring during the peri- and post-natal periods.

   In a study with intraperitoneal administration of menatrenone to pregnant mice, the authors concluded that menatrenone did not induce any teratogenic effects, mortality or inhibition of fetal growth, and, postnatally, did not affect development or weaning (Suzuki et al., 1971).

4. **Mutagenicity and Genotoxicity Studies.** Several mutagenicity and genotoxicity studies have been conducted on various forms of vitamin K. These results appear in Table 10.

Table 10. Mutagenicity and Genotoxicity Studies on Various Forms of Vitamin K

Study	In Vivo / In Vitro	System	Author Conclusion	Results and Remarks
Cook et al., 1991	*In Vitro*	SOS chromotest	Positive	SOS inducing potencies varied by a factor of 2 between replicate experiments, consistent SOS induction occurred with vitamin K micelles and increased in a dose dependent manner. The SOS inducing potency factor in the two sets of results show 0.02 and 0.04 respectively. It is generally accepted that inducers of the SOS response in bacteria are considered genotoxins.
D'Ordorico et al., 1997	*In Vitro*	HT-29 (colon carcinoma cell line)	Negative (K_1 and K_2) Positive (K_3)	Vitamin K and K_2 did not cause significant single strand breaks in the presence or absence of dicoumarol as compared to the control (untreated cells). The authors conclude that the natural forms of vitamin K (K_1 and K_2) do not cause DNA damage in HT-29 cells while the synthetic form of vitamin K (K_3 or menadione) does cause dose-dependent DNA damage.
Israels et al., 1987	*In Vivo*	Sister Chromatid Exchange	Inconclusive	Fetal sheep were given a 1 mg dose of vitamin K1 into the femoral vein via a catheter. The mean number of SCEs per metaphase increased from 3.94 + 0.15 at 15 minutes pre-injection to 5.38 + 0.23 at 24 hours post-injection. The increase in SCE was low but was stated to be statistically significant.
Israels et al., 1987	*In Vivo*	Sister Chromatid Exchange	Inconclusive	*In vitro* SCE induction was investigated in both fetal and adult sheep leukocytes incubated with vitamin K1. The doses tested ranged from 0.1 nM to 1 M. At 0.1 nM the number of SCEs in the adult cells (from the dam) was not significantly different from the solvent control. The number of SCEs in the fetal cells was significantly increased compared to the controls. The increase in SCEs in the adult cells became significant at 10 nM.
Edenharder et al., 1999	*In Vitro*	Ames Salmonella	Significant anti-mutagenic effect	Vitamins K1, K3, K4 and 1,4 naphthoquinone caused a concentration-related reduction in the mutagenicity of six heterocyclic amines in the Ames *Salmonella* assay.
Tikkanen et al., 1983	*In Vitro*	Ames Salmonella	Vitamin K_1 negative; positive	Vitamin K_1 was not mutagenic in the presence and absence of metabolic activation systems in TA98, TA 100 and TA2637 strains of salmonella. Menadione was mutagenic in strain TA2637.

D. Other Studies in Animal Models

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Several other studies with laboratory animals which primarily studied the nutritional efficacy of vitamin K_2 have some relevance to the safety review. In general, these studies were 2-8 weeks in duration and involved diets with varying levels of minerals or other vitamins. In these studies, the authors did not note any adverse effects attributable to vitamin K_2 (Tomiuga et al., 1994; Akiyama et al., 1999; Kobayashi et al., 2002; Iwamoto et al., 2005).

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E. Epidemiology Studies

Several retrospective studies have examined the relationship of dietary vitamin K and health. These studies have shown possible benefits in bone metabolism (Feskanich et al., 1999; Ikeda et al., 2006) and cardiovascular health (Geleijnse et al., 2004). No adverse effects were identified in these investigations that could be associated with vitamin K levels in the diet.

IARC (2000) reviewed the hypothesis that vitamin K therapy in infants may be associated with childhood cancer. A total of 7 epidemiology studies, and other information related to carcinogenicity, including mutagenicity studies were reviewed. Only 2 of the studies showed a possible association with increased incidence of cancer when given by the intramuscular route. None of the studies that followed administration by the oral route showed any association with increase of incidence of cancer. IARC concluded that vitamin K therapy in infants was not classifiable for carcinogenicity since the evidence was inadequate.

F. Clinical Studies on Effects of Vitamin K to Patients on Anticoagulant Therapy

Schurgers et al. (2004) studied the effect of vitamin K intake on the action of anticoagulants in healthy subjects. Twelve volunteers (6 men aged 26 - 30 and 6 women aged 25 - 31) were anticoagulated with acenocoumarol for 4 weeks in order to become stabilized. After this time, incremental doses of vitamin K supplements were administered daily for 7 days, starting with 50 μg of vitamin K supplement at week 1 (week 5 of the study) and ending with 500 μg of vitamin K supplement at week 7 (week 11 of the study). Blood samples were taken 4 days a week after an overnight fast. During the 2 weeks following the highest dose of vitamin K supplement (weeks 12 and 13), the participants experienced a wash-out period in which they did not receive any vitamin K treatments, but still remained on acenocoumarol to remove all traces of vitamin K from their systems. Four weeks following this period (weeks 14 through 17), the participants ingested meals rich in vitamin K once a week (on Mondays), beginning with spinach, and continuing with broccoli, curd cheese, and finally natto. Blood was collected prior to and after ingesting the meals and at 24 hour intervals during the subsequent week. Plasma concentrations of vitamin K, as well as vitamin K content of food items, were measured with liquid chromatography, and the amounts of circulating K-dependent coagulants (II, IV, IX, and X) were measured by coagulometer. The internal normalized ratio (INR), a coagulation value, was calculated using a pre-established formula. Results showed that there was a significant decrease in INR at 150 μg of vitamin K per day in women and 200 μg of vitamin K per day in men. Meals high in vitamin K provided little, if any, change. Ultimately, this studied demonstrated that supplements providing up to 100 μg/day of vitamin K do not significantly interfere with oral anticoagulant therapies.

Schurgers et al. (2007a) compared the absorption and efficacy of vitamin K_1 and menaquinone-7 (MK-7) in healthy volunteers aged 25-35. In the first study, 15 volunteers received 100 μg each of vitamin K_1 and MK-7. Blood was taken at baseline, 2, 4, 6, 8, 24, 48, 72, and 96 hours post intake. In the second study, 10 volunteers received 1 mg/L of vitamin K_1 and MK-7 in increasing doses of 50, 100, 150, 200, 250, 300, and 500 μg per day with a wash out period of 2 weeks between doses. Blood was taken at baseline, 4, and 24 hours post intake. In the third study, 18 volunteers received either 0.22 μmol/day of vitamin K_1 or MK-7 for 6 weeks. Blood was drawn in the morning on days 3, 7, 14, 21, 28,

35, and 42 in order to measure vitamin K and osteocalcin levels. In the fourth study, 12 volunteers were treated with acenocoumarol for about 3 weeks to stabilize them at an international normalized ratio (INR) value of 2. Upon stabilization, 110 nmol vitamin K_1 or 150 nmol MK-7 was taken every day for a week and then increased by 110 nmol or 150 nmol, respectively. Blood was taken at the end of each week for analysis. All blood samples from the four experiments were processed, and the serum levels of vitamin K_1 and MK-7 were analyzed by HPLC. Osteocalcin was quantified using molecular methods and anticoagulants were measured using a coagulometer. Results from the various studies showed that both vitamin K_1 and MK-7 levels peaked 4 hours after ingestion and rapidly declined thereafter. MK-7 displayed a longer half-life than vitamin K_1, as evidenced by increased serum levels at up to 5 days post ingestion, and was more effective at catalyzing osteocalcin and counteracting anticoagulants. Because of these factors, the authors concluded that doses greater than 50 µg/day may interfere with anticoagulant therapy treatment in a clinically relevant way.

G. Adverse Events and Contraindications

A case of persistent indurated erythema, at the site of intramuscular injection of a menatetrenone preparation, was reported in a 48-year-old woman diagnosed with liver cirrhosis (Yamamoto et al., 1982). The patient was subsequently subjected to intracutaneous testing with a vitamin K_2 preparation. Evaluated at 48 and 72 hours, positive reactions were observed and were accompanied by an acute flare-up of lesions on the buttocks as well as generalized pruritic-scattered erythema.

NattoPharma is not aware of any adverse reactions reported for ingestion of levels of MK-7 in the range of 500 - 1000 µg day from use of MenaQ7. Furthermore, reviews of the literature indicate no adverse reactions to consumption of natto in Japan at levels of up to 100 g/day or to MK-4 therapy for osteoporosis at levels of 45 - 90 mg/day.

VI. Discussion of Reviewed Information and GRAS Criteria

A. GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as

> "...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance."[10]

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance, and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that

> "...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food."

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following components:[11]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and

- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA[12] and the National Academy of Sciences.

The apparent imprecision of the terms "appreciable", "at the time" and "reasonable certainly" demonstrates that the FDA recognizes the impossibility of providing absolute safety, in this or any other area (Lu 1988; Renwick 1990).

B. Analysis of Safety Information Pertaining to MenaQ7 and Vitamin K

MenaQ7 is the ethanol extract of fermented soybeans and is subsequently formulated with sunflower oil. According to the product analysis, the extracted soybean components are tocopherol and menaquinones. All the raw materials used to formulate MenaQ7 products

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[10] See 21 CFR 170.3(i).
[11] See Footnote 1.
[12] Joint FAO/WHO Expert Committee on Food Additives.

listed in Section II.D, as well as extracted tocopherol, are considered to be GRAS. Other forms of *Bacillus* are considered to be GRAS as determined by FDA (FDA, 2001). The Panel, therefore, focused on the menaquinone components to ascertain the safety of MenaQ7.

The physiological necessity of vitamin K has become more understood in the last two decades. The function found first was as a coenzyme in the production of several cofactors in the blood clotting mechanism. More recently, studies suggest that vitamin K has been found to possibly have an important role in bone metabolism and cardiovascular health (EVM, 2003).

Vitamin K is a family of molecules known as naphthylquinones. Phylloquinone is known as vitamin K_1 and is a natural form found predominately in leafy green vegetables. Various forms of menaquinone constitute vitamin K_2. MenaQ7 is rich in the MK-7 form of vitamin K_2.

The importance of vitamin K_2 has been the subject of some scientific debate. In the most recent reports, expert bodies on nutrition in the US (NAS, 2000) and in the UK (EVM, 2003) did not give a firm opinion on whether or not vitamin K_2 is nutritionally important. Booth and Suttie (1998) speculated that, based on the unique tissue distribution of menaquinones in humans, they are likely to be nutritionally important. In Japan, many clinical studies demonstrate the improvements in patients with osteoporosis who are given large doses of MK-4. This therapy is now commonly used in Japan for osteoporosis. Other studies suggest several health benefits to consumers of natto, a rich source of MK-7 eaten regularly by many consumers in Japan. MK-4 and MK-7 have been found to function as a cofactor for the enzyme γ-glutamyl carboxylase, the same pathway modulated by phylloquinone. It is the opinion of the Panel that, although the absorption and distribution of all forms of vitamin K are not totally understood, there is sufficient evidence that forms of vitamin K_2 are absorbed, albeit slowly at normal dietary levels, and they contribute to maintaining function of vitamin K-reliant pathways.

Despite the uncertainty about the nutritional importance of vitamin K_2, the Panel agrees that there is no concern about the safety of the closely related vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinones) chemical structures for the healthy adult population at the exposure levels under consideration.

NAS (2000) has indicated that the daily recommended intake (DRI) for vitamin K ranges according to age and population groups from 75 -120 µg/day (for a 60 kg adult) and 30 - 75 µg/day for children, and 2.0 - 2.5 µg/day for infants. NAS reported documented consumption levels of 340 - 370 µg/day (5.7 - 6.2 µg/kg for a 60 kg adult). There were no adverse effects associated with this level of consumption. NAS did not set an upper limit (UL) for vitamin K consumption. EVM (2003) indicated that adverse effects would not be expected with doses as high as 1 mg/day.

Some researchers have suggested that even higher levels are needed to prevent osteoporosis and to maintain cardiovascular health. The Council for Responsible Nutrition has set an ULS (Upper Level for Supplements) at 10 mg/day (Hancock, 2004). Vermeer et al. (2003) states that daily intakes of between 200 and 500 µg/day of dietary vitamin K may be required for optimal carboxylation of extrahepatic GLA-proteins, which may have benefits for primary or secondary disease prevention. In a separate publication, Vermeer (2003b) recommended that there would be better public health benefits if a greater

proportion of supplements contained vitamin K_2 forms rather than vitamin K_1 forms and noted that natto is the richest source of vitamin K_2.

It is important to note that, unlike other forms of fat-soluble vitamins, vitamin K is not stored very well in human tissues, and appreciable amounts are lost in the urine and feces on a daily basis (Wildman and Medeiros, 2000).

IARC (2000) reviewed the hypothesis that vitamin K therapy in infants may be associated with childhood cancer. A total of 7 epidemiology studies, and other information related to carcinogenicity, including mutagenicity studies, were reviewed. Only 2 of the studies showed a possible association with increased incidence of cancer when given by the intramuscular route. None of the studies that followed administration by the oral route showed any association with increase of incidence of cancer. IARC concluded that vitamin K therapy was not classifiable for carcinogenicity based on existing evidence.

The no effect levels found in the two longest term, i.e., one year, toxicology studies conducted on MK-4 were 20 mg/kg in rats (Hosakawa et al., 1995) and 200 mg/kg in dogs (Vanatta et al., 1995). These no effect levels are several orders of magnitude higher than the most exaggerated consumption level predicted for total vitamin K.

High doses of MK-4 have been well tolerated in several clinical studies. A single 24-week was conducted with doses of 90 mg/day. Many other studies were done using a dose of 45 mg/day most notably for durations of six months (Somekawa et al., 1999) and for two years in two studies (Iwamoto et al., 2005; Sato et al., 2005). It is common for osteoporosis patients in Japan to be prescribed to take 45 - 90 mg/day of MK-4. This therapeutic regimen has been in place for at least a decade without report of adverse effects (Orimo et al., 1998). Several studies have been done with MK-7. Up to 3 servings per week of natto (equivalent to 525 µg/wk of MK-7) were well tolerated in a one year study (Katsuyama et al., 2004).

By considering the composite studies cited, the Panel concluded that all forms of vitamin K have a low order of toxicological potential. To be sure, it was noted that the recommended dietary intake is several orders of magnitude lower than doses considered in the cited studies. Comparison of the chemical structures of MK-7 to MK-4 and phylloquinone provided further support for the safety conclusion. As previously reported, MK-4 differs in structure from phylloquinone only by the presence of three unsaturated carbon-carbon bonds in the side chain. MK-7 has three additional units on the side chain, and it is not likely to more toxic than MK-4. Much clinical data on MK-4 indicate that it is safe at doses of 45 mg/day and higher. The maximum daily intake of MK-7 from the designated food uses of MenaQ7 proposed by NattoPharma is 50 µg/day.

The Panel has considered the evidence on the mutagenicity and genotoxicity of various forms of vitamin K but is more influenced by the several epidemiology studies on cancer incidence and the review by IARC which indicates that there is no indication of a cancer risk from vitamin K at the low levels required in the diet.

The Panel has considered the potential interference of increased dietary vitamin K in patients on anticoagulant therapy. Vitamin K therapy is useful in treating patients where the anticoagulant therapy has resulted in blood levels which are too high. Vitamin K_2 may also prevent the side effect of anticoagulant therapy of calcification of blood vessel walls. Still there is the possibility that too much vitamin K may counteract the benefits of anticoagulant therapy. The study by Schurgers et al. (2004) concluded that vitamin K_1 at dietary levels up

to 100 µg/day would not interfere with anticoagulant therapy. The same group concluded in a subsequent study (Schurgers et al., 2007a) that, because of the greater half life of MK-7, doses greater than 50 µg/day should be avoided by patients on anticoagulant therapy.

The Panel is of the opinion that 50 µg/day of MK-7 is rather conservative. FDA considered the need for a warning label directed at these patients on vitamin K-enriched foods containing approximately 40 µg/serving of phylloquinone and decided that requiring a warning label was not necessary.[13] These patients are actively monitored for blood levels of anticoagulants, and physicians can readily adjust their dosages and diets.

The Panel concluded that MK-7 is safe at a level of 1 mg per day or more for healthy adults. Because of the possible interference with anticoagulant therapy, the Panel recommends a conservative consumer exposure to assure that consumption does not consistently exceed 50 µg/day. Based on the desire of NattoPharma to supply 10 µg per serving in dairy products and butter and margarine substitutes, and by considering the USDA food intake survey data referenced in Section IV.B., the Panel is satisfied that consumers are not likely to consume more than 5 servings of these foods per day. The Panel agrees that the proposed limitation to use in dairy products and butter and margarine substitutes will adequately limit consumption of MK-7 which obviates the need for a warning label for consumers on anticoagulant therapy.

If a consumer were to eat on a chronic basis a combination of foods that are naturally high in vitamin K, along with foods fortified with MenaQ7, as well as ingestion of a dietary supplement containing vitamin K, it is difficult to imagine that the total vitamin K consumption would exceed 300 µg/day (5.0 µg/kg for 60 kg adult). Even so, this level of consumption would remain within the range which NAS considered to be safe. In view of the variability of natural vitamin K in the diet, it is not likely that levels this high could be sustained over long periods. The Panel agrees that levels of MK-7 up to 100 µg/day would be safe even with patients on anticoagulant therapy. However, NattoPharma has adopted the prudent practice of limiting the addition to foods as noted elsewhere to keep consumption levels at or below 50 µg/day.

The Panel has reviewed the other ingredients used in the manufacture and formulation of MenaQ7. As indicated in Table 4, all ingredient used in the formulation are adequately covered by applicable FDA clearances. The Panel concludes that consumption of these ingredients at the levels present in a total of 5 servings of dairy products and butter and margarine substitutes combined is safe.

C. Common Knowledge Element of GRAS Review

As discussed in Section VI.A, the two components comprising the common knowledge requirement must be satisfied for any GRAS determination.

Regarding the first element, the Panel recognizes that the vast majority of the studies relied upon in the GRAS evaluation has been published and is available in the open scientific literature. The information on the definition of natto, its common use in Japan and other information on fermented soybean products is also readily available on the internet and other public sources.

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[13] See 68 FR 43692, 2003.

The Panel recognizes that consensus exists in the scientific community on the importance of vitamin K_2 in maintaining good health. More importantly for the GRAS designation is the convergence of scientific opinions on the safety aspects of vitamin K and its principal components, vitamin K_1, MK-4, and MK-7 with supportive positions having been proclaimed by NAS, EVM, and IARC. The Panel cited several publications where scientists made favorable comments on the need for vitamin K_2 in the diet (Hollander et al., 1976; Booth and Suttie, 1995; Vermeer et al., 2003; Johnson, 2005; Schurgers et al., 2007a). The review by the well-respected IARC committee (2000) adds substantially to the scientific consensus that natural forms of vitamin K are safe and do not constitute a carcinogenic concern.

VII. Conclusions[14]

Expert panels of scientists have indicated that no upper limit is needed for vitamin K consumption, and other experts in the field have recommended an increased consumption of vitamin K_2 to enhance bone and cardiovascular health. Ample scientific evidence exists on MenaQ7 and MK-7 to conclude that the anticipated human food use as a nutrient supplement in dairy products and butter and margarine substitutes when consumed at daily levels up to 50 µg/day by adults is generally recognized as safe.

The preponderance of the scientific evidence has established that FDA's food ingredient safety standard of "reasonable certainty of no harm under the intended conditions of use" has been met for menaquinone-7 and MenaQ7; consequently, MenaQ7 has attained GRAS status and is exempt from premarket approval requirements.

In conclusion, adult human exposure to MenaQ7 as an ingredient added to dairy products and butter and margarine substitutes at levels up to 10 mg per serving (to provide an equivalent of up to 10 µg of MK-7 per serving) is generally recognized as safe (GRAS). This conclusion requires that MenaQ7 is produced in accordance with Good Manufacturing Practices and that the finished product meets appropriate food grade specifications.

Richard C. Kraska, Ph.D., DABT	February 22, 2008 Date
Robert S. McQuate, Ph.D.	February 22, 2008 Date
Stanley T. Omaye, Ph.D.	February 22, 2008 Date

[14] Appendix B contains the educational and professional backgrounds for Richard C. Kraska, Ph.D., DABT, Robert S. McQuate, Ph.D., and Stanley T. Omaye, Ph.D. Each has extensive technical background in the evaluation of food ingredient safety. Drs. Kraska and McQuate each worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this are in the private sector. Dr. Omaye is a professor of nutrition at the University of Nevada, Reno and has published extensively on the nutritional and toxicological aspects of food ingredients. Dr. Kraska served as Chair of the Panel.

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Appendix A

Product Specifications

MenaQ7 Oil M 1500

MenaQ7 Powder P 1000

Certificate of Analysis

Certification for Non-GMO Status

MenaQ7 Oil M 1500

MenaQ7 Powder P 1000

PRODUCT: MenaQ7 OIL M 1500

PRODUCT INFORMATION DATASHEET

NattoPharma®

FOOD SUPPLEMENT/DIETARY & FORTIFIED FOOD VITAMIN INGREDIENT

SECTION 1- PRODUCT IDENTITY AND FORM

Product name	MenaQ7 OIL M 1500
Chemical name	Menaquinone-7
Common name	Vitamin K2
Vitamin concentration	1500 ppm
Product form	Sunflower Oil formulation
General product information	Natural Vitamin K2 extracted from *Bacillus subtilis* fermented soybeans (natto)
Scope of product information	**Product evaluation documents**

SECTION 2- MANUFACTURING AND QUALITY ASSURANCE/QUALITY CONTROL INFORMATION

Name and address of manufacturing site	J-Oil Mills Inc, Japan
Manufacturing	Fermentation, sterilization, extraction, filtration, evaporation, filtration
GMP compliance production	ISO 9001, ISO 14001
Other QA/QC systems	Production according to Japanese Food Sanitation Law. Inspection by Japan Public Health Institute
Non-GMO ingredients	Using only non-GMO ingredients under identity preserved (IP) conditions
Safety of *Bacillus subtilis*	*Bacillus subtilis* is a bacterium which is used extensively for many industrial products for human uses with GRAS status (http://vm.cfsan.fda.gov/~rdb/opa-gras.html; File://K:\Literature\2004\Schallmey et al 2004-1.pdf) and in EU (J Oficiale de la Republic Francaise; Dec. 2, 2006, page 169: Decrets, arrêtes, circulaires)
Safety, health and environment	Concept followed (incl ISO 14001), QA/QC in line with HACCP procedures
Known or potential impurities	No ink, grease, or glass used during production. Double 10 micrometers filters used before and after packaging eliminating the risk for metal or foreign bodies
Irradiation	No irradiation used during production
Traceability system for product identity	In place
Certificate of analysis	Batch specific
Material Safety Data Sheet (MSDS)	MSDS attached
Last revision of QA/QC systems	January 2008

SECTION 3 – PRODUCT DEVELOPMENT & REGULATORY AFFAIRS - DISTRIBUTORS

Name of product developer and marketing, and IPR holder	NattoPharma ASA Lysaker Torg 5 P.O.Box 397 1326 Lysaker, Norway Telephone: + 47 22 55 36 30 Fax: +47 22 55 36 31 info@nattopharma.com www.nattopharma.com
Name of company responsible for nutraceutical regulatory affairs	NattoPharma ASA Lysaker Torg 5 P.O.Box 397

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	1326 Lysaker, Norway Telephone: + 47 22 55 36 30 Fax: +47 22 55 36 31 info@nattopharma.com www.nattopharma.com
European Union: **Regulatory status EU Food supplement**	Pending amended to ANNEX II in Commission Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 relating to food supplements
European Union: **Regulatory status Fortified Food**	Pending amendment to ANNEX II in Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods
European Union: **Regulatory status Novel Food**	Pending Novel Food status according to Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel food and novel food ingredients.
European Union: **Regulatory status food for specific nutritional purposes (PARNUTS)**	Pending amendment to Commission Directive 2001/15/EC of 15 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional purposes
EU legislation	
Regulation (EC) No 1829/2003 of European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (GMO)	MenaQ7 product complies
Council Directive 1999/EC and 1999/3/EC of 22 February 1999 on the approximation of the laws of the Member States concerning food and food ingredients treated with ionizing radiation	MenaQ7 product complies
Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuff (Heavy metals; Toxins)	MenaQ7 product complies
Council Directive 76/895/EEC of 23 November 1976 relating to the fixing of maximum levels for pesticides in and on fruit and vegetables – with added directives CD 86/362/EEC of 24 July 1986; CD 86/363/EEC of 24 July 1986; CD 90/642/EEC of 27 November 1990; CD 2007/27/EC of 15 May 2007	MenaQ7 product complies
Council Directive 88/344/EEC of 13 June 1988 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients	MenaQ7 product complies
North America	
United States: Food/dietary supplement:	Freely sold
United States: Fortified food **Generally Recognized As Safe (GRAS) with FDA**	Self -affirmed GRAS for dairy products January 2008 FDA filing February 2008
Canada: Natural Health Products Directorate; Bureau of Product Review and Assessment	Product license obtained 2007.
Exclusive distributor North America	P L Thomas Inc, 119 Headquarters Plaza, Morristown, NJ 07960 USA www.plthomas.com Contact: eric@plthomas.com Telephone: +1 973 984 0900 , ex 215

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SECTION 4 – PRODUCT SPECIFICATIONS: List of product ingredients and origin-see certificates

Name ingredient	CAS #	Composition	Origin	GMO status
Menaquinone-7 (Vegetable)	2124-57-4	Not less than 1500 ppm	Produced by *B. subtilis natto* (Japan & USA: soybean and corn)	Non GMO for *B. subtilis natto* and for bacterial substrates soybeans and corn starch
Menaquinone-6 (Vegetable)	84-81-1	Not more than 100 ppm	Produced by *B. subtilis natto*	Non GMO for *B.subtilis natto* and for bacterial substrates soybeans and corn starch
Sunflower oil (Vegetable)	8001-21-6	>97 %	From sunflower plant seeds (Helianthus annuus- Japan)	Non GMO sunflower plants
Product certificate of analysis	Separate document following each batch			
Control of impurities and batch to batch variations	Vitamin K2 content is certified using accredited HPLC analytical method			
The product does not contain	*Bacillus subtilis*, Hormones, Nattokinase, Gluten, and is free from BSE/TSE			

SECTION 5- PRODUCT INFORMATION HEAVY METALS, TOXINS AND PESTICIDES

Compound	Analytical results	Method of analysis [1]
Arsenic as As_2O_3	Contains < 0,5 ppm	AOAC 952.13. Silver Diethyldithiocarbamate method
Cadmium- Cd	Contains < 0,5 ppm	AOAC 952.13 Atomic Absorption method
Lead- Pb	Contains < 0,5 ppm	AOAC 973.35 Atomic Absorption method
Total Mercury - Hg	Contains < 0,1 ppm	AOAC 971.21 Atomic Absorption method
Chromium- Cr	Not determined	APHA Diphenylcarbazid colorimetric method
Mycotoxins Aflatoxin B1+B2+G1+G2	Contains less than 10 parts per billion (ppb)	AOAC 990.33 HPLC method
Specific toxins: Dioxins	Contains less than 0.75 pg WHO-PCDD/F-TEQ/g [2]	Gas Chromatography - Mass Spectrometric methods (GS-MS method)
Pesticides 350 different pesticides analyzed annually.	All < 0,01 ppm	Gas Chromatography - Mass Spectrometric methods (GS-MS method)

1: Association of Official Analytical Chemistry (http //www.aoac.org)
2: Limits for dioxins are measured in WHO-PCDD/F-TEQ, where PCDD means Poly Chlorinated Dibenzo-p-Dioxins, the common name for the group of dioxins, and where F- TEQ means Fixed Toxic Equivalents decided by WHO.

SECTION 6 – PRODUCT INFORMATION ALLERGENS

MenaQ7 OIL M-1500 Allergen list below	No animal allergens are used in the production, neither any of the following big allergens: Milk (casein, lactose); eggs; nuts (peanuts, tree nuts); seafood; shellfish; wheat (gluten).
Soy protein	Soy protein extracts and Corn starch are used as

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substrate for *B. subtilis natto* are removed during the final extraction, purification and filtration processes. Sum of soy and corn proteins are analyzed by fluorescence spectrophotometric method and are less than 5 ppm.
After extensive validation of the production method for MenaQ7 Oil M-1500 soy protein content is controlled by annual QC/QA testing

Raw material/allergen	Presence in MenaQ7 product	Presence on production line	Presence in factory	Measures implemented to reduce contamination
Nuts & nut oils				
Almonds *(Amygdalus communis)*	No	No	No	Yes
Brazil nut *(Bertholletia excels)*	No	No	No	Yes
Cashew nut *(Anacadium ccidentale)*	No	No	No	Yes
Hazel nut *(Coryllus avellana)*	No	No	No	Yes
Hazel nut oil	No	No	No	Yes
Peanut	No	No	No	Yes
Refined peanut oil Fediol	No	No	No	Yes
Refined peanut oil no Fediol	No	No	No	Yes
Pecan nut *(Carya Illinoi)*	No	No	No	Yes
Pistachio nut *(Pistcia vera)*	No	No	No	Yes
Sesam	No	No	No	Yes
Sesam oil	No	No	No	Yes
Queensland or Macadamia nut	No	No	No	Yes
Walnut *(Juglans regal)*	No	No	No	Yes
Walnut oil	No	No	No	Yes
Milk components				
Milk	No	No	No	Yes
Lactose	No	No	No	Yes
Casein or Caseinate	No	No	No	Yes
Concentrated milk minerals	No	No	No	Yes
Flour & Grains				
Barley	No	No	No	Yes
Gluten	No	No	No	Yes
Kamut	No	No	No	Yes
Oat	No	No	No	Yes
Rye	No	No	No	Yes
Spelt	No	No	No	Yes
Wheat	No	No	No	Yes
Whey	No	No	No	Yes
Soya products	No	No	No	Yes
Soya	(Yes)	Yes	Yes	Yes
Soya lechtin	No	No	No	Yes
Egg and egg				

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components				
Egg	No	No	No	Yes
Egg lyzozyme	No	No	No	Yes
Fish and shellfish				
Crustacea	No	No	No	Yes
Fish	No	No	No	Yes
Fish gelatin	No	No	No	Yes
Fish oil	No	No	No	Yes
Mollusc	No	No	No	Yes
Vegetables and spices				
Celery	No	No	No	Yes
Mustard	No	No	No	Yes
White lupin	No	No	No	Yes
Other substances				
Sulfites and anhydrous sulfurous	No	No	No	Yes
Tree and grass pollen	Not applicable			

SECTION 7 – PRODUCT INFORMATION ORGANIC SOLVENTS

Chemical	Presence in final product
Ethanol	Used in early step in production process but later removed completely by prolonged distillation at 170°C

SECTION 8 – PRODUCT INFORMATION MOULD, YEAST & BACTERIA

Species	Analytical method	Product specifications: Colony forming units/sampling weight	Typical test results
Total aerobic count	Method of AOAC [1]No 966.23	Not more than 300/g	Less than 300/g
Mould	Method of AOAC No 997.07	Not more than30/g	Less than 30/g
Yeast	Method of AOAC No 997.07	Not more than30/g	Less than 30/g
E. coli	Method of AOAC No 991.14	Less than 10/2,2g	Absent/2,2 grams
Salmonella sp	Method of AOAC No 967.25	Absence in 10 g	Absent/ 10 grams
Staphylococcus aureus	Method of Japan Food Hygiene Association	Absence in 0,01 g	Absent/0,01 grams
Other bacteria, including *Bacillus subtilis*	Not applicable		

[1]: Association of Official Analytical Chemistry (http://www aoac org)

SECTION 9 – PRODUCT STORAGE, SHELF-LIFE & LABELLING INFORMATION

Product name	Packaging size and labelling
MenaQ7 OIL M 1500	1 kg Oil & 5 kg Oil in cans
Packaging material	Epoxy resin-coated cans
Packaging waste material handling	Please see national/local regulations for packaging waste material handling
Transport conditions	Transport as tempered goods

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Storage conditions	Recommended storage temperature is 10°- 15° Celsius and protected from UV light, lamp and direct sunlight. The product is very light sensitive and exposure may deteriorate K2 activity considerably
Expiration date	Shelf life 36 months from date of production
Batch/Lot numbering system	Year/Month/Day- serial number batch that date
Nutritional/Supplement facts	Nutritional profile inserted below
Required finished product label statement	The conditions for use of MenaQ7 logo is outlined in customers contract with NattoPharma ASA
Recommended warnings on labels	Patients on anti-coagulant therapy must consult their physician
Other (usage, cautionary, safety or other warning statements)	Vitamin K is safe for human consumption, and presently no internationally upper tolerable level is established. Information on MenaQ7 bioavailability; MenaQ7 recommended dosage; MenaQ7 and safety;, MenaQ7 and effects on the coagulation system is available as NattoPharma ASA partner
Material Safety Data Sheet (MSDS)	MSDS attached

MenaQ7™
Natural Vitamin K2

SECTION 10 – NUTRITIONAL PROFILE

NA: Not applicable, nd: Not detected

	Unit	Detection limit
Total amount of calories pr 100g	**900 kcal/100 g**	
Calories from fat pr 100g	**900 kcal/100 g**	
Total amount of protein	**0%**	
Total fat –	100%/100g	
including specified amounts of:		
Saturated	8,0%	
Poly unsatured	5,2%	
Mono unsatured	82,6%	
Trans Fatty Acids	0,19%	
Phospholipids	nd	10mg/100g
Milk fat	NA	
Cholesterol	nd	1 mg/100g
Total Carbohydrates –	0%	
including specified amounts of:		
Dietary fibre	nd	0,5 g/100g
Soluble fibre	NA	
Insoluble fibre	NA	
Sugars	NA	
Lactose	NA	
Saccharose	NA	
Glucose	NA	
Glucose syrup	NA	
Polyols	NA	
Sugar alcohol	NA	
Other carbohydrates	NA	
Minerals		
Potassium (K)	nd	1mg/100g
Sodium (Na)	nd	1mg/100g
Calcium (Ca)	nd	1mg/100g
Phosphorous (Ph)	nd	1mg/100g
Magnesium (Mg)	nd	0,1mg/100g

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Iron (Fe)	nd	0,1mg/100g
Vitamins	g/100g	
Vitamin A	nd	
Vitamin C	nd	
Vitamin E	38mg/100g	
Vitamin D	nd	
Other components		
Alcohol (Ethanol)	0%	
Caffeine	0%	
Theobromine	0%	
Phenolics	0%	
Moisture	0%	
Ashes	0%	
Hormones	0%	

SECTION 11: PHYSICAL & CHEMICAL CHARACTERIZATION

Appearance	Clear or hazy/cloudy, odor less oil solution
Water solubility	Not soluble
Stability higher temperatures	Sustains 100° C for up to 24 hours
Light exposure	Must be protected from UV and lamplight
pH	Not applicable
Particle size	Not applicable

SECTION 12: SUITABILITY FOR HUMAN USE

Suitable for human foods	Yes
Suitable for vegetarians	Product does not contain any material of animal origin
Suitable for lacto-ovo-vegetarians	Product does not contain any material of animal origin
Suitable for celiac patients	Product does not contain any gluten
Certified as organic	No
Certified as kosher food	Yes
Certified as halal food	No
Animal testing	Product is not tested in animals

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PRODUCT: MenaQ7 POWDER P 1000

PRODUCT INFORMATION DATASHEET

NattoPharma®

FOOD SUPPLEMENT/DIETARY & FORTIFIED FOOD VITAMIN INGREDIENT

SECTION 1- PRODUCT IDENTITY AND FORM	
Product name	MenaQ7 POWDER P 1000
Chemical name	Menaquinone-7
Common name	Vitamin K2
Vitamin concentration	1000 ppm
Product form	Powder formulation: K2 from stock Sunflower Oil dispensed in dextrin powder formulation
General product information	Natural Vitamin K2 extracted from *Bacillus subtilis* fermented soybeans (natto)
Scope of product information	**Product evaluation documents**

SECTION 2- MANUFACTURING AND QUALITY ASSURANCE/QUALITY CONTROL INFORMATION	
Name and address of manufacturing site	J-Oil Mills Inc, Japan
Manufacturing	Fermentation, sterilization, extraction, filtration, evaporisation, filtration
GMP compliance production	ISO 9001, ISO 14001
Other QA/QC systems	Production according to Japanese Food Sanitation Law. Inspection by Japan Public Health Institute
Non-GMO ingredients	Using only non-GMO ingredients under identity preserved (IP) conditions
Safety of *Bacillus subtilis*	*Bacillus subtilis* is a bacterium which is used extensively for many industrial products for human uses with GRAS status (http://vm.cfsan.fda.gov/~rdb/opa-gras.html; File://K:\Literature\2004\Schallmey et al 2004-1.pdf) and in EU (J Oficiale de la Republic Francaise, Dec. 2, 2006, page 169: Decrets, arrêtes, circulaires)
Safety, health and environment	Concept followed (incl ISO 14001), QA/QC in line with HACCP procedures
Known or potential impurities	No ink, grease, or glass used during production. Double 10 micrometers filters used before and after packaging eliminating the risk for metal or foreign bodies
Irradiation	No irradiation used during production
Traceability system for product identity	In place
Certificate of analysis	Batch specific
Material Safety Data Sheet (MSDS)	MSDS is worked out and follows this product information sheet
Last revision of QA/QC systems	January 2008

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SECTION 3 – PRODUCT DEVELOPMENT & REGULATORY AFFAIRS - DISTRIBUTORS	
Name of product developer and marketing, and IPR holder	NattoPharma ASA Lysaker Torg 5 P.O.Box 397 1326 Lysaker, Norway Telephone: + 47 22 55 36 30 Fax: +47 22 55 36 31 info@nattopharma.com www.nattopharma.com

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Name of company responsible for nutraceutical regulatory affairs	NattoPharma ASA Lysaker Torg 5 P.O.Box 397 1326 Lysaker, Norway Telephone: + 47 22 55 36 30 Fax: +47 22 55 36 31 info@nattopharma.com www.nattopharma.com
European Union: **Regulatory status EU Food supplement**	Pending amended to ANNEX II in Commission Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 relating to food supplements
European Union: **Regulatory status Fortified Food**	Pending amendment to ANNEX II in Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods
European Union: **Regulatory status Novel Food**	Pending Novel Food status according to Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel food and novel food ingredients.
European Union: **Regulatory status food for specific nutritional purposes (PARNUTS)**	Pending amendment to Commission Directive 2001/15/EC of 15 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional purposes
EU legislation	
Regulation (EC) No 1829/2003 of European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (GMO)	MenaQ7 product complies
Council Directive 1999/EC and 1999/3/EC of 22 February 1999 on the approximation of the laws of the Member States concerning food and food ingredients treated with ionizing radiation	MenaQ7 product complies
Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuff (Heavy metals; Toxins)	MenaQ7 product complies
Council Directive 76/895/EEC of 23 November 1976 relating to the fixing of maximum levels for pesticides in and on fruit and vegetables -- with added directives CD 86/362/EEC of 24 July 1986; CD 86/363/EEC of 24 July 1986; CD 90/642/EEC of 27 November 1990; CD 2007/27/EC of 15 May 2007	MenaQ7 product complies
Council Directive 88/344/EEC of 13 June 1988 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients	MenaQ7 product complies
North America	
United States: Food/dietary supplement:	Freely sold
United States: Fortified food **Generally Recognized As Safe (GRAS) with FDA**	Self -affirmed GRAS for dairy products January 2008 FDA filing February 2008
Canada: Natural Health Products Directorate; Bureau of Product Review and Assessment	Product license obtained 2007.
Exclusive distributor North America	P L Thomas Inc, 119 Headquarters Plaza, Morristown, NJ 07960 USA www.plthomas.com Contact: eric@plthomas.com Telephone: +1 973 984 0900 , ex 215

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SECTION 4 – PRODUCT SPECIFICATIONS: List of product ingredients and origin/function- see certificates				
Name ingredient (Origin)	CAS #	Composition	Origin/Function	GMO status
Menaquinone-7 (Vegetable)	2124-57-4	Not less than 1000 ppm	Produced by *B. subtilis natto* (Japan& USA: Soybeans and corn)	Non GMO for *B. subtilis natto* and for bacterial substrates soybeans and corn starch
Menaquinone-6 (Vegetable)	84-81-1	Not more than 50 ppm	Produced by *B. subtilis natto*	Non GMO for *B.subtilis natto* and for bacterial substrates soybeans and corn starch
Dextrin (Vegetable)	NA	Ca 60- 65 %	Delivery vehicle for K2.(Japan)	Derived from Non GMO starch
Sunflower oil (Vegetable)	8001-21-6	Ca 20 -25 %	Delivery vehicle for K2. From sunflower plant seeds (Helianthus annuus- from Japan)	Non GMO sunflower plants
Sodium casein (Animal)	NA	Ca 10%	Powderization material. From milk from Danish cows. Free from BSE (Denmark)	GMO status not relevant for casein proteins
Starch sodium octenylsuccinate (Vegetable)	NA	Ca 2%	Powderization material (USA)	Derived from Non GMO starch
Glycerol Fatty Acid Ester (Vegetable)	NA	Ca 1%	Emulsifier (Japan)	GMO status not relevant
L-Ascorbyl Palmitate (Vegetable)	NA	Ca 0,5%	Antioxidant (Denmark)	GMO status not relevant
Aqueous extract of rosemary (Vegetable)	NA	Ca 0,1%	Stability improver (Japan)	GMO status not relevant
Product certificate of analysis	Separate document following each batch			
Control of impurities and batch to batch variations MenaQ7 content	Vitamin K2 content is certified using accredited HPLC analytical method			
The product does not contain	*Bacillus subtilis*, Hormones, Nattokinase, Gluten; Sulfites, and is free from BSE/TSE			

NA: Not Applicable

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SECTION 5- PRODUCT INFORMATION HEAVY METALS, TOXINS AND PESTICIDES		
Compound	Analytical results	Method of analysis[1]
Arsenic as As_2O_3	Contains < 0,5 ppm	AOAC 952.13. Silver Diethyldithiocarbamate method
Cadmium- Cd	Contains < 0,5 ppm	AOAC 952.13 Atomic Absorption method
Lead- Pb	Contains < 0,5 ppm	AOAC 973.35 Atomic Absorption

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		method
Total Mercury - Hg	Contains < 0,1 ppm	AOAC 971.21 Atomic Absorption method
Chromium- Cr	Not determined	APHA Diphenylcarbazid colorimetric method
Mycotoxins **Aflatoxin B1+B2+G1+G2**	Contains less than 10 parts per billion (ppb)	AOAC 990.33 HPLC method
Specific toxins: **Dioxins**	Contains less than 0.75 pg WHO-PCDD/F-TEQ/g[2]	Gas Chromatography - Mass Spectrometric methods (GS-MS method)
Pesticides **350 different pesticides analyzed annually**	All < 0,01 ppm	Gas Chromatography - Mass Spectrometric methods (GS-MS method)

1: AOAC: Association of Official Analytical Chemistry (http://www.aoac.org)
2 Limits for dioxins are measured in WHO-PCDD/F-TEQ, where PCDD means Poly Chlorinated Dibenzo-p-Dioxins, the common name for the group of dioxins, and where F- TEQ means Fixed Toxic Equivalents decided by WHO

SECTION 6 – PRODUCT INFORMATION ALLERGENS AND COMPONENTS CAUSING INTOLERANCES	
MenaQ7 POWDER P 1000 **Relevant allergens/intolerance components** **Allergen list below**	Content of allergenic material in products supplemented with 45 µg MenaQ7 POWDER P1000 (recommended daily dose)
Milk : Allergens due to casein content	Ca 4,5 milligrams of casein/dose The content of casein in a 250 ml glass of milk is approximately 2,5-3,0 g.
Soy proteins	Soy protein extract that is used as substrate for *B. subtilis natto* is removed during the final extraction, purification and filtration processes. Proteins are analyzed by fluorescence spectrophotometric method and are less than 5 ppm; This is equivalent to <225nanogram per dose MenaQ7. After extensive validation of the production method for MenaQ7 Powder P-1000 soy protein content is controlled by annual QC/QA testing
Corn	Starch sodium octenylsuccinate is derived from corn, and may contain traces of corn protein from 1-100ppm; This is equivalent to 45 nanogram – 4.5 µg per dose MenaQ7; values are calculated based upon raw material information
Lactose	< 9 µg of lactose per dose MenaQ7. In a 250 ml glass of milk there is approximately 12 grams of lactose.

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Raw material/allergen	Presence in MenaQ7 product	Presence on production line	Presence in factory	Measures implemented to reduce contamination
Nuts & nut oils				
Almonds *(Amyg dalus communis)*	No	No	No	Yes
Brazil nut ***(Bertholletia excels)***	No	No	No	Yes
Cashew nut *(Anacadium occidentale)*	No	No	No	Yes
Hazel nut ***(Corylus avellana)***	No	No	No	Yes
Hazel nut oil	No	No	No	Yes
Peanut	No	No	No	Yes
Refined peanut oil Fediol	No	No	No	Yes

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Refined peanut oil no Fediol	No	No	No	Yes
Pecan nut *(Carya Illinoi)*	No	No	No	Yes
Pistachio nut *(Pistcia vera)*	No	No	No	Yes
Sesam	No	No	No	Yes
Sesam oil	No	No	No	Yes
Queensland or Macadamia nut	No	No	No	Yes
Walnut *(Juglans regal)*	No	No	No	Yes
Walnut oil	No	No	No	Yes
Milk components	No	No	No	Yes
Milk	Yes	Yes	Yes	Yes
Lactose	Yes	Yes	Yes	Yes
Casein	Yes	Yes	Yes	Yes
Caseinate	Yes	Yes	Yes	Yes
Concentrated milk minerals	No	No	No	Yes
Flour & Grains	No	No	No	Yes
Barley	No	No	No	Yes
Corn- see comments	*(Yes)*	Yes	Yes	Yes
Gluten	No	No	No	Yes
Kamut	No	No	No	Yes
Oat	No	No	No	Yes
Rye	No	No	No	Yes
Spelt	No	No	No	Yes
Wheat	No	No	No	Yes
Whey	No	No	No	Yes
Soya products	No	No	No	Yes
Soya- see comments	*(Yes)*	Yes	Yes	Yes
Soya lechtin	No	No	No	Yes
Egg and egg components	No	No	No	Yes
Egg	No	No	No	Yes
Egg lyzozyme	No	No	No	Yes
Fish and shellfish				
Crustacea	No	No	No	Yes
Fish	No	No	No	Yes
Fish gelatin	No	No	No	Yes
Fish oil	No	No	No	Yes
Mollusc	No	No	No	Yes
Vegetables and spices	No	No	No	Yes
Celery	No	No	No	Yes
Mustard	No	No	No	Yes
White lupin	No	No	No	Yes
	No	No	No	Yes
Other substances	No	No	No	Yes
Sulfites and anhydrous sulfurous	No	No	No	Yes
Tree and grass pollen	Not applicable			

SECTION 7 - PRODUCT INFORMATION ORGANIC SOLVENTS	
Chemical	Presence in final product
Ethanol	Used in early step in production process but later removed completely by prolonged distillation at 170°C

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SECTION 8 – PRODUCT INFORMATION MOULD, YEAST & BACTERIA			
Species	Analytical method	Product specifications: Colony forming units/sampling weight	Typical test results
Total aerobic count	Method of AOAC No	Not more than 3000/g	Less than 3000/g

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	966.23		
Mould	Method of AOAC No 997.07	Not more than300/g	Less than 300/g
Yeast	Method of AOAC No 997.07	Not more than300/g	Less than 300/g
E. coli	Method of AOAC No 991.14	Less than 10/2,2g	Absent/2,2 grams
Salmonella sp	Method of AOAC No 967.25	Absent in 10 grams	Absent in 10 grams
Staphylococcus aureus	Method of Japan Food Association	Absent in 0,01 g	Absent in 0,01 g
Enterobacter spp	Method of AOAC 2003.01	Less than 10/g	Less than 10/g
Other bacteria, including *Bacillus subtilis*	Not applicable		

SECTION 9 – PRODUCT STORAGE; SHELF-LIFE ; PACKAGING & LABELLING INFORMATION	
Product name	Packaging size and labelling
MenaQ7 POWDER P 1000	1 kg or 5 kg in bags. Packed in clean rooms
Packaging material and waste material handling	Sealed Al laminated bags. Please see national/local regulations for packaging waste material handling
Transport conditions	Transported as tempered goods
Storage conditions	Recommended storage temperature is 10°- 15° Celsius and protected from UV light, lamp and direct sunlight. The product is very light sensitive and exposure may deteriorate K2 activity considerably
Expiration date	Shelf life 36 months from date of production
Batch/Lot numbering system	Year/Month/Day- serial number batch that date
Nutritional/Supplement facts	Nutritional profile inserted below
Required finished product label statement MenaQ7™ Natural Vitamin K2	The conditions for use of MenaQ7 logo is outlined in customers contract with NattoPharma ASA
Recommended warnings on labels	Patients on anti-coagulant therapy must consult their physician
Other (usage, cautionary, safety or other warning statements)	Vitamin K is safe for human consumption, and presently no internationally upper tolerable level is established. Information on MenaQ7 bioavailability; MenaQ7 recommended dosage; MenaQ7 and safety;, MenaQ7 and effects on the coagulation system is available as NattoPharma ASA partner
Material Safety Data Sheet (MSDS)	MSDS attached

SECTION 10 – NUTRITIONAL PROFILE		
NA: Not applicable **nd: Not detected**		
	Unit	**Relevant detection limit**
Total amount of calories pr 100g	498 kcal/100 g	
Calories from fat pr 100g	213 kcal/100 g	
Total amount of protein	8,8 %	
Total fat – including specified amounts of:	23,7%/100g	
Saturated	1,9 %	
Poly unsatured	1,2%	

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Mono unsatured	19,6%	
Trans Fatty Acids	nd	0,1 %
Phospholipids	18 mg/100g	10mg/100g
Milk fat	NA	
Cholesterol	3 mg/100g	1 mg/100g
Total Carbohydrates	62,3%	
Dietary fibre	nd	0,5 g/100g
Soluble fibre	NA	
Insoluble fibre	NA	
Sugars	NA	
Lactose	NA	
Saccharose	NA	
Glucose	NA	
Glucose syrup	NA	
Polyols	NA	
Sugar alcohol	NA	
Other carbohydrates	NA	
Minerals		
Potassium (K)	4,3 mg/100g	1mg/100g
Sodium (Na)	133 mg/100g	1mg/100g
Calcium (Ca)	7,6 mg/100 g	1mg/100g
Phosphorous (Ph)	73 mg/100 g	1mg/100g
Magnesium (Mg)	1,2 mg/100 g	0,1mg/100g
Iron (Fe)	nd	0,1mg/100g
Vitamins	g/100g	
Vitamin A	nd	
Vitamin C	nd	
Vitamin E	9,3 mg/100g	0,1 mg/100g
Vitamin D	nd	
Other components		
Alcohol (Ethanol)	0%	
Caffeine	0%	
Theobromine	0%	
Phenolics	0%	
Moisture	4, 8 %	105°C in 4 hours
Ashes	0%	0,5 %
Hormones	0%	

SECTION 11- PHYSICAL & CHEMICAL CHARACTERIZATION	
Appearance	White or light yellowish powder; odorless
Powder density	Bulk: 0,30 – 0,35 w/v Tapped: 0,40-0,45 w/v
Particle size	Average 133 µm, SD 110 µm, all particles pass 16mesh (991µm)
Effect of drying	Not more than 8% upon drying at 105°C for 4 hours
Water solubility	Not soluble
pH	Not applicable
Stability higher temperatures	Sustains 120° C for up to 20 mins
Light exposure	Must be protected from UV and direct lamplight Storage for 24 hours in daylight: ca 10% loss of K2 activity

SECTION 12- SUITABILITY FOR HUMAN USES	
Suitable for human foods	Yes
Suitable for vegetarians	Product contains casein – proteins from bovine milk (

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	Danish Holstein), but no other material of animal origin
Suitable for lacto-ovo-vegetarians	Product contains casein – proteins from bovine milk (Danish Holstein), but no other material of animal origin
Suitable for celiac patients	Product does not contain any gluten
Certified as organic	No
Certified as kosher food	Yes
Certified as halal food	No
Animal testing	Product is not tested in animals

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January 2008

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Certificate of Analysis

9 January, 2008

	Analytical Data				Detection limit	Method	Specification of the product
Product	M-1500	M-1500	P-1000	P-1000			
Batch (Lot)	060609-1	070809-1	070117-3	070206-2			
Arsenic (As_2O_3)	nd	nd	nd	nd	0.2ppm	AOAC* 952.13 Silver Diethyldi-thiocarbamate method	not more than 0.5ppm
Cadmium (Cd)	nd	nd	nd	nd	0 01ppm	AOAC* 974.27 Atomic Absorption method	not more than 0.5 ppm
Lead (Pb)	nd	nd	nd	nd	0.05ppm	AOAC* 973.35(Atomic absorption method)	not more than 0 5 ppm
Total Mercury (Hg)	nd	nd	nd	nd	0.01ppm	AOAC* 971.21 (Atomic Absorption method)	not more than 0.1ppm

*AOAC: Official methods of analysis of AOAC international
nd: not detected

Quality Control Division
Shizuoka Site
J-OIL MILLS, INC.

Asaba Factory, J-OIL MILLS, INC.
1746 Nakashinden, Fukuroi-city
Shizuoka 437-1111 Japan

J-OIL MILLS

MenaQ7™
Natural Vitamin K2

To WHOM IT MAY CONCERN

GMO notification

We refer to your request for GMO information on the critical raw materials used in the production as well as in the final formulation of the products that we currently supply to you.
We hereby confirm the following·

Product Name: MenaQ7 OIL M 1500

Ingredient	GMO Information of critical raw materials
Sunflower oil	Sunflower oil is manufactured from sunflower. Sunflower· A genetically modified sunflower is not being grown and not available in the crops market. Therefore, the sunflower oil does not originate from genetically modified products.
"Vitamin K2 (Menaqunone-7) (extracted from Bacillus subtilis natto)"	Soybean protein concentrate and corn starch are used for fermentation of Bacillus subtilis natto. Soybean protein concentrate: Soybean protein concentrate is derived from non-genetically modified soybean which is handled under identity preserved conditions (IP). Corn starch: Corn starch is derived from non-genetically modified corn which is handled under identity preserved conditions (IP).

The final product does not contain any detectable amount of DNAs from GMOs.

Date: 05-Feb-2008

Satoshi Kiyama
Quality Control Manager

J-OIL MILLS

Quality Control Division Shizuoka Site
J-OIL MILLS INC.
2 Shinminato , Shimizu-ku
Shizuoka-city 424-0823 Japan

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MenaQ7™
Natural Vitamin K2

To WHOM IT MAY CONCERN

GMO notification

We refer to your request for GMO information on the critical raw materials used in the production as well as in the final formulation of the products that we currently supply to you
We hereby confirm the following

Product Name MenaQ7 POWDER P 1000

Ingredient	GMO Information of critical raw materials
Dextrin	Dextrin is manufactured from corn starch, sweat potato starch and potato starch Corn starch Corn starch is derived from non-genetically modified corn which is handled under identity preserved conditions (IP) Sweat potato starch A genetically modified sweat potato is not being grown and not available in the crops market Therefore, the sweat potato does not originate from genetically modified products Potato starch Potato starch is derived from non-genetically modified corn which is handled under identity preserved conditions (IP)
Sunflower oil	Sunflower oil is manufactured from sunflower Sunflower A genetically modified sunflower is not being grown and not available in the crops market Therefore, the sunflower oil does not originate from genetically modified products
Starch sodium octenylsuccinate	Starch sodium octenylsuccinate is manufactured from corn starch Corn starch Corn starch is derived from non-genetically modified corn which is handled under identity preserved conditions (IP)
Glycerol fatty acid ester	Glycerol fatty acid ester is manufactured from palm and coconuts A genetically modified palm and coconuts are not being grown and not available in the crops market Therefore, the glycerol ester fatty acid does not originate from genetically modified products
"Vitamin K2 (Menaquinone-7) (extracted from Bacillus subtilis natto)"	Soybean protein concentrate and corn starch are used for fermentation of Bacillus subtilis natto Soybean protein concentrate Soybean protein concentrate is derived from non-genetically modified soybean which is handled under identity preserved conditions (IP) Corn starch Corn starch is derived from non-genetically modified corn which is handled under identity preserved conditions (IP)
L-ascorbyl palmitate	L-ascorbyl palmitate is manufactured from palm and tapioca A genetically modified palm and tapioca are not being grown and not available in the crops market Therefore, the L-ascorbyl palmitate does not originate from genetically modified products
Rosemary extract	Rosemary extract is manufactured from rosemary A genetically modified rosemary is not being grown and not available in the crops market Therefore, the rosemary extract does not originate from genetically modified products

The final product does not contain any detectable amount of DNAs from GMOs.

Date 05 Feb 2008

Satoshi Kiyama
Quality Control Manager

J-OIL MILLS

Quality Control Division Shizuoka Site
J-OIL MILLS INC
2 Shinminato , Shimizu-ku
Shizuoka-city 424-0823 Japan

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Appendix B

Qualifications of Expert Panel Members

Richard C. Kraska, Ph.D., DABT

Robert S. McQuate, Ph.D.

Stanley T. Omaye, Ph.D.

Richard C. Kraska
Chief Operating Officer and Co Founder
GRAS Associates, LLC
12068 Via Cercina Dr
Bonita Springs, FL 34135

Curriculum Vitae

EDUCATION B S , Chemistry, Providence College
Ph D , Pharmacology, University of Minnesota

PROFESSIONAL CERTIFICATION Diplomate, American Board of Toxicology

EXPERIENCE 29 year in toxicology and regulatory affairs for industry and government in broad aspects of the chemical industry including food additives, foods, food contact materials, cosmetics, lubricants and fuels, coatings, defoamers, anti-microbial pesticides and pharmaceuticals

GRAS ASSOCIATES, LLC
Bonita Springs, FL (2006 to Present)
Chief Operating Officer and Co Founder

- Serve as Lead Scientist and Panel Chair for GRAS determinations
- Coordinate drafting and report review by chemists, toxicologists and scientists of other disciplines as needed
- Ingredients reviewed include natural antioxidants, novel sources of dietary fiber, fats and oils and extracts from exotic fruit

KRASKA CONSULTANTS, INC
Bonita Springs, FL (2004 to Present)
Vice President and Principal

- Toxicology and Regulatory Consultant for a variety of lubricant, chemical, food processing companies and trade associations
- Offer services in Toxicology and Product Safety including FDCA, TSCA and FIFRA regulations and filings, International Hazard Communication Support, Product Stewardship, Expert Witness and Litigation Support
- Founder and Technical Consultant for the Defoamer Industry Trade Association
- Toxicology Consultant for the Independent Lubricant Manufacturers Association

THE LUBRIZOL CORPORATION
Wickliffe, OH (1987 to 2004)
MANAGER OF SPECIAL TOXICOLOGY AND REGULATORY PROJECTS (2001 to 2004)

- Toxicology and regulatory consultant for organic growth initiatives and new acquisitions
- Coordinating $2 8 million inhalation toxicology program on engines emissions with a novel diesel fuel formulation for registration with EPA under the Clean Air Act
- Coordinating world wide implementation of compliance with revised European hazard communication regulations
- Consultant to Lubrizol defoamer, coating, process chemical, metalworking and lubricant businesses on regulations and toxicology
- Team member studying and planning implementation of sustainable development at Lubrizol

MANAGER OF TOXICOLOGY AND RISK ASSESSMENT (1987 – 2001)

- Provided leadership and management for corporate toxicologists and product safety specialists
- Direct responsibility for toxicology testing and evaluation of all Company specialty chemicals and products
- Manage $1 million annual toxicology and environmental testing budget for regulatory approvals and product stewardship
- Lead consultant for business units on novel regulatory approvals, product stewardship and risk evaluation
- Developed and institutionalized product risk assessment process for all Lubrizol businesses
- Provide leadership role representing Company on trade association task groups involved in legislative and regulatory advocacy
- Co-team leader for development and implementation of award -winning expert system for writing MSDSs from a product safety database

BP AMERICA INC (formerly THE STANDARD OIL CO)
Cleveland, OH (1985-1987)
MANAGER OF PRODUCT SAFETY AND REGULATORY COMPLIANCE

- Assumed responsibility for assuring all Company products complied with federal regulations (TSCA, FIFRA, FDCA, USDA).
- Coordinated and expedited all regulatory submissions for premarket approval, reporting rules and rulemaking comment.
- Conscientiously developed Company Product Safety Policies and Manual.
- Critically evaluated Corporate Hazard Communication Program in a decentralizing company.
- Successfully initiated labeling program to comply with OSHA Hazard Communication Standard.

AMERICAN CYANAMID COMPANY, CHEMICALS GROUP
Wayne, NJ (1983-1985)
MANAGER OF TOXICOLOGY PROGRAMS

- Wide range of responsibility for recommending, contracting, monitoring and evaluating mammalian, genetic and aquatic toxicology studies for chemical products.
- Responsible for $250,000 total contract value for testing, quality assurance and consultants.
- Effectively guided regulatory staff in strategy and data requirements for premarket approvals.
- Successfully orchestrated targeted research programs for mechanistic studies on key chemicals for aquatic and mammalian toxicity.
- Actively represented Company in a wide spectrum of trade association activities.

FOOD AND DRUG ADMINISTRATION
Washington, DC (1977-1983)
GRAS Review Branch
Division of Food and Color Additives
SUPERVISORY CONSUMER SAFETY OFFICER
(1981-1983)

- Successfully managed group of 3-4 professionals in regulatory program to implement expert panel reviews of GRAS list food ingredients.
- Projects of responsibility included salt, caffeine, BHA, BHT, cellulose, enzymes, rapeseed oil, vitamins, iron, manganese and zinc salts.
- Co-directed agency expertise on toxicology, chemistry, law and policy to propose regulatory action on food uses of DSS. Negotiated consistency with Bureau of Drugs proposal on OTC and Rx uses.
- Advised Branch Chief in matters of policy, consistency and personnel.
- Interacted with industry regarding regulatory opinions and new product approvals.

Petitions Control Branch
Division of Food and Color Additives
CONSUMER SAFETY OFFICER (1977-1981)

- Coordinated scientific review and regulatory response to review food additive petitions submitted by industry for direct additives and food packaging materials.
- Scientific and historical expert for General Counsel, U. S. Attorney and Department of Justice for legal proceedings on cyclamate.
- Expert on food/drug interface of vitamins and dietary supplements.
- Analyzed quality of critical studies on aspartame and served on GLP review committee
- Served as Bureau representative in Interagency Regulatory Liaison Group on phthalate plasticizers.
- Assistant to Bureau Director on advocacy activities on behalf of U.S. industry for WHO programs

PUBLICATIONS

Reed, MD, Blair LF, Burling K, Daly I, Gigliotti AP, Gudi R, Mercieca MD. McDonald JD, O'callaghan JP, Seilkop, SK, Ronsko NL, Wagner VO, Kraska RC Health effects of subchronic exposure to diesel-water-methanol emulsion emissions Toxicology & Industrial Health Vol 22 In Press

Reed, MD, Blair LF, Burling K, Daly I, Gigliotti AP, Gudi R, Mercieca MD. McDonald JD, Naas DJ, O'callaghan JP, Seilkop, SK, Ronsko NL, Wagner VO, Kraska RC Health effects of subchronic exposure to diesel-water emulsion emissions. Inhal Toxicol 17: 851-70 (2005)

Kraska, RC , Industrial Chemicals. Regulation of new and existing chemicals. In: Gad S.C. editor. *Regulatory Toxicology.* Taylor and Francis Ltd. London 2001.

Kraska, RC . and Hooper DH, Industrial Chemicals. Hazard Communication, exposure limits, labeling and other workplace and transportation requirements under OSHA, DOT, and similar authorities around the world. In: Gad S.C. editor. *Regulatory Toxicology.* Taylor and Francis Ltd. London 2001.

Strother, DE, Mast RW, Kraska RC, Frankos V Acrylonitrile as a carcinogen. Research needs for better risk assessment. Ann NY Acad Sci 534:169-78 (1988)

Petersen DW, Kleinow KM, Kraska RC, Lech JJ Uptake, disposition and elimination of acrylamide in rainbow trout Toxicol Appl Pharmacol 80: 58-65 (1985)

Mast RW, Jeffcoat AR, Sadler BM, Kraska RC and Friedman MA Metabolism, disposition and excretion of [C14] melamine in male Fischer 344 rats. Food Chem Toxicol 21: 807-810 (1983)

SPEAKER

Talks given on following topics at national meetings, seminars and workshops
- GRAS Criteria
- REACH and GHS Regulations
- HPV Toxicology Testing
- Risk Assessment and Risk Management
- Lubricant Additive Safety
- Trade Association Environmental Activism
- Product Deselection Lists
- MSDS Expert Systems
- Confidential Business Information under TSCA
- TSCA Section 12(b) Compliance

TRAINING COURSES

Training courses given to business, research and legal groups at Lubrizol
- General Regulatory Overview
- TSCA New Chemicals
- FDA Food Additive Requirements
- Product Regulatory Law Course (TSCA, FDCA, OSHA)

Trainer, Toxicology Module, Metalworking Fluids Certificate Course (2005-2006)

TRADE ASSOCIATION ACTIVITIES

Chemical Reporting Task Group (1983-1998)
Chemical Manufacturers Association Chairperson (1997-1998)
Safety, Health, Environmental and Regulatory Affairs Committee, Independent Lubricant Manufacturers Association (1997 to present)
- Vice chairperson (2001-2002)
- Chairperson (2003-2004)
- Toxicology consultant (2006)

Oversight Committee, Metalworking Fluid Product Stewardship Group, Independent Lubricant Manufacturers Association (1997-2004)
Health Environmental and Regulatory Task Group, Petroleum Additives Panel (1997-2002)
- Chairperson, Sensitization Work Group (1999 to 2002)

Biocides Panel, AEATF II Protocol Committee and Technical Committee (2003-2006) Team Leader for Metalworking Study (2005-2006)
Defoamer Industry Trade Association, Founder and Technical Consultant (2005-2006)

PROFESSIONAL SOCIETY MEMBERSHIPS

Society of Toxicology (SOT)
American Standards and Testing Methods (ASTM)
Society of Tribology and Lubrication Engineers (STLE)
Regulatory Affairs Professionals Society (RAPS)
Roundtable of Toxicology Consultants (RTC)

ROBERT S. MCQUATE, Ph. D.

WORK HISTORY

2006 - Present	CEO, and Co-Founder, GRAS Associates, LLC, Bend, OR
1988 - Present	President & CEO, R. S. McQuate & Associates, Inc., Bend, OR
2005 - 2006	Chemistry Instructor, Truckee Meadows Community College, Reno, NV
2000 - 2005	Senior Vice President, Scientific & Regulatory Affairs, AminoPath Labs, LLC, Portland, OR
1998 - 2002	Board Member & Business Consultant, National Institute of Standards & Technology, Advanced Technology Program, Gaithersburg, MD
1986 - 1996	Executive Director, Advanced Science & Technology Institute, Eugene & Corvallis, OR
1991 - 1992	Adjunct Professor, Food Science & Technology, Oregon State University, Corvallis, OR
1983 - 1986	Science Director, National Soft Drink Association, Washington, DC
1980 - 1983	Senior Regulatory Scientist and Group Leader of Regulatory and Nutrition, The Dial Company, Inc., Scottsdale, AZ
1977 - 1980	Consumer Safety Officer, Food and Drug Administration, Center for Food Safety & Applied Nutrition, Division of Food and Color Additives, Washington, DC
1974 - 1977	Assistant Professor of Chemistry, Willamette University, Salem, OR

EDUCATION

Postdoctoral Research Fellow with Professor R. G. Wilkins, New Mexico State University, Las Cruces, NM
Ph.D. in Chemistry, The Ohio State University, Columbus, OH
B.S. in Chemistry with Honors, Lebanon Valley College, Annville, PA

PROFESSIONAL EXPERIENCE

CONSULTING SERVICES

CEO, GRAS Associates, LLC; President & CEO, R. S. McQuate & Associates, Inc.

- Provide food ingredient safety evaluations, focusing on independent GRAS evaluations.
- Provide broad-based business consulting services to universities & companies involved in technology commercialization.
- Rapid assimilation of technical and business background for use in formulating commercialization strategies.
- Critically evaluate new technologies and business plans compared to competitive firms and products for economic potential.
- Implement marketing activities to establish strategic alliances and/or licensing agreements.
- Facilitate start-up ventures, including drafting of business plans.
- Utilize negotiation skills to achieve successful execution of deals.

UNIVERSITY EXPERIENCE

Executive Director, Advanced Science & Technology Institute

- Managed industry-university interface program on behalf of University of Oregon, Oregon State University, Oregon Health Sciences University and Portland State University.
- Strategic planning and program implementation; managed staff of up to 8.
- Facilitated linkages between university research community and private sector, working with over 500 faculty members to yield consulting contracts, industrial research sponsorship, technology licensing and business start-ups.
- Aggressively marketed faculty expertise, universities' technologies, and research capabilities through network of contacts, Internet, publications, and conferences.
- Represented universities in broad-based statewide and regional economic development initiatives.

Faculty, Willamette University, Oregon State University, & Truckee Meadows Community College

- Presented introductory and upper level chemistry lecture and laboratory courses.
- Conducted independent research in molecular biology, enzymology, and metal ion catalysis.
- Generated external grant funding to support six research students & acquire equipment.
- Provided food safety guidance to industry.

- Various scientific and chemical education publications.

PRIVATE SECTOR EXPERIENCE

Technical Management, The Dial Company & National Soft Drink Association

- Managed 5-person technical regulatory group with corporate responsibility for compliance with FDA, USDA, EPA, FTC, OSHA, CPSC, and NRC.
- Creatively interpreted regulations to favorably impact company revenues by over $4.2 M annually.
- Special focus on product and ingredient safety; formulated regulatory strategies in anticipation of and in response to agency positions; applied quantitative risk analysis to product safety considerations.
- Provided regulatory support and training to Manufacturing and QA on Good Manufacturing Practices requirements.
- Teamed with Marketing by evaluating advertising, product claims, and labeling for compliance.
- Assessed university research proposals in response to industry solicitations for funding.
- Served as liaison for industry interests on food ingredient safety before FDA officials.
- Served as industry spokesperson with media on technical topics such as NutraSweet addition to soft drinks.

GOVERNMENT EXPERIENCE

Staff, Food & Drug Administration

- FDA representative with regulated food industry officials.
- Managed safety evaluations of food and color additives and GRAS ingredients among FDA scientific divisions and with legal staff.
- Generated food safety notices, proposals, and regulations.
- Evaluated complex net weight food labeling and compliance issues and formulated agency position for Commissioner.
- Participated on special FDA Food Labeling Task Force to develop total food label requirements.
- Formulated recommended agency policy on iron bioavailability nutritional concerns.

PROFESSIONAL AFFILIATIONS

American Chemical Society
Institute of Food Technologists
Licensing Executives Society
Regulatory Affairs Professional Society

BOARD AND COMMITTEE MEMBERSHIPS

External Evaluator, Kansas Technology Enterprise Corporation, Higuchi Biosciences Center (2001)
Judge, Ohio State University Business Plan Competition (2001)
Board of Directors - Universal Pulping, Inc. (1996 - 2004)
Scientific Advisory Committee - Bainbridge Technology Group, Ltd. (1991 - 2000)
Board of Directors - Regional Council of Project SBIR West (1994 - 1996)
Board of Directors - Oregon Environmental Technology Association (1994 - 1995)
Co-Director – Oregon Governor's Task Force on Technology Transfer (1991 - 1992)
Board of Directors - LEAP, Inc. (1988 - 1994)
Board of Directors - Oregon Biosciences Association (1991 - 1993)
Board of Directors - BioForum (1988 - 1991)
Oregon Governor's Biotechnology Industry Advisory Council (1988)

HONORS AWARDS AND FELLOWSHIPS

Governor Barbara Roberts Certificate of Appreciation - Task Force on Technology Transfer (1993)
Governor Neil Goldschmidt Letter of Commendation - Biotechnology Industry Advisory Council (1988)
FDA Award of Merit from FDA Commission Jere Goyan (1980)
Letter of Commendation from FDA Commissioner Donald Kennedy (1979)
Seven Research Grants Awarded as Faculty Member at Willamette University (1974 - 1977)
National Science Foundation - Graduate Research Fellowship, The Ohio State University (1971 - 1973)
Graduated with Honors, Lebanon Valley College (1969)
Petroleum Research Fund - Undergraduate Research Fellowship, Lebanon Valley College (1967 and 1968)
Dean's List Student, Lebanon Valley College (1966 - 1969)
Salutatorian, South Lebanon High School, Lebanon, PA (1965)

Stanley T. Omaye, Ph.D.

Biography

For over 30 years, Dr. Omaye has been at the forefront of research focusing on environmental and nutritional interactions. His goal has been to identify nutrient interactions with environmentally induced disorders and to understand the mechanisms of such interactions and their effects on human health and welfare. Currently, Dr. Omaye's research and educational efforts are targeting: Nutrition and aging - efficacy of antioxidant compounds on atherosclerosis and the oxidation of low-density lipoproteins; Environmental health - health effects of air pollutants, environmental tobacco smoke, and mercury/arsenic; Health promoting bioactive compounds - elected phytochemicals and conjugated linoleic acid; and Improving food safety for vulnerable populations of Nevada. Thus, Dr. Omaye is often called upon to serve regionally, nationally, and internationally as an expert in food, nutrition and toxicology.

FDA Experience

- Past Academic Chairperson and currently Secretary, Nevada Food Safety Task Force – FDA initiated state project which has the task of improving food safety in Nevada, 2002 - present
- Appointed Consultant for the Institute of Medicine, Academy of Sciences, Washington D.C. Work involves the assessment the safety of supplement ingredients, FDA & USDA, 2002.
- USA advisor to the University of Hong Kong regarding, "An Investigation of Nutritional Imbalance and Carcinogenicity", at the request of FDA, 1987-1989.
- PI/CoPI, DOA GLP Compliant studies of new antidotal compounds used in the treatment of various biological and chemical military relevant compounds, 1987-1991.

Education

- Postdoctoral Fellow in Pulmonary Biochemistry and Toxicology; California Primate Research Center, Davis, California, 1976
- Ph.D., Biochemistry/Nutrition, University of California, Davis, California, 1975
- M.S., Pharmacology/Physiology, University of the Pacific, Stockton, California, 1972
- B.A., Chemistry, Sacramento State College, Sacramento, California, 1968

Professional Experience

- Professor, Department of Nutrition, College of Agriculture, Biotechnology and Natural Resources, University of Nevada, Reno. 1991 – present; Department Chair, Department of Nutrition, 1991-1996.
- Letterman Army Institute of Research, Research Toxicologist and Team Leader, Military Trauma Research Division, Presidio of San Francisco, CA. 1989 - 1991.
- Letterman Army Institute of Research, Assistant Chief of Toxicology Division and Research Chemist, Presidio of San Francisco, California. Toxicology and safety evaluation (Under Good Laboratory Practices) of military relevant products. 1987 - 1989.
- U. S. Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, Presidio of San Francisco, California. Project Leader and Research Chemist, Bioanalytical Research Unit (Oct. 1983 - April 1984) and Biochemistry Research Unit (April 1984 - April 1987).
- U. S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Berkeley, California. Project Leader and Research Nutritionist, Nutrients Research Unit. 1980 - 1983.
- Letterman Army Institute of Research, Chief of Applied Nutrition Branch and Research Chemist, Biochemistry Division, Department of Nutrition, Presidio of San Francisco, California. 1976 - 1980.
- California Primate Research Center, Pulmonary Biochemistry Section, Postdoctoral fellow. 1975 - 1976.
- Quality Control Chemist, Campbell Soup Company, Sacramento, California, June 1969 - Sept 1969.

Awards

- Fellow, Academy of Toxicological Sciences, 1987. Recertification granted, 1991, 1997, and 2002.
- Fellow, American College of Nutrition, 1993
- Certified Nutrition Specialist, 1993
- Awarded Sabbatical Leave, University of Nevada, Reno, 2001.
- Recipient of Exemplary Service Plaque for efforts as Division Councilor, Toxicology and Safety Evaluation Division, IFT, 1995-1998.
- Recipient of Leadership Award from the Food Safety Specialty Section, Society of Toxicology, 1995.
- Exemplary Service Plaque from the Toxicology and Safety Evaluation Division of the Institute of Food Technologists, 1994.

- Special Act Award, Letterman Army Institute of Research, Presidio of San Francisco, CA., 1989..
- Letter of Commendation of Commendable Performance of Duties by the Institute Commander, Letterman Army Institute of Research, 1976.
- Recipient of NIH Trainee ship Award in Pulmonary Biochemistry and Inhalation Toxicology (Nat'l. Heart, Lung and Blood Institute), 1975.
- RhoChi (National Honor Pharmacy Society), 1972.
- Recipient of Mary Aaron Scholarship, 1963 - 1965.

Membership

- Editorial Board Member/Associated Editor, Toxicology, Elsevier, Amsterdam, 1998-Present
- Editor/Co-Founder, Environmental and Nutrient Interactions, Taylor & Francis, 1997
- Editorial Board Member, Society for Experimental Biology and Medicine, New York, NY, 2002-2005
- Editorial Board Member, Nutritional and Environmental Medicine, Taylor & Francis, 2000-Present
- Editorial Board Member, Inhalation Toxicology, Taylor & Francis, 2002-2004.
- Editorial Board Member, Journal of Food Protection, 2003-Present.
- Member, Board of Scientific Advisors of the American Council on Science and Health, 1989-present
- Electronic Journal Editorial Boards: American J. Food Technology; Asian J of Clinical Nutrition; Asian J of Epidemiology; Asian J of Scientific Research; J Pharmacology and Toxicology; Research J of Environmental Sciences; Research J of Environmental Toxicology

Professional Society Memberships, Elected Positions, Advisory and Consulting Activities

- Elected to Councilor, Food Safety Specialty Section, Society of Toxicology, 2007-2010.
- Appointed Food safety Specialty Section Newsletter Editor, Society of Toxicology, 2007-2010.
- Appointed Panel Member, IOM, National Academy of Sciences, 2004-06.
- Invited Panel Member, CSREES Review, Department of Food Science and Toxicology, University of Idaho, 2005.
- Panel member, study group for nutrition and health, USDA CSRS NRI competitive grants, 1996, 2002-2005.
- Panel Member for reviewing grants, USDA, SBIR, 2004
- Invited Speaker Chairperson, Food Safety and Nutraceuticals, Society of Toxicology Annual Meeting, Baltimore, Maryland, 2004.
- Invited Speaker, "Food Toxicology", International Food Protection Conference, Phoenix, AZ, 2004.
- Invited Symposium Chair, 12th World Food Science and Technology Congress. An International Meeting scheduled for 2003 in Chicago, ILL.
- Member or Review Panel, Research Competitiveness Subprogram (RCS) of the Louisiana Board of Regents' R & D Program, 2001 – Present
- Member of Review Panel, Midwest Advanced Food Manufacturing Alliance, 2000 – Present
- Appointed to Assessment Panel for Washoe District Department Health Officer Candidates, Reno/Sparks, Washoe County, Nevada, 1998 and 1999.
- Appointed to the Mead Johnson Award Selection Committee, American Society for Nutritional Sciences, 1998-99.
- Appointed to the Expert Panel on Food Safety and Nutrition, Institute of Food Technologists (three year term), 1998.
- Elected member of the Certification Board for Nutrition Specialists (three year term, 1997.
- Appointed to the Committee for Divisions and Sections, Institute of Food Technologists (three year term), 1996.
- Appointed as Alternative Executive Committee Member, Toxicology and Safety Evaluation Division, Institute of Food Technologists, 1995.
- Nominated to run as Treasurer, Executive Committee, Northern California Chapter of the Society of Toxicology, 1995.
- Appointed to the Program Committee for the Society of Toxicology (three year term), 1994.
- Advisory Committee member for National Association of State Universities and Land-Grant Colleges (NASULGC). Committee authored a position paper on, "Food Safety, Diet, Nutrition and Health", 1994.
- Study Group member/reviewer for SOH/DRG/NIH competitive grants for three years (1993 - 1996).
- Ad Hoc Committee member for NIH Nutrition Study Section, 1993.
- Elected President, Food Safety Specialty Section, Society of Toxicology, 1993 - 1994.
- Elected Chairman, Division of Toxicology and Safety Evaluation, Institute of Food Technologists for 1992 - 1993.
- Nominated to run for the Executive Committee, American College of Toxicology, 1993.

- Member of Team for the evaluation of the Interdepartmental Graduate Toxicology Program, Iowa State University, Ames, Iowa, 1993.
- Technical Advisor, Toxa Chemica, International, Rockville, Maryland. 1993 - 1998.
- Appointed to the Program Committee for the Institute of Food Technologists (three year term), 1991.
- Appointed by the Board of Directors to the Board of scientific Advisors of the American Council on Science and Health, New York, NY, 1989 - present.
- Elected to executive board of the Toxicology and Safety Evaluation Division of IFT in 1989. Elected to two year term.
- Elected Treasurer of the Northern California Chapter of the Society of Toxicology, 1989 (two year term).
- Consultant to the Departments of Community Medicine and Biochemistry, University of Hong Kong for investigations regarding the effect of diet on heavy metal toxicity, 1987 - 1991.
- Provided consultation to the Permanent Senate Subcommittee investigating nutritional quackery, Washington, D. C., 1985.
- Member of program committee for the annual meeting of the Gene and Environmental Toxicologist Society, 1988.
- Member of program committee for the annual meeting of the Northern California Society of Toxicologist, 1988 & 1989.
- Nomination to standing "Membership Committee" of the American College of Toxicology, 1988.
- Nominated to membership committee, Association of Government Toxicologists, Washington DC, 1988.
- Appointed representative from SOT to the Joint Societies Workshop on Food Safety. Served as Chair for writing the position paper on, Naturally Occurring Toxicants. 1988.
- Editor of IFT Toxicology and Safety Evaluation Division Newsletter, 1988 - 1993. Associate Editor between 1986 - 1988.
- Appointed participant sponsor for National Research Council Fellowship, 1987-88. Appointed participant sponsor for 2nd National Research Council Fellowship, 1988-89.
- At the request of FDA acting as USA advisor to the University of Hong Kong regarding, "An Investigation of Nutritional Imbalance and Carcinogenicity", 1987-1989.
- Elected Secretary of CSRS Project W-143 Committee on Nutrient Bioavailability--A Key to Human Nutrition, 1986-1987.
- Appointed to the Interim Executive Committee for the Northern California Chapter of the Society of Toxicology, 1986.
- Appointed to the Committee on making recommendations for the Institute of Food Technologists' on our present state of knowledge of the nutritional problems facing our aged population.
- Appointed to the Committee for National Seminars on "Diet and Health". Held at the University of California, Davis, Sept. 1985.
- Society of Toxicology`s designated liaison representative to the Institute of Food Technologists; 1982 - 1998.
- Society of Toxicology`s designated liaison representative to the American Institute of Nutrition; 1982 - 1998.
- Peer Review Panel/Research Personnel Evaluation, U. S. Department of Agriculture, Agricultural Research Service, Western Region; 1982 - 1987.
- Biological Safety Committee, U.S. Department of Agriculture, Agriculture, Agricultural Research Service, Western Regional Research Center, 1982-1983.
- Internal Grant Review Committee, U.S. Department of Agriculture, Agricultural Research Service (Review application for new and established investigator awards); 1981-1982.
- Served on special committee on the revision of USDA Directive and Manual 232.2: "Laboratory Use of Chemical Substances of Potential Carcinogenic Risk", 1980.

Publications (partial list since 2000):

(a) In Refereed Journals (92):

- Chen, L., Yang, W., Jennison, B. L. and **Omaye, S. T.** Air particulate pollution and hospital admissions for chronic obstructive pulmonary disease in Reno, Nevada. *Inhalation Toxicol* 12: 281-298, 2000.
- Zhang, P. and **Omaye, S. T.** $-Carotene and protein oxidation: Effect of ascorbic acid and "-tocopherol. *Toxicology* 146: 37-48, 2000.
- Chen, L., Jennison, B. L., Yang, W., and **Omaye, S. T.** Air pollution and elementary school absenteeism. *Inhalation Toxicol,* 12: 997-1016, 2000.
- Zhang, P. and **Omaye, S. T.** DNA strand breakage and oxygen tension: Effects of $-carotene, "-tocopherol and ascorbic acid. *Food Chem Toxicol* 39: 239-246, 2001.

- Zhang, P. and **Omaye, S. T.** Antioxidant and prooxidant roles for $-carotene, "-tocopherol and ascorbic acid in human lung cells. *Toxicol in vitro* 15: 13-24, 2001.
- Zhang, P. and **Omaye, S. T.** $-Carotene: Interactions with "-tocopherol and ascorbic acid in microsomal lipid peroxidation. *J Nutri Biochem* 12: 38-45, 2001.
- Chen, L., Yang, W., Jennison, B. L., Goodrich, A. and **Omaye, S. T.** Using two different statistical models to compare the associations between PM10 and hospital admissions for COPD. *Toxicol Meths,* 11: 233-246, 2001.
- Chen, L., Yang, W., Jennison, B. L., Goodrich, A. and **Omaye, S. T.** Air pollution and birth weight in Northern Nevada, 1991-1999. *Inhalation Toxicol* 14: 141-158, 2002.
- Nakamura, Y. K. and **Omaye, S. T.** Age-related changes of serum lipoprotein oxidation in rats. *Life Sciences* 74: 1265-1275, 2004.
- Elsayed, N. M. and **Omaye, S. T.** Biochemical changes in mouse lung after subcutaneous injection of the sulfur mustard 2-chloroethyl 4-chlorobutyl sulfide. *Toxicology* 199: 194-206, 2004.
- Flintoff-Dye, N. L. and **Omaye, S. T.** Antioxidant effects of conjugated linoleic acid isomers in isolated human low-density lipoproteins. Nutr Res 25: 1-12, 2005
- Nakamura, Y. K., Read, M. H., Elias, J. W., and **Omaye, S. T.** Oxidation of serum low-density lipoprotein and antioxidant status in young and elderly humans. *Arch Gerotol Geriatrics* 42: 265-276, 2006.
- Cirico, T. L., and **Omaye, S. T.** Additive or synergetic effects of phenolic compounds on human low density lipoprotein oxidation. *Food Chem Toxicol* 44: 510-516, 2006.
- Badr El-Din, N. and Omaye, S. T. Concentration dependent antioxidant activities of conjugated linoleic acid and alpha-tocopherol in corn oil. *J Sci Food Agriculture* (In press), 2007.

(b) Books, Book Chapters and Reviews Articles (34):

- Bidlack, W. R., **Omaye, S. T.**, Meskin, M. S. and Jehner, D. Phytochemicals As Bioactive Agents. Technomic Publishing Co., Inc. Pennsylvania, 2000.
- Dubick, M. A. and **Omaye, S. T.** Modification of atherogenesis and heart disease by grape wine and tea polyphenols. In: Nutraceuticals Handbook, (Wildman, R., Ed.) CRC press, pp. 235-260, 2001.
- Chen, L. and **Omaye, S. T.** Air pollution and health effects in northern Nevada. *Rev Environ Health* 16:51-67, 2001.
- Dubick, M. A. and **Omaye, S. T.** Evidence for grape, wine and tea polyphenols as modulators of atherosclerosis and ishemic heart disease in humans. *Nutraceuticals, Functional & Medical Foods* 3: 67-93, 2001
- Meskin, M. S., Topham, D. K. W., **Omaye, S. T.**, Bidlack, W. R. Phytochemicals in Nutrition and Health, CRC Press, Boca, Raton, FL, 2002.
- **Omaye, S. T.** Metabolic modulation of carbon monoxide toxicity. *Toxicology* 180: 139-150, 2002.
- Hussein, H. S., and **Omaye, S. T.** Introduction to the food safety concerns of Verotoxin-producing *Escherichia coli. Exp Biol Med* 228: 331-332, 2003.
- **Omaye, S. T.** Introduction to food toxicology. In: *Pesticides, Veterinary and Other Chemical Residues in Food.* Watson, D., Ed., Woodhead Publishing Ltd., pp, 1-26, 2004.
- **Omaye, S. T.** *Food and Nutritional Toxicology*, CRC Press, 2004.
- Todd, S. J. T. and **Omaye, S. T.** Maternal environmental tobacco exposure and in utero and adverse birth outcomes. *Inhalation Toxicology*, 2nd edition, Salem, H. and Katz, S. A., Eds., pp, 875-912, CRC Press, Roco Ratan, FL, 2006.
- Dubick, M. A., and **Omaye, S. T.** Grape wine and tea polyphenols in the modulation of atherosclerosis and heart disease. In: Nutraceutical Handbook, 2nd edition (Wildmann, R., Ed.) CRC Press, 2007.

(c) Other Professional Publications (41):

- **Omaye, S. T.**, and Elsayed, N. M. Editorial: A new beginning. *Toxicology*, 48: 81-83, 2000.
- Bendich, A., Elsayed, N. M., and **Omaye, S. T.** Editorial: Lawrence J. Machlin, Ph.D., FACN, 1927 - 2000. *Toxicology* 159: 117-118, 2001
- **Omaye, S. T.** Biotechnology and sustainable agriculture. *Food Techn* 55 (February): 23, 2001.
- **Omaye, S. T.** Preventing BSE in the U.S. *Food Techn* 55 (April): 26, 2001.
- **Omaye, S. T.** Shark-fin soup and methylmercury: To eat or not to eat. *Food Techn* 55 (October): 26, 2001.
- **Omaye, S. T.** Cooperating to meet food safety challenges. *Food Tech* 57: 24, 2003.

(d) Published Abstracts (88):

- Dubick, M. A., Williams, C., **Omaye, S. T.**, Villarreal, R. L., Herndon, D. N., Goodwin, C. W., and Kramer, G. C. High dose ascorbic acid infusion reduces net fluid balance in sheep subjected to a 40% TBSA burn. *J Burn Care & Rehabilitation*, 21: S144, 2000

- Chen, L., Jennison, B. L., Yang, W., and **Omaye, S. T.** Air pollution and elementary school absenteeism. *Toxicologist* 54: 15, 2000
- Chen, L., Jennison, B. L., Yang, W., and **Omaye, S. T.** Particulate air pollution and hospital admissions for chronic obstructive pulmonary disease in Reno, Nevada. *Toxicologist* 54:15, 2000
- Zhang, P. and **Omaye, S. T.** Oxygen tension and DNA strand breakage: Effects of antioxidants. *American College of Nutrition Annual Meeting*, Las Vegas, NV, 2000.
- Chen, L., **Omaye, S. T.**, Yang, W., and Jennison, B. L. Comparisons between ARIMA and GAM applications in the same data analysis. *Twelfth Conference of the International Society of Environmental Epidemiology*, University at Buffalo, New York., 2000
- Chen, L., **Omaye, S. T.**, Yang, W., and Jennison, B. L. Particulate air pollution and hospital admissions for COPD: Two statistical models. one result. *Toxicologist* 60: 190, 2001.
- Carughi, A., **Omaye, S**. and Furst, A. Carotenoid levels before and after supplementation with a mixture of vegetable extracts. *FASEB*, 2001.
- Carughi, A., and **Omaye, S**. Plasma carotenoid levels before and after supplementation with mixture of vegetable oleoresins. *Ventura Gordon Conference*, Ventura, California, 2001.
- Yamauchi, M., Fernandez, G. C. J., and **Omaye, S. T.** Multiple linear regression analysis of total mercury levels in blood and urine samples. SAS Annual Meeting, San Diego, CA, 2002.
- Cheng, H., **Omaye, S. T.,** Yang, W., and Calderon, R. Mortality rates of Churchill county, rural Nevada, and Nevada state. Fifth International Conference on Arsenic Exposure and Health Effects, San Diego, CA, 2002.
- Maher, T., **Omaye, S. T.**, Nagle, D., Wren, A. F., Geraci, C. L., Zumwalde, R. D., Miller, A. L., and Hoover, M. D. A case study in partnering to develop a nanotechnology occupational safety program in a nanotechnology manufacturing environment. Second International Symposium on Nanotechnology and Occupational Health, Minneapolis, Minnesota, Oct, 2005.
- Elsayed, N. M., and **Omaye, S. T.** Pulmonary biochemical alterations induced by systemic administration of nitrogen mustard. Society of Toxicology Annual Meeting and published in the "Toxicologists", San Diego, California, April, 2006.
- Wilson, M. P., **Omaye, S. T.**, and Surrendera-Babu, A. Food handling practices and eating preferences of child and adult care providers. Society for Nutrition Education, Annual Meting, San Francisco, California, 2006.

SUBMISSION END

AM

GRAS ASSOCIATES, LLC

Generally Recognized As Safe

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RECEIVED
APR 10 2008
BY: (b)(6)

RECEIVED
APR 01 2008
BY (b)(6)

March 24, 2008

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Dr. Robert L. Martin

Re: GRAS Notification for Menaquinone-7

Dear Dr. Martin:

On March 6, 2008, we received a telephone inquiry from Dr. M. Ramon-Valle regarding the need for clarification of the foods to which Menaquinone-7 is to be added as described in the above-referenced GRAS Notification.

We have addressed this aspect in more detail within the enclosed information.

1. Dr. Richard Kraska, who chaired our Expert Panel, has compiled additional information on the anticipated consumer exposure based on the specific foods that constitute dairy products. His enclosed letter, including Table 1 and component sections from the USDA survey data, summarizes these findings which are supportive of the estimated daily exposure as found in our initial submission.

2. Those pages from our original GRAS Notification that have been modified in response to your March 6, 2008 inquiry to identify the individual food types are also provided.

With this additional information, we hope you will determine that the content supplied is sufficient to proceed with the filing of our GRAS Notification of Menaquinone-7.

We look forward to your feedback as your review progresses.

Thank you.

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Sincerely,

(b)(6)

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com

Enclosures:

Four Replacement Pages for Menaquinone-7 GRAS Notification (in triplicate)
Letter and Table with Supporting References for Alternative Dietary Exposure Calculations

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GRAS ASSOCIATES, LLC
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March 24, 2008

Dr. Moraima Ramos-Valle
US Food and Drug Administration
Center for Food Safety & Applied Nutrition (HFS-25)
Harvey W. Wiley Federal Building
5100 Paint Branch Parkway
College Park, MD 20740-3835

RE: Menaquinone-7 GRAS Notification

Dear Dr. Ramos-Valle:

Based on your March 6, 2008 telephone inquiry regarding the food categories and uses of menaquinone-7 that are contained in the subject GRAS Notification, we have prepared alternate dietary intake calculations as summarized in attached Table 1 for your consideration. This representation will hopefully supply you with additional detail on the anticipated consumer exposure levels to menaquinone-7.

The dietary exposure calculations contained in our original submission were based on USDA pyramid data for dairy products. The calculations found in Table 1 were derived from USDA survey data for all individuals, as opposed to eaters-only. Copies of Table 1 and the underlying tables from the USDA survey are enclosed. We believe the pyramid data and the mean data based on all individuals provide reliable estimates for the dietary intake due to the fact that the proposed food uses essentially blanket the dairy products category and its component foods and that consumers, as we stated in our original submission, on a daily basis will likely consume one to three servings of dairy products from the wide variety of choices.

From our calculations, eaters-only data yield results that exceed the estimate based on pyramid data. Using data from all individuals yields results that are compatible with FDA guidance as found in Appendix B, page 20, of the guidelines found on the agency website (http://www.cfsan.fda.gov/~dms/opa2cg8.html). In example 3 for an emulsifier as used in a wide variety of foods, the guidelines state:

> Intake of the emulsifier from the regulated uses was calculated using food consumption data for the total sample, rather than for the eaters-only population, because of the high probability that the entire population would consume some foods containing the emulsifier. Given the likelihood that the number of eaters of at least one of the specified foods would be close to 100%, the choice to use total sample is reasonable.

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Our Panel determination of 50 µg per day consumption of menaquinone-7 for a high user of these products is likely to be an overestimate of daily per person exposure for two reasons: (1) The guidelines state that the "simple sum" of a "point estimate" is probably an overestimate of consumption as opposed to more sophisticated statistical methods using a distributional analysis that more closely reflects actual food consumption patterns; and (2) This analysis makes no attempt to exclude that portion of the diet that is eaten away from home, and, according to NattoPharma, market penetration of menaquinone-7 for products to be served in restaurants and cafeterias, for example, is expected to be very low.

If there are remaining questions on the approach that our Panel has taken in assessing consumer exposure, please feel free to contact us.

Sincerely yours,

(b)(6)

Richard C. Kraska, Ph.D., DABT
Chief Operating Officer
GRAS Associates, LLC

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Table 1

Dietary Estimate for Consumption of MK-7 in Proposed Foods

Food Category[a]	USDA Category	Use Level MK-7 micrograms per serving	Serving Size (grams)	USDA Mean Grams of Food Consumed (Users Only)	Fraction (decimal) of Population represnting users	USDA Mean[b] Grams of Food Consumed (All Individuals)	Mean Micrograms MK-7 Consumed (All Individuals)	Mean x 2 Micrograms MK-7 Consumed (All Individuals)	Refernce Number, Page Number
Hard and soft cheeses including low fat cheese, cheese spreads, cottage and cream cheese	Total Cheese	10	43			16	3.72	7.44	Reference 2, Table 9.4 p. 29
Ice Cream	Ice Cream and Ice Milk	10	157	28	0.166	4.648	0.30	0.59	Reference 1, Table 2.049, p.168
Milk and milk drinks	Fluid milk, total	10	244			194	7.95	15.90	Reference 2, Table 9.4 p. 29
Cream and cream substitutes	Total Fluid Cream	10	15	15	0.592	8.88	5.92	11.84	Reference 1, Table 2.081 p.200
Yogurt and Yogurt Beverages	Yogurt	10	245			8	0.33	0.65	Reference 2, Table 9.4 p. 29
Yogurt (frozen)	Milk Desserts	10	245			27	1.10	2.20	Reference 2, Table 9.4 p. 29
Butter and margarine	Table Fats	10	5			4	8.00	16.00	Reference 2, Table 9.6, p. 31
Total							**27.32**	**54.63**	

[a] MK-7 to be added to these foods or similar subsitutes to the extent not precluded by a food standard

[b] Mean grams food consumed for all individuals taken from Reference 2 or calculated from Reference 1

Reference List

1 Foods Commonly Eaten in the United States
Quantities Consumed Per Eating Occasion and in a Day, 1994-96
Helen Smiciklas-Wright, Diane C. Mitchell, Sharon J. Mickle, Annetta J. Cook, Joseph D. Goldman
http://www.ars.usda.gov/SP2UserFiles/Place/12355000/pdf/Portion.pdf.

2 DATA TABLES: Results from the USDA's
1994-96 Continuing Survey of Food Intakes by Individuals and
1994-96 Diet and Health Knowledge Survey Table Set 10
Food Surveys Research Group, Beltsville Human Nutrition Research Center,
Agricultural Research Service, U.S. Department of Agriculture, 10300 Baltimore Ave.,
Bldg. 005, Rm 102, BARC-West, Beltsville, Maryland 20705-2350
http://www.barc.usda.gov/bhnrc/foodsurvey/home.htm

Table 9.4.--Milk and milk products: Mean quantities (in grams) consumed per individual, by sex and age, 1 day, 1994-96

Sex and age (years)	Percentage of population	Total	Milk, milk drinks, yogurt						Milk desserts	Cheese
			Total	Fluid milk				Yogurt		
				Total	Whole	Low fat	Skim			
	Percent	*Grams*								
Males and females										
Under 1	1.1	749	743	57	39	† 16	0	†*	† 4	† 1
1-2	3.1	474	449	412	262	135	11	11	14	10
3-5	4.7	392	354	319	142	149	22	8	24	12
5 and under	8.9	465	436	318	170	128	15	8	18	10
Males:										
6-11	4.6	448	400	334	117	174	33	6	35	11
12-19	5.8	409	358	303	100	157	40	† 3	29	19
20-29	7.3	240	192	176	59	81	33	4	22	21
30-39	8.3	262	201	172	61	82	25	10	33	21
40-49	7.0	254	197	178	52	82	40	7	34	18
50-59	4.6	223	172	153	33	76	41	5	31	14
60-69	3.4	238	182	166	41	76	47	5	35	15
70 and over	3.4	263	205	187	46	97	41	3	37	14
20 and over	33.9	248	193	172	52	82	36	6	31	18
Females:										
6-11	4.4	382	337	285	107	138	28	3	30	13
12-19	5.6	268	219	189	67	91	30	† 4	29	14
20-29	7.0	208	172	145	58	44	41	7	17	16
30-39	8.8	198	156	128	30	64	31	13	21	17
40-49	6.9	179	134	116	31	50	34	10	25	14
50-59	5.2	195	149	120	20	46	50	17	27	16
60-69	4.1	194	149	128	24	57	43	11	26	14
70 and over	4.9	209	165	149	30	73	45	7	28	11
20 and over	36.8	197	154	131	33	55	39	11	23	15
All individuals	100.0	274	227	191	65	88	35	8	27	16

† See "Statistical notes," appendix B.
* Value less than 0.5 but greater than 0.
Excludes breast-fed children.
SOURCE: USDA Continuing Survey of Food Intakes by Individuals, 1994-96.

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Table 9 6.--Eggs; legumes; nuts and seeds; fats and oils; sugars and sweets. Mean quantities (in grams) consumed per individual, by sex and age, 1 day, 1994-96

Sex and age (years)	Percentage of population	Eggs	Legumes	Nuts and seeds	Fats and oils			Sugars and sweets		
					Total	Table fats	Salad dressings	Total	Sugars	Candy
	Percent	*Grams*								
Males and females										
Under 1	1 1	4	153	†*	†*	†*	†*	† 1	†*	†*
1-2	3 1	16	18	3	3	1	1	17	*	4
3-5	4.7	12	10	5	4	2	2	33	1	8
5 and under	8.9	12	31	4	3	2	1	23	1	6
Males										
6-11	4.6	12	12	5	8	3	4	40	1	13
12-19	5.8	22	17	5	12	3	9	35	2	13
20-29	7.3	27	28	4	15	3	10	23	4	9
30-39	8.3	21	41	4	19	5	10	25	4	6
40-49	7 0	23	29	4	19	5	11	23	5	7
50-59	4.6	24	30	5	20	5	11	25	6	5
60-69	3.4	27	41	4	19	6	10	26	6	5
70 and over	3.4	21	33	4	16	5	8	21	4	3
20 and over	33.9	24	33	4	18	5	10	24	5	7
Females										
6-11	4.4	11	12	4	7	3	4	42	1	12
12-19	5.6	13	14	3	10	2	7	31	2	12
20-29	7.0	16	20	2	14	2	10	20	4	6
30-39	8.8	16	23	3	15	3	9	20	5	5
40-49	6 9	16	19	3	17	3	11	21	4	7
50-59	5.2	16	22	3	18	4	11	20	4	6
60-69	4.1	18	23	2	16	4	9	17	3	3
70 and over	4 9	14	21	3	14	5	7	20	3	3
20 and over	36.8	16	21	3	16	4	10	20	4	5
All individuals	100.0	18	25	4	14	4	8	25	3	7

† See "Statistical notes," appendix B
* Value less than 0 5 but greater than 0.
Excludes breast-fed children
SOURCE USDA Continuing Survey of Food Intakes by Individuals, 1994-96

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Table 2.081. Total Fluid Cream: Percentage of persons using food in 2 days and quantities consumed in a day.

Statistic	All individuals age 2 and over	Age (years) and sex									
		2-5	6-11	12-19		20-39		40-59		60 and older	
		Males and females	Males and females	Males	Females	Males	Females	Males	Females	Males	Females
Number in sample	14,262	2,109	1,432	696	702	1,543	1,449	1,663	1,694	1,545	1,429
Percent of persons using	%										
at least once in 2 days	59.2	60.3	61.2	66.5	59.1	66.7	57.6	58.1	55.0	55.7	51.1
on 1 of 2 days	39.4	40.8	41.2	45.0	40.2	41.1	38.7	40.0	37.3	34.8	36.3
on both days	19.8	19.5	19.9	21.5	18.9	25.6	18.9	18.2	17.6	20.9	14.8
Quantity consumed in a day (1 tablespoon = 15 g)	g										
Mean	15	9	13	14	15	16	13	19	16	22	16
SEM	#	1	1	1	2	1	1	1	1	1	1
5th percentile	1	#	1	1	1	1	#	1	#	1	#
10th percentile	1	1	1	1	1	1	1	1	1	1	1
25th percentile	1	1	1	1	1	2	1	2	1	2	1
50th percentile	3	2	3	3	2	4	3	5	4	10	8
75th percentile	19	12	16	10	15	20	18	29	17	29	22
90th percentile	44	26	32	44	45	46	36	59	41	47	36
95th percentile	60	32	47	73	63	62	49	68	59	76	58

* Indicates a statistic that is potentially unreliable because of small sample size or large coefficient of variation.
\# Indicates a percentage that is greater than 0 but less than 0.05 or a mean, SEM, or percentile that is greater than 0 but less than 0.5.
— Indicates a percentile that could not be estimated
Note: See "Table notes."
Source: USDA's Continuing Survey of Food Intakes by Individuals, 1994-96, 2 days.

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Table 2.049. Ice Cream and Ice Milk. Percentage of persons using food in 2 days and quantities consumed in a day.

Statistic	All individuals age 2 and over	Age (years) and sex									
		2-5	6-11	12-19		20-39		40-59		60 and older	
		Males and females	Males and females	Males	Females	Males	Females	Males	Females	Males	Females
Number in sample	14,262	2,109	1,432	696	702	1,543	1,449	1,663	1,694	1,545	1,429
Percent of persons using	%										
at least once in 2 days	16.6	18.4	21.1	14.2	15.2	14.7	13.6	18.0	14.2	22.7	18.9
on 1 of 2 days	14.2	15.6	18.9	12.4	13.7	13.2	12.5	15.3	12.1	17.1	14.2
on both days	2.4	2.8	2.2	1.8 *	1.4 *	1.5	1.1	2.8	2.1	5.5	4.8
Quantity consumed in a day (1/2 cup hard ice cream = 67 g)	g										
Mean	157	96	140	229	206	202	138	184	145	144	112
SEM	4	4	5	13	20	20	7	8	8	5	5
5th percentile	44	33	51	63 *	39 *	44 *	39 *	64	43 *	33	30
10th percentile	66	35	66	66 *	58 *	66	66	69	50	66	43
25th percentile	74	61	83	132	98	132	68	131	66	66	66
50th percentile	132	66	132	198	134	172	132	133	132	132	100
75th percentile	199	132	171	293	265	265	163	264	197	198	133
90th percentile	266	175	248	385 *	363 *	398	251	304	265	265	198
95th percentile	370	199	265	477 *	398 *	422 *	297 *	396	275 *	293	232

* Indicates a statistic that is potentially unreliable because of small sample size or large coefficient of variation.
\# Indicates a percentage that is greater than 0 but less than 0.05 or a mean, SEM, or percentile that is greater than 0 but less than 0.5.
— Indicates a percentile that could not be estimated.
Note: See "Table notes."
Source: USDA's Continuing Survey of Food Intakes by Individuals, 1994-96, 2 days.

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D. Conditions of Intended Use in Food

Menaquinone-7, in the form of selected MenaQ7 products, is intended to be added to designated foods as a nutrient supplement as defined in 21 CFR 170 3(o)(20) The per serving levels added to dairy products (see Section IV A for the specific foods constituting the dairy products category) and butter and margarine substitutes range from 6 7 – 10 mg of MenaQ7 to yield a total daily dietary consumption of 50 µg of menaquinone-7 as a source of vitamin K_2

E. Basis for the GRAS Determination

Pursuant to 21 CFR 170 30, menaquinone-7 as MenaQ7 has been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR 97702-3074

IV. Intended Food Use and Dietary Estimates

A. Intended Use of MenaQ7 in Food

NattoPharma intends to add the two existing MenaQ7 products to selected processed food categories as a nutrient supplement as defined in 21 CFR 170.3(o)(20). The MenaQ7 products will be added at levels ranging from 6.7 - 10 mg/serving to deliver 10 µg of MK-7 per serving of food. NattoPharma is developing other MenaQ7 products of varying concentrations of MK-7 for formulation flexibility for the food industry

The foods to which MenaQ7 will be added include dairy products and butter and margarine substitutes The specific foods constituting the dairy products category consist of the following hard and soft cheeses including low fat cheese, cheese spreads, cottage and cream cheese, ice cream; milk and milk drinks; cream and cream substitutes, yogurt and yogurt beverages; and yogurt (frozen) NattoPharma recognizes that certain foods may have compositional restrictions because of specified food standards, for those foods MenaQ7 can only be added if allowed by the food standards or if incorporated into substitute or alternate foods that are not subject to the food standards limitations

B. *Estimated Dietary Intake for the United States*

USDA survey data indicate that the average consumption of total dairy products for Americans is 1 5 servings per day.[8] Based on FDA guidance, a level of twice the average (or 3 servings per day) can be considered an overestimate of the 90th percentile of total dairy products (FDA, 2006)

USDA survey data indicate that the average consumer consumes 4 g/day of table fats, the category which includes butter and margarine products [9] Given the serving size of 5 g, this equates to 0.8 servings per day for the butter and margarine type products, at twice the consumption of the mean to estimate the 90th percentile consumer, we obtain the estimated consumption of butter and margarine products to be about 1.6 servings per day

To deliver 10 µg of MK-7 per serving of food, 6 7 mg per serving of MenaQ7 Oil M 1500 (which contains 0 15% MK-7) will be added to the designated food When adding MenaQ7 Powder P 1000 (which contains 0.10% of MK-7), 10 mg per serving will be added to the designated food

Thus, a total daily dietary intake of MK-7 using either MenaQ7 Oil M 1500 or MenaQ7 Powder P1000 as described above provides 50 µg of MK-7. Since the MenaQ7 Oil M 1500 is 95+% sunflower oil, 5 total servings of the designated foods would provide about 35 mg/day of sunflower oil to the diet. At 5 servings per day, the consumption of added components of MenaQ7 Powder P 1000 products will be as represented in Table 7

---

[8] USDA, 1999 Table 4a Pyramid Servings Data for Dairy Products

[9] USDA, 1997 Table 9 6 Eggs, legumes; nuts and seeds, fats and oils, sugars and sweets Mean quantities (in grams) consumed per individual, by sex and age, 1 day, 1994-95.

to 100 µg/day would not interfere with anticoagulant therapy The same group concluded in a subsequent study (Schurgers et al , 2007a) that, because of the greater half life of MK-7, doses greater than 50 µg/day should be avoided by patients on anticoagulant therapy.

The Panel is of the opinion that 50 µg/day of MK-7 is rather conservative. FDA considered the need for a warning label directed at these patients on vitamin K-enriched foods containing approximately 40 µg/serving of phylloquinone and decided that requiring a warning label was not necessary [13] These patients are actively monitored for blood levels of anticoagulants, and physicians can readily adjust their dosages and diets.

The Panel concluded that MK-7 is safe at a level of 1 mg per day or more for healthy adults. Because of the possible interference with anticoagulant therapy, the Panel recommends a conservative consumer exposure to assure that consumption does not consistently exceed 50 µg/day. Based on the desire of NattoPharma to supply 10 µg per serving in dairy products as defined in Section IV A and butter and margarine substitutes, and by considering the USDA food intake survey data referenced in Section IV B , the Panel is satisfied that consumers are not likely to consume more than 5 servings of these foods per day. The Panel agrees that the proposed limitation to use in dairy products and butter and margarine substitutes will adequately limit consumption of MK-7 which obviates the need for a warning label for consumers on anticoagulant therapy.

If a consumer were to eat on a chronic basis a combination of foods that are naturally high in vitamin K, along with foods fortified with MenaQ7, as well as ingestion of a dietary supplement containing vitamin K, it is difficult to imagine that the total vitamin K consumption would exceed 300 µg/day (5 0 µg/kg for 60 kg adult). Even so, this level of consumption would remain within the range which NAS considered to be safe. In view of the variability of natural vitamin K in the diet, it is not likely that levels this high could be sustained over long periods. The Panel agrees that levels of MK-7 up to 100 µg/day would be safe even with patients on anticoagulant therapy. However, NattoPharma has adopted the prudent practice of limiting the addition to foods as noted elsewhere to keep consumption levels at or below 50 µg/day.

The Panel has reviewed the other ingredients used in the manufacture and formulation of MenaQ7. As indicated in Table 4, all ingredient used in the formulation are adequately covered by applicable FDA clearances The Panel concludes that consumption of these ingredients at the levels present in a total of 5 servings of dairy products and butter and margarine substitutes combined is safe

C. Common Knowledge Element of GRAS Review

As discussed in Section VI.A, the two components comprising the common knowledge requirement must be satisfied for any GRAS determination

Regarding the first element, the Panel recognizes that the vast majority of the studies relied upon in the GRAS evaluation has been published and is available in the open scientific literature. The information on the definition of natto, its common use in Japan and other information on fermented soybean products is also readily available on the internet and other public sources

[13] See 68 FR 43692, 2003

VII. Conclusions[14]

Expert panels of scientists have indicated that no upper limit is needed for vitamin K consumption, and other experts in the field have recommended an increased consumption of vitamin K_2 to enhance bone and cardiovascular health. Ample scientific evidence exists on MenaQ7 and MK-7 to conclude that the anticipated human food use as a nutrient supplement in dairy products as defined in Section IV.A and butter and margarine substitutes when consumed at daily levels up to 50 µg/day by adults is generally recognized as safe.

The preponderance of the scientific evidence has established that FDA's food ingredient safety standard of "reasonable certainty of no harm under the intended conditions of use" has been met for menaquinone-7 and MenaQ7; consequently, MenaQ7 has attained GRAS status and is exempt from premarket approval requirements.

In conclusion, adult human exposure to MenaQ7 as an ingredient added to dairy products and butter and margarine substitutes at levels up to 10 mg per serving (to provide an equivalent of up to 10 µg of MK-7 per serving) is generally recognized as safe (GRAS). This conclusion requires that MenaQ7 is produced in accordance with Good Manufacturing Practices and that the finished product meets appropriate food grade specifications.

(b)(6)

February 22, 2008

Richard C. Kraska, Ph.D., DABT — Date

(b)(6)

February 22, 2008

Robert S. McQuate, Ph.D. — Date

(b)(6)

February 22, 2008

Stanley T. Omaye, Ph.D. — Date

[14] Appendix B contains the educational and professional backgrounds for Richard C. Kraska, Ph.D., DABT, Robert S. McQuate, Ph.D., and Stanley T. Omaye, Ph.D. Each has extensive technical background in the evaluation of food ingredient safety. Drs. Kraska and McQuate each worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this are in the private sector. Dr. Omaye is a professor of nutrition at the University of Nevada, Reno and has published extensively on the nutritional and toxicological aspects of food ingredients. Dr. Kraska served as Chair of the Panel.

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Generally Recognized As Safe
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June 2, 2008

Ms. Blondell Anderson
Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-265)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification 245 --- Menaquinone-7

Dear Ms Anderson:

On behalf of NattoPharma ASA, we request that you cease the evaluation of the above-referenced GRAS notice addressing menaquinone-7.

We may elect to resubmit a modified notification at a future date.

Thank you.

Sincerely,

(b)(6)

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
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541-678-5522
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SU

GRAS ASSOCIATES, LLC

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September 12, 2008

Ms. Blondell Anderson
Office of Food Additive Safety (HFS-265)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

RE: NattoPharma, ASA
Prenotice Communication (GRN)

Dear Ms. Anderson:

GRAS Associates, LLC has submitted GRN 000245 on behalf of NattoPharma, ASA regarding the the intended food uses of menaquinone-7 (MK-7) in dairy products, butter and margarine. This notification was withdrawn from FDA review as requested in our letter of June 2, 2008. NattoPharma intends to resubmit a modification of this notice in the near future. Consequently, we request a meeting with FDA staff to discuss issues and concerns raised during the conference call held on May 29. We are providing you with additional information for agency review and discussion at the meeting.

We wish to discuss the following issues at the meeting:

1. **The natural occurrence of MK-7 and other menaquinones in the human diet.** Attachment A shows that there is a significant amount of naturally occurring Menaquinones (specifically including MK-6 to MK-8) in western diets---including the US-- which likely fulfills at least a moderate level of the dietary supplement of vitamin K. Significant MK-4 is provided by eggs and significant MK-8 and MK-9 are provided by various cheeses. Much higher levels of MK-7 have been shown in diets in Japan, especially for people eating natto, a fermented soybean product. Although we are not asserting that MK-7 is GRAS based on history of use in the US, we believe this dietary experience warrants consideration as a factor in the scientific judgement required in the safety assessment.

2. **Bioavailability and Safety of MK-7.** Attachments B and C discuss the relative bioavailability of MK-7 to MK-4. Attachment B estimates that MK-7 is 6-10 fold more bioavailable than MK-4 and phylloquinone. MK-4 and phylloquinone are similar in molecular weight and are likely to exhibit similar bioavailabilities. We assert that there is ample safety data---and 20 years of clinical experience---on MK-4 to show that 45 mg/day is safe and that these data can be applied to assess the safety of low levels of structurally related menaquinones such as MK-7

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and MK-6. In addition, the existing safety data on MK-7, including several clinical studies, additional data found on the Japanese government website for its National Institute of Health (Attachment D), including a 90-day rat study on a natto product where the highest dose tested was equivalent to 13 µg/kg of MK-7, and the level of its natural occurrence, collectively demonstrate that MK-7 is safe at the proposed usage level of 50 µg/day. In view of the approximate 1000-fold difference between the safe level of MK-4 to the proposed use level of structurally related MK-7, there is an ample margin of safety to account for a 6-10-fold higher bioavailability of MK-7.

3. **Safety of MK-6.** NattoPharma's product contains up to 6% MK-6. Attachment A shows that MK-6 naturally occurs with MK-7 and is normally present in natto, which is the raw material for NattoPharma's product and is the food source which accounts for the high levels of MK-7 in the diet of Japanese people. Since MK-6 is less lipophilic than MK-7, it is expected to be less bioavailable than MK-7. Further arguments are included in Attachment C. It should be noted that although some forms of natto contain more MK-8 than MK-6, there is no measurable MK-8 in NattoPharma's product.

4. **Reproductive effects of MK-4.** FDA noted potential reproductive toxicity in one of the studies provided (Minaki et al.) FDA was particularly concerned about the data in Table 8 in the publication of this study concerning neonatal death. The number of neonatal deaths observed from a total of 24 dams at each dose was as follows:

Dose Group	Number of Neonatal Deaths Observed Within 4 days of birth	Percentage of Total Neonates
Control	9	2.9%
10 mg/kg	30	10.1%
100 mg/kg	7	2.3%
1000 mg/kg	21	6.6%

Clearly, this effect is not dose related and likely indicates uncontrolled sources of variability in this one study. There was no evidence of any neonatal deaths with MK-4 in mice or rats (Suzuki et al. 1971), no teratology or effect on the neonate in rats (Goto et al., 1986) and no teratology in the rabbit (Ohsumi et al., 1985). We have reexamined the literature on the potential effects of MK-4 on reproductive performance and the neonate and have found some additional references. It is our opinion based on our review of these studies that there is no indication that menaquinones as a class of compounds present any hazard to reproductive performance or to the neonate. A complete list of these references can be found in Attachment E. English translations are also attached for those studies which were published in Japanese.

5. **Concern about patients on anti-coagulant therapy.** We note FDA's concern on this issue from the conference call, and we would would like to discuss possible alternative approaches with FDA at the meeting. This issue is also addressed in Attachment C by Dr. Leon Schurgers. Two important studies that will be discussed are Schurgers et al. (2004, 2007).

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We would like to suggest meeting with you in mid-November and would like to discuss possible dates as soon as possible so that our team can make travel plans, which include traveling from Norway and the Netherlands. If there are any questions, please contact me at the phone number or email address listed above.

Sincerely,

(b)(6)

Richard C. Kraska, Ph.D., DABT
Chief Operating Officer and Co-Founder
GRAS Associates, LLC

RCK:lsm

Enclosures: Attachments A through E

000087.003

ATTACHMENT A

The Natural Occurrence of MK-7 and Other Menaquinones in the Human Diet

Menaquinones in the US diet

A summary of US cheese and egg consumption and consequences for intake of higher menaquinones in the US population

Prepared for obtaining a "Non Comment " letter from Food and Drug Administration, US as a response to GRAS filing for the products branded MenaQ7 from NattoPharma ASA, Norway

By Anne Bjørnebye Vik, PhD, MBA

Vice President Research and Development NattoPharma ASA

INTRODUCTION

Vitamin K2 as natural menaquinone-7 (Branded as MenaQ7 from NattoPharma) – is submitted to FDA for GRAS evaluation. FDA has questioned the content of other menaquinones (MKn) than MK-7 in the product, especially MK-6 and MK-8 for potential safety risks.

In this paper we have highlighted the facts that

- ✓ MK-6 and MK-8 are natural menaquinones present in fermented products, especially in fermented cheese that are sold in large quantities all over the world, included in the US
- ✓ The doses consumed by US citizens of MK-6 and/or MK-8 are comparable to cheese consuming populations in other parts of the world
- ✓ There are no adverse reactions associated with the daily dietary intakes of products containing MK-6 or MK-8
- ✓ Our MenaQ7 products contain MK-7 with 1-3 % of MK-6 and no detectable MK-8
- ✓ MK-7 in MenaQ7 products originates from Natto food. Natto foods have safely been consumed by Asian populations, especially Japanese people in Japan but also Japanese populations in western countries like the US, UK, Germany and elsewhere in hundreds of years.

This paper makes use of official statistics and published papers to support the above statements. The following data is used for the purposes outlined below:

- Cheese production in the Us and annual consumption/capita – ref USDA statistics
- Egg production and consumption/capita

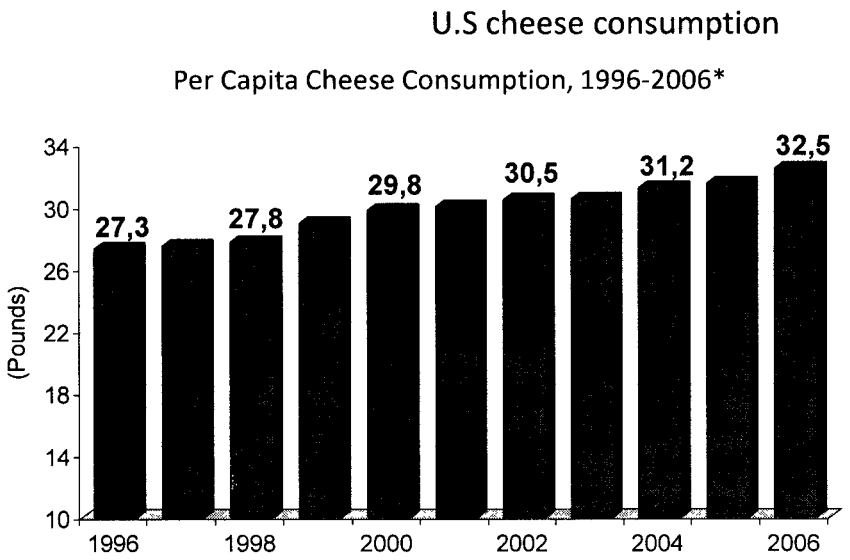
- Vitamin K1 (phylloquinone) and vitamin K2 (menaquinones- 4 to menaquinone -8) content of ordinary foods bought in supermarkets in Europe
- Estimates of content of vitamin K1 and vitamin K2 – as MK-4 to MK-8 - in US consumed cheese and eggs/day based upon Dutch vitamin K measurements
- Comparison of cheese consumption/capita in the US and in the Netherlands
- Comparison dietary intake of vitamin K2 as MK-4 to MK-8 in the US and in the Netherlands; and comparison to the dietary intake of vitamin K1 and vitamin K2 in the Rotterdam study from 2004
- Consumption of menaquinone-7 from Natto food in Japan
- Safety consideration for menaquinone-7 from the Japanese Health Authorities
- Batch to batch variation of content of MK-7 and MK-6 in MenaQ7 products

References cited in this review are listed at the end of Attachment 1.

US cheese consumption 1981 to 2006

Menaquinones are naturally derived components from bacterial fermentation of cheeses and other products like the Japanese dish Natto. To evaluate the consumption of such naturally occurring menaquinones, cheese consumption has been evaluated in the US and elsewhere. Based upon laboratory analyses of menaquinone content in various cheeses, we have calculated the average US citizen menaquinone consumption.

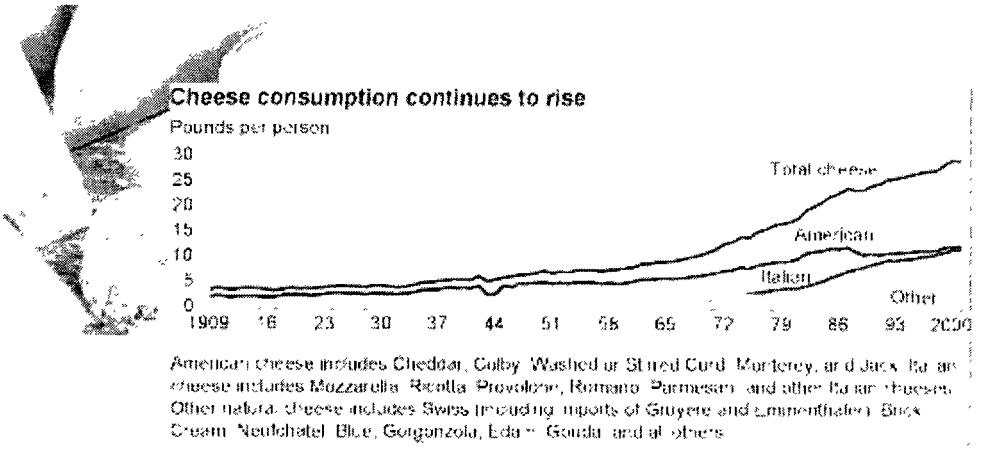
Fig 1: US cheese consumption [1]

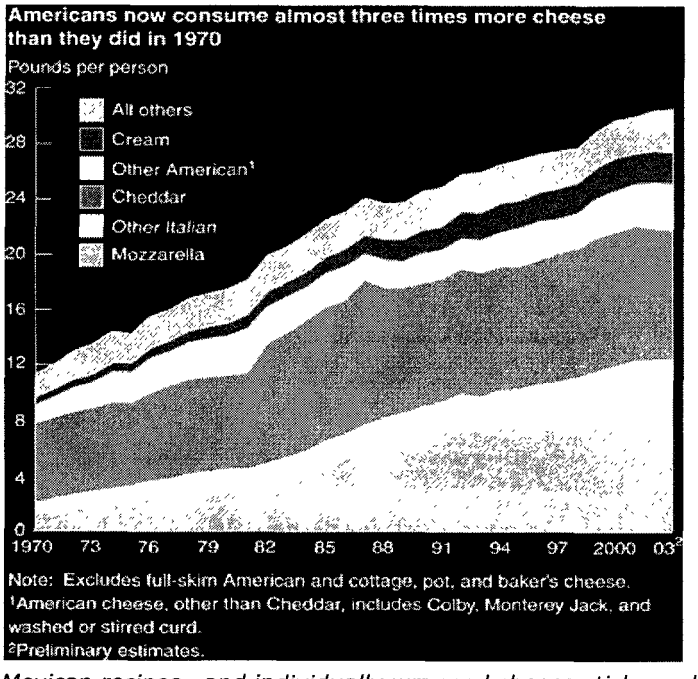


** Preliminary estimate.*

Source USDA/ERS, Livestock, Dairy, and Poultry Outlook.

Statistics from United States Department of Agriculture, Economic Research Service (ERS)[2] show consumption of a large selection of food products over the years in the US. Data for cheese[3] and egg consumption is used. Fig 2 shows statistics for cheese consumption from 1909 to 2003, Fig 3 a more detailed picture of the various kinds of cheeses consumed.



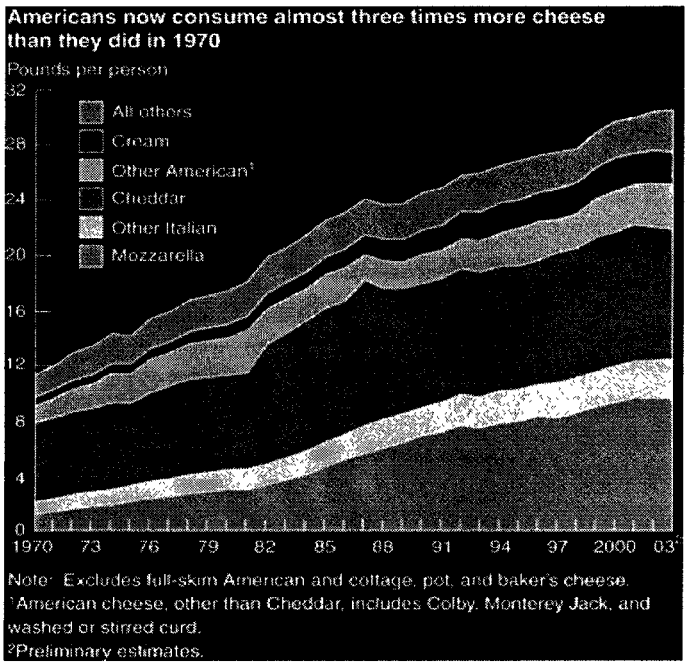


According to Jean Buzby [4](Amber Waves – The Economics of Food, Farming, Natural Resources, and Rural America) the market can be described as:

"Demand for easy, time-saving strategies and cheese's utility in adding rich flavor to quick-serve meals (like soups, salads, pastas, sandwiches, cooked vegetables, eggs, and countless other dishes) are driving the rise in cheese consumption since 1970. The boom in eating out and ordering in parallels cheese's use as a major ingredient in food manufacturing (roughly 60 percent of our cheese now comes through these channels). For at-home consumption, re-sealable bags of shredded cheeses—including cheese blends tailored for use in Italian and Mexican recipes—and individually wrapped cheese sticks and baby Goudas have made cheese even more of a household staple. Average U.S. cheese consumption nearly tripled between 1970 and 2003, from 11 pounds per person to 31 pounds."

In this paper we have selected examples of hard and soft cheeses. Detailed information on the US consumption of various cheeses per capita per year is found in ref [5]

3



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In this paper we have selected examples of hard and soft cheeses. Detailed information on the US consumption of various cheeses per capita per year is found on the web page: http://future.aae.wisc.edu/data/annual_values/by_area/2191?tab=sales.

Fig 4 show the distribution of cheeses consumed in 2006 (amounts consumed in US pounds).

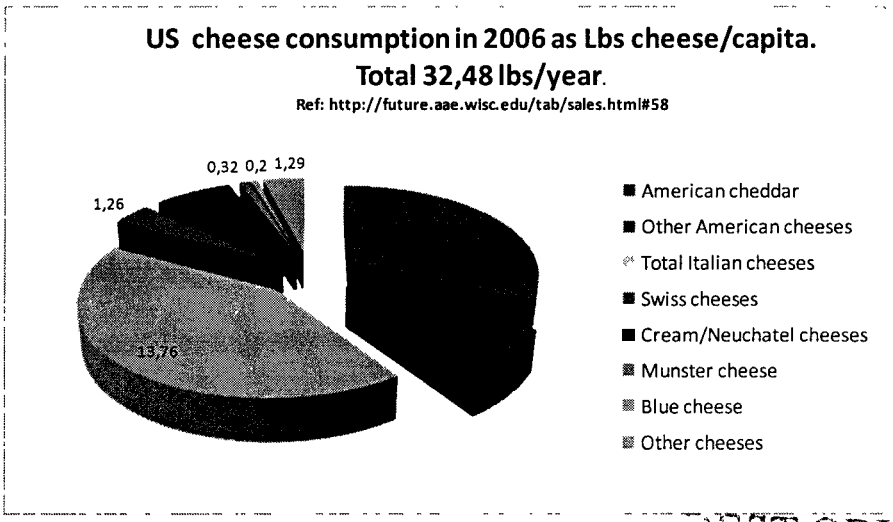

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Table 1 gives the figures in kilograms.

We have used cheddar, Swiss and cream cheeses in this paper. These contribute about 44 % to the total cheese consumption in the US. *(Must check the MK content in Italian cheeses- these represent 42, 4 % of total consumption)*

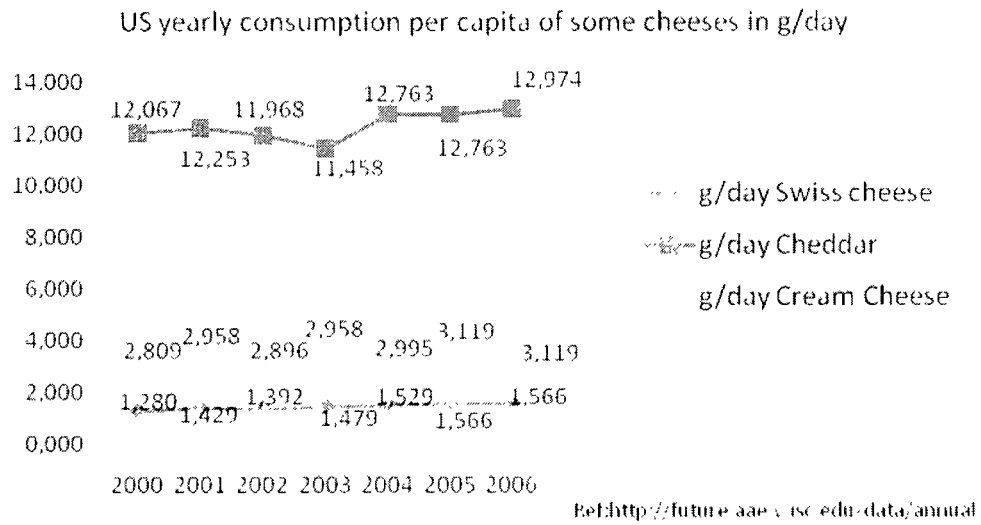


Figure 5

The cheeses illustrated give an average daily consumption per capita (all year around= 365 days) of 16-18 grams/day[6]. As previously shown this is about 44% of the total daily consumption per capita.

If Italian cheeses were included (17, 10 g/day) – the daily cheese consumption (Cheddar, Swiss, cream and Italian cheeses) would be (12,97g +1, 57g+3, 12g+17,1g) = 34, 76 g/day which is 86% of total consumption/capita- ref fig 1 and fig 4 and table 1.

Egg statistics from the US can be traced back to 1909[7] and shows that the annual production of eggs per capita has changed over time: From 292 eggs in 1909 to all time high in 1945 with 421 eggs/capita to the 2006 figures of 251 (ref 1) – 256 eggs/capita/year(ref 2) respectively.

In the following calculations we have used 250 eggs/capita/year for 2006 as well as that the weight of an egg is 58 grams.

To estimate the content of vitamin K1 and menaquinones we have used the data published by Schurgers[8]. Another table for content of Menaquinones is found in an article by Sakano et al[9]

The full table from Schurgers covering K1, MK-4, MK-5,MK-6,MK-7 and MK-8 in 51 different food and drink products is attached (Attachment 1). From this table values found for K1 and menaquinones in hard (Cheddar and Swiss cheese) and soft cheeses (Cream cheese /Neufchatel) are used in the following sections. As can be seen in fig 6 there is hardly any menaquinones in yoghurt. Yoghurts are thus not included in the following.

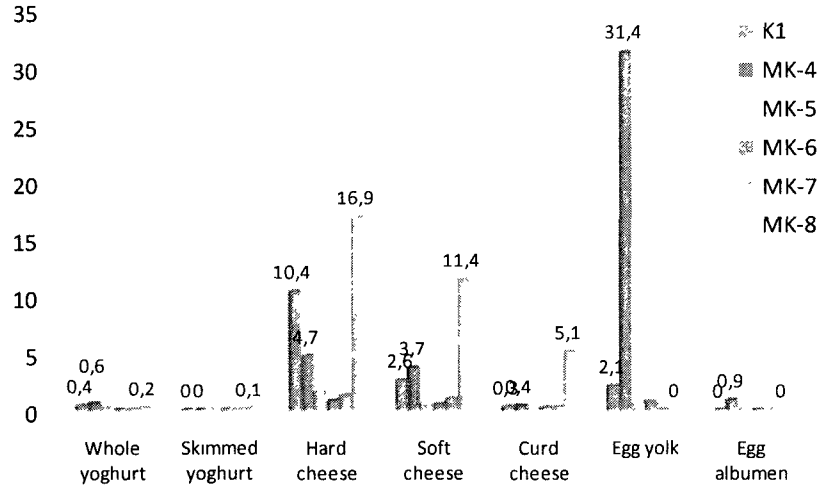


Figure 6

From fig 6 it can be calculated that on average 100 grams of hard cheese bought in the supermarket contains about 20 ug Vitamin K2 (sum of MK5 to MK-8); while soft cheese contains a bit less, approximately 13 ug of vitamin K2 (sum of MK-5 to MK-8). In addition these cheese products contain some MK-4.

Table 2

	ug/100 g K1	ug/100 g MK-4	ug/100 g MK-5	ug/100 g MK-6	ug/100 g MK-7	ug/100 g MK-8	ug/100 g Sum MK-5-8
Whole yoghurt	0,4	0,6	0,1	0	0	0,2	0,3
Skimmed yoghurt	0	0	0	0	0	0,1	0,1
Hard cheese	10,4	4,7	1,5	0,8	1,3	16,9	20,5
Soft cheese	2,6	3,7	0,3	0,5	1	11,4	13,2
Curd cheese	0,3	0,4	0,1	0,2	0,3	5,1	5,7

Eggs contain approximately 30 – 32 ug/100g of vitamin K2 as MK-4- the majority of which is found in the egg yolk (see table 3).

Measurements from Schurgers et al show the following vitamin K content of eggs

Table 3	ug/100 g	ug/100 g	ug/100 g	ug/100 g	ug/100 g	ug/100 g	ug/100 g
	K1	MK-4	MK-5	MK-6	MK-7	MK-8	Sum MK-5-8
Egg yolk	2,1	31,4	0	0,7	0	0	34,2
Egg albumen	0	0,9	0	0	0	0	0,9

To estimate the daily intake of vitamin K2 per capita per day in the American population we have used the consumption of 3 kinds of cheeses and 1 egg. The amount of the different menaquinones (MK-4 through MK-8) are measured by Schurgers (2002) for hard and soft cheeses and eggs bought in the supermarkets in the Netherlands.

These values are shown in table 4.

Table 4

Analytical values of vitamin Ks		**MK-4 ug**	**MK-5 ug**	**MK-6 ug**	**MK-7 ug**	**MK-8 ug**
Hard cheese per 100 g	Ref Schurgers , 2002	4,7	1,5	0,8	1,3	16,9
Soft cheese per100 g	Ref Schurgers , 2002	3,7	0,3	0,5	1	11,4
Egg per 100g	Ref Schurgers , 2002	31,4	0	0,7	0	0
Albumen per 100g	Ref Schurgers , 2002	0,9	0	0	0	0

Table 5 shows the dietary intake from 44% of a per capita cheese consumption and 1 whole egg. Fig 7 shows the same distribution.

Table 5: Estimation of vitamin K2 in cheeses and 1 egg consumed in the US

Food	**Amount- grams**	**MK-4 ug**	**MK-5 ug**	**MK-6 ug**	**MK-7 ug**	**MK-8 ug**	**Total K2 ug**
Cheddar- grams	12,32	0,58	0,18	0,10	0,16	2,08	3,10
Cream Cheese - grams	2,98	0,11	0,11	0,01	0,03	0,34	0,60
Swiss cheese - grams	1,46	0,07	0,07	0,01	0,02	0,25	0,41
Egg yolk- one egg	58	18,21	0,00	0,41			18,62
Egg albumen- one egg	58	0,52					0,52
Total each MK		**19,49**	**0,36**	**0,53**	**0,21**	**2,67**	**23,26**

Consuming 16,76 grams of cheeses and one egg daily equals intake of 23,3 ug of vitamin K2. As can be seen the major contribution comes from the egg and MK-4, while the rest is a mix of the higher menaquinones.

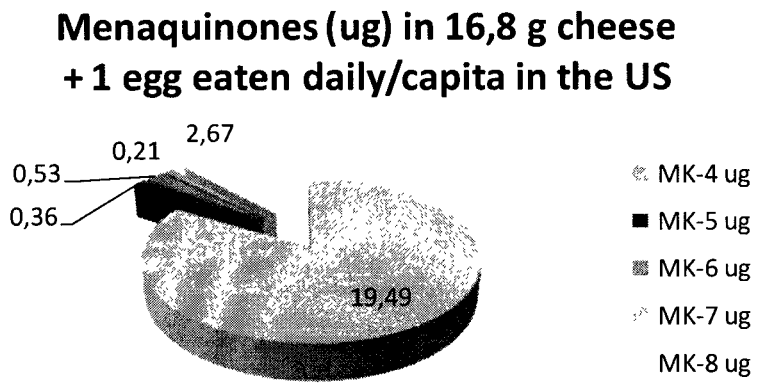


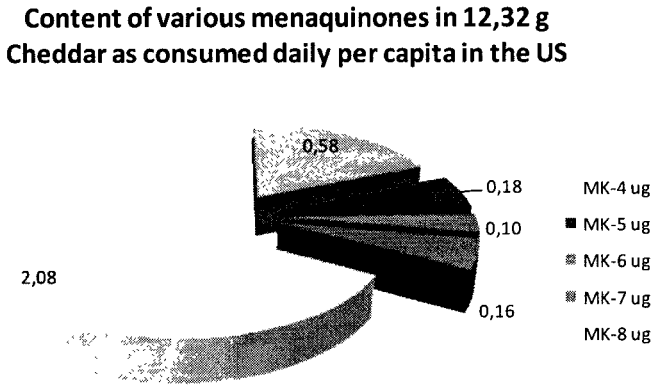
Fig 7.

Each American is consuming 9,53 pounds(lbs) or 4,32 kg Cheddar cheese /year. This equals to 12,32 grams/day. According to analysis performed in VitaK (Schurgers 2002) hard cheeses like cheddar contains several of the menaquinones as shown in table 6 and Fig 8 for cheddar cheese below.

Table 6 : Dietary consumption of menaquinones from 16,76 grams of cheeses

Food	Amount-grams	MK-4 ug	MK-5 ug	MK-6 ug	MK-7 ug	MK-8 ug	Total Vit K2
Cheddar	12,32	0,58	0,18	0,10	0,16	2,08	3,10
CreamCheese/Neufchantal	2,98	0,11	0,11	0,01	0,03	0,34	0,60
Swiss cheese	1,46	0,07	0,07	0,01	0,02	0,25	0,41
Total	16,76	0,76	0,36	0,13	0,21	2,67	4,12

Fig 8: Menaquinones in Cheddar cheese – a best seller in the US with market share of >32 % (ref fig 4)



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The average weight of an egg is 58 grams. The US annual consumption is 250 eggs

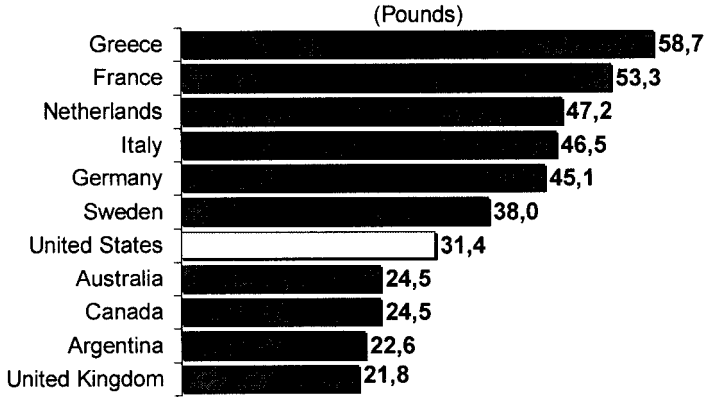
Table 7 : Eggs	K1 ug	MK-4 ug	MK-5 ug	MK-6 ug	MK-7 ug	MK-8 ug
Vitamin K per egg in ug	1,218	18,212	0	0,406	0	0
Total Vitamin K in 250 eggs	304,5	4553	0	101,5	0	0

The average US consumer digests vitamin K2 in the form of 4,5 mg MK-4 and 0,101 mg MK-6 per year from eggs.

Fig 9 shows the comparable cheese consumption in the US and several European countries as well as Australia, Canada and Argentina[10]. As can be seen Greece consumes almost twice as much cheese as the US, while the Netherlands consume 1,24 times more than in the US.

International Cheese Consumption

Per Capita Consumption, Selected Countries, 2005



Source USDA/FAS, Production, Supply and Distribution Online Database; EuroStat Yearbook, US Census Bureau, International Database; USDA/ERS, Livestock, Dairy and Poultry Outlook.

It can be seen from this figure that the per capita cheese consumption in the US: 31,4 lbs or 14,24 grams is comparable to the previous estimates in table 5 of 16-17 grams; the latter giving a total of 4 ug higher menaquinones/day/capita when eating 3 different kinds of cheese.

In the Netherlands the consumed amount of cheese (47,2 lbs or 21,4 grams/day) is estimated in table 8.

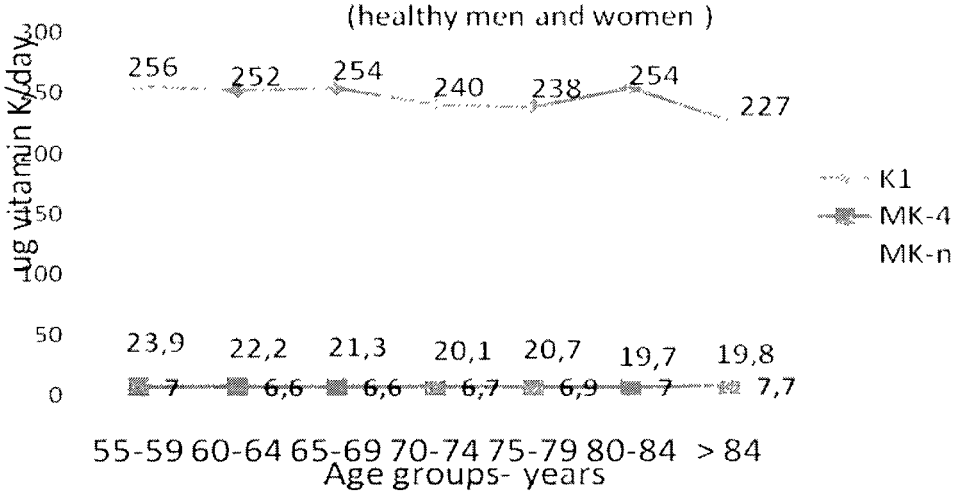
Table 8

Consumption Netherlands	MK-4 ug	MK-5 ug	MK-6 ug	MK-7 ug	MK-8 ug	Total Vit K2
Hard cheese 100 g	4,7	1,5	0,8	1,3	16,9	
Hard cheese 21 g	0,987	0,315	0,168	0,273	3,549	5,292
Soft cheese 100 g	3,7	0,3	0,5	1	11,4	
Soft cheese 21 g	0,777	0,063	0,105	0,21	2,394	3,549

According to figure 9 the Dutch population eats an average of 21 g cheese daily. If this is hard cheese the vitamin K2 intake is approximately 5ug/day- if it is soft cheeses- the intake is in the range 3-4 ug/daily per capita.

Very few laboratories analyze food samples for vitamin K1 and menaquinone content. As previously referred to, Dr. Schurgers et al has published a list of vitamin Ks in 51 food and drink products. These analysis are also part of a large population based study in the Rotterdam area in the Netherlands[11]. Dietary information from 5435 healthy subjects has been collected, and the various foods have been analyzed for vitamin K content using standard HPLC method.

Vitamin K as K1(phylloquinone) and vitamin K2 as MK-4 and sum of MK- 5 to MK-8 in the Dutch population enrolled in the Rotterdam study - a total of 5435 subjects (healthy men and women)



It can be seen from fig 10 that the adult population in the Rotterdam area consumes an average of 20 ug/day of the higher menaquinones – MKn (sum of MK-5 to MK-8). In addition they consume approximately 7 ug MK-4/day. Thus this cohort of people consumes 4-5 times as much menaquinones from cheese as the average cheese consumption/capita.

Conclusion

The evaluation of menaquinone intake from a diet of small amounts of cheese (44% of annual consumption per capita) and one egg in the US states that:

- All forms of menaquinones – from MK-4 through MK-8- is present – and has been present for very many years in high volume dairy products in the US market
- The average US citizen consumes all forms of menaquinones – from MK-4 through MK-8 in ordinary cheese products. MK-8 concentration is especially high compared to the other menaquinones.
- Estimates of the amount of menaquinones in American dairy products – included eggs- show that the US citizen consume about the same amount of these vitamin K2 derivatives (MKn) as Dutch population
- A large population based study from the Netherlands- the Rotterdam study – show that adults consume 4-5 times as much menaquinones of all types compared to the US average

citizen. No adverse effects from eating such food products have been recorded- on the contrary- the higher the consumption of menaquinones in this study, the better cardiovascular health.

- Table 9 summarizes the findings based upon official statistics and published studies.

Table 9

Dietary intake Menaquinone from cheese

	MK-4 ug	MK-5 ug	MK-6 ug	MK-7 ug	MK-8 ug	Total Vit K2
US: 44% of annual cheese consumption	0,76	0,36	0,13	0,21	2,67	4,13
Netherland: 21 g soft cheese	0,99	0,32	0,17	0,27	3,55	5,30
Netherland: 21 g hard cheese	0,78	0,06	0,11	0,21	2,39	3,55
Rotterdam study: Average consumption	7					20

Menaquinones from Natto food

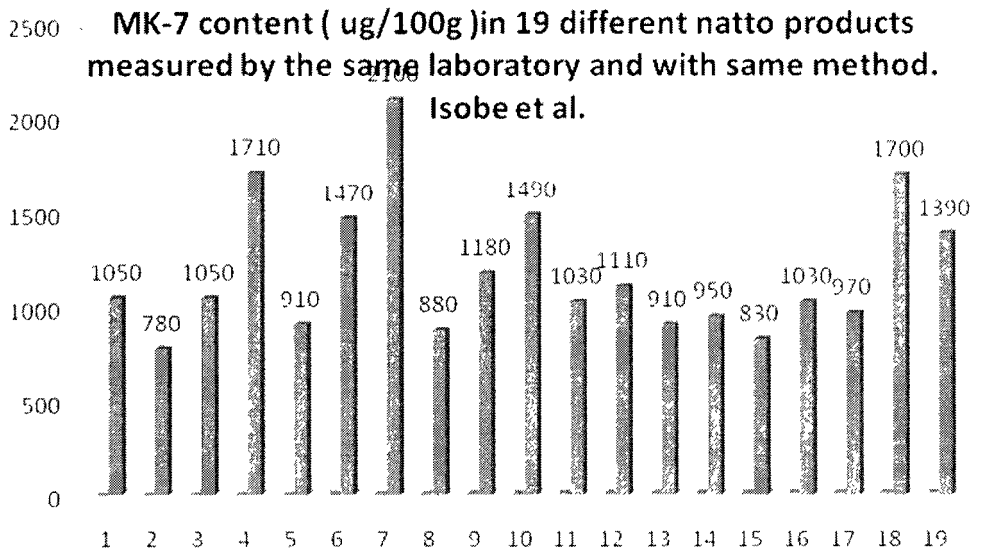
Natto food has a very long tradition in Asia and especially in Japan, where it has been consumed for hundreds of years. Many commercial products are on the Japanese market. Natto food contains about 1,4% MK-8 (personal communication Prof C. Vermeeer). This means that MK-8 has been consumed in relatively large amounts also in Japan.

Table 10 shows references to studies using commercial Natto products where the content of MK-7 has been analyzed.

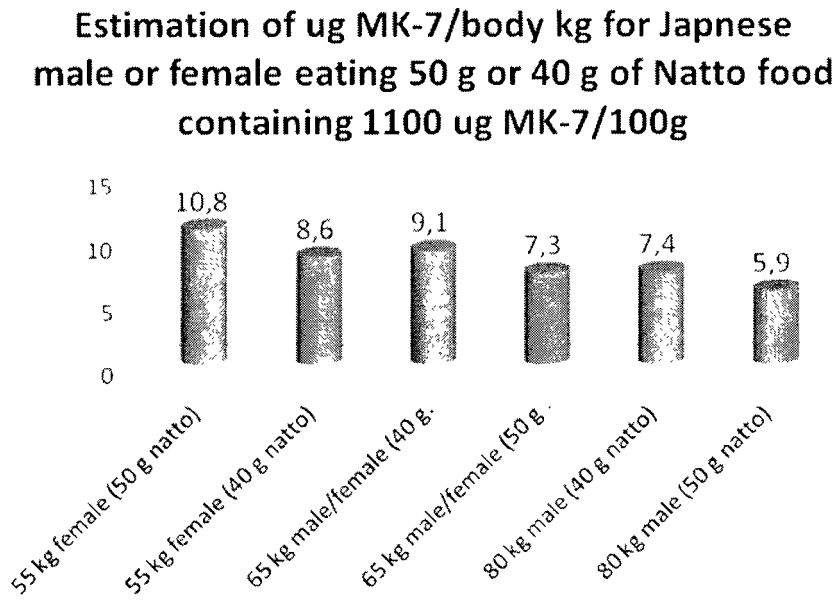
Table 10 MK-7 Content in Commercial Natto Products Sold in Japan

Reference	Natto product	µg MK-7/100g Natto
Schurgers, LJ Thesis, 2002, p. 37	Unknown, product from supermarket Maastricht	993
Sumi, H, 1999. Food Sci Tech Res vol 5	B subtilis Miyagino strain	1230
Tsukamoto et al 2000, J.Health Sci 46	Natto from Asahi Freshia Co Ltd	865
Tsukamoto et al 2000 J Bone Min Meta	Natto from Asahi Freshia Co Ltd	775
Kanki et al 2001.Nutrition vol 17	Natto from Shoujikimura Ltd	1398
Katsuyama et al 2004 J Nutr Sci Vitam 50	Natto - unknown source	900
Kamao en al 2007 J Nutr Sci Vitaminol	Natto - unknown source	939
Isobe,et al: Natto and vitamin K. in Basic and Clinical Aspects of Japanese Traditional Food Natto. Ed Sumi, Hiroyuki. Page 73-82. 1995. Japanese Technology Transfer Association.	19 different commercial Natto products (average MK-7 value)	1186

Fig 11 shows the distribution of MK-7 content in 19 different commercial natto food products sold in Japan[12] .



The average MK-7 content is approximately 1100 µg/100 gram Natto. Intake of MK-7 from one serving of Natto (which is approximately 40 g to 50 g) has been estimated for different body weights. Body weights were estimated from several published Japanese studies, including persons in the Japanese Population-Based Osteoporosis (JPOS) study [13]. Low body weight men (65 kg) are comparable to high body weight women (65 kg), while average body weight for Japanese women in several studies (see also Ikeda *et al.*, 2006) is set to be 55 kg. The resulting daily intakes of MK-7 in ug/kg body weight are shown in Fig 12



No adverse effects have been reported from daily Natto consumption in hundreds of years. Furthermore, the safety of Natto has been evaluated by the National Institute of Health in Japan.

Safety Evaluation of Natto by Japanese Authorities

The National Institute of Health in Japan has evaluated the safety of the Natto product "Kinnotsubu", containing 650 µg MK-7/50 g (http.//hfnet nih.go jp/contents/detail405.html).

Unofficial summary translation:

"[Human test]
Twelve (12) healthy adults (age 29 to 50) took 150 g portion of the Natto (equivalent to 1.95 mg/kg body weight/day MK-7) for 7 days. Serum parameters and coagulating system parameters have not been changed compared to the values before starting this test.

[Animal test]
(Acute toxicological study)
LD_{50} of this Natto is over 2000 mg/kg. (which is equivalent to 26 µg MK-7) in 5-week-old male rats.
(Subchronic toxicological study)
Four-week-old male and female rats were used. Each group were divided into groups (n=5) tested for 13 weeks Result: No animal was dead and behaved abnormally. Examinations of haematology and biochemistry values in blood and urine revealed no abnormality. Also, any histopathological abnormality has not been seen. Thus, none toxicological dose of this Natto was concluded to be over 1000 mg/kg body weight/day (which is equivalent to 13 µg/kg body weight/day MK-7)."

Menaquinone -7 from NattoPharma – branded as MenaQ7

The product MenaQ7 is routinely checked for content of menaquinones and several other potential components like heavy metals, toxins, bacteria, mould, yeast etc. See appendix 1 and 2 for full technical product descriptions.

Of special interest is the content of menaquinones. Menaquinones are analysed using a HPLC method, a method which gives the full range of different menaquinones. Each batch of MenaQ7 products is analysed for content of menaquinones, and specifications are set accordingly. Fig shows the content of MK-7 and MK-6 in 25 different batches of the MenaQ7 P-1000 product. No other menaquinones are detected in the MenaQ7 products but MK-7 and

The content of MK-6 and MK-7 in MenaQ7 Powder formulation P-1000 product is shown in Fig. 13

Batch-to-batch variation in Menaquinone-6 and Menaquinone-7 for the product MenaQ7Powder P-1000

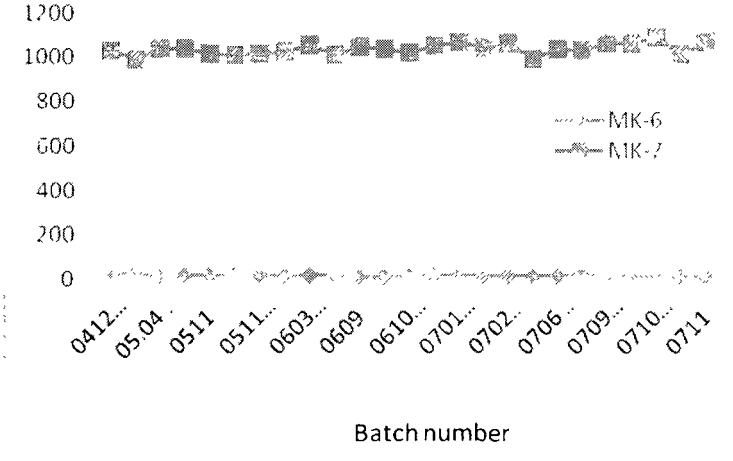
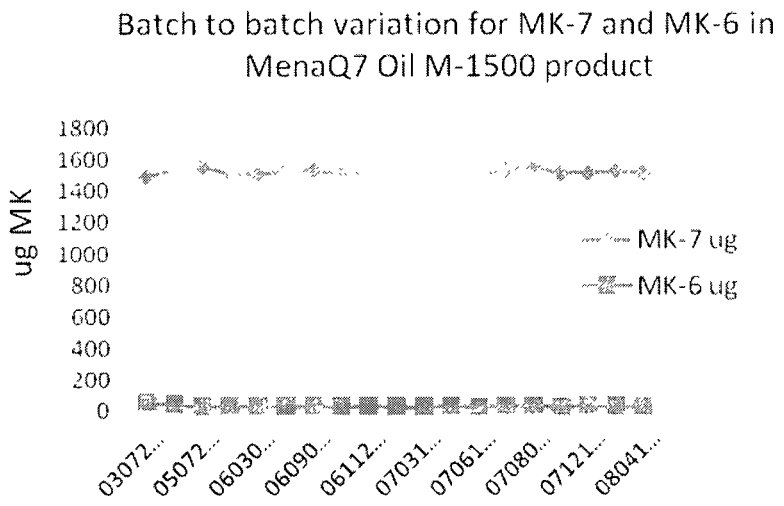


Fig 14 shows the content of MK-6 and MK-7 in MenaQ7 Oil formulation M-1500 product



Both figure show small batch to batch variation – and with 1,4-3,4 % of total menaquinone content representing MK-6 . The data in these two figures are also tabulated as Tables 11 and 12.

Table 11: Batch to batch variation for MenaQ7 M 1500 oil product.

MenaQ7 M-1500 Oil

Lot No	MK-7 ug	MK-6 ug
030724-1	1493,8	53,1
041203-1	1539,7	42,2
050728-1	1559,2	26,8
060223-1	1511,2	31,0
060303-1	1510,4	30,6
060609-1	1529,5	27,0
060908-1	1534,6	29,4
061006-1	1508,5	26,6
061122-1	1519,1	28,3
070208-1	1544,1	27,5
070316-1	1521,5	27,5
070530-1	1573,5	29,0
070612-1	1501,3	25,7
070801-1	1539,2	30,6
070809-1	1560,0	29,7
071012-1	1515,8	24,6
071217-1	1517,6	29,1
080116-1	1529,4	26,7
080416-1	1513,8	25,7

Table 12:

Batch to batch variation for MenaQ7 P 1000 powder product.

MenaQ7 P 1000 powder

Lot No	MK-6 ug	MK-7 ug
041213-1	19,7	1032,6
041025-1	27,6	992,1
05.0411-1	15,4	1041,4
050613-1	19	1040,3
051108-2	20,6	1018,4
050822-1	21,1	1014,6
051108-2	20,6	1018,4
060116-1	20,1	1031
060301-3	19,9	1056,9
060417-1	19,2	1012,8
060928-3	15,9	1053,2
061018-3	15,3	1038,5
061019-3	16,9	1023,9
070115-3	17,9	1056,2
070117-3	18,9	1070,7
070206-2	19,2	1046,6
070206-3	18,8	1066,7
070424-3	16,7	1000
070619-1	17,1	1039,3
070823-3	24,5	1035,3
070906-3	21,3	1064,9
070818-3	17,6	1065,5
071003-3	17,8	1094,5
071112-1	14,3	1017,1
071122-3	15,5	1072,5

PARTIAL LIST OF REFERENCES

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Vermeer, C. Personal Communication.

Attachment 1:

[illegible]

REF: L. Schurgers 2002: Thesis, Maastricht University

Table 1: K-vitamins (µg/100 g) in various foods

- Means NOT detectable

Type of food	n= samples		K_1	MK-4	MK-5	MK-6	MK-7	MK-8	MK-9	MK-10
Beef	7	0.6	1.1	-	-	-	-	-	-	
Chicken	7	-	8.7	-	-	-	-	-	-	
Pork	7	0.3	2.1	-	-	-	-	-	-	
Animal liver	7	0.2	0.3	-	-	0.5	1.1	-	-	
Hare	7	4.8	0.1	-	-	-	-	-	-	
Deer	7	2.0	0.7	-	-	-	-	-	-	
Goose	10	4.1	31	-	-	-	-	-	-	
Duck	7	1.9	3.6	-	-	-	-	-	-	
Whole milk	6	0.5	0.8	0.1	-	-	-	-	-	
Skimmed milk	6	-	-	-	-	-	-	-	-	
Buttermilk	6	-	0.2	0.1	0.1	0.1	0.6	1.4	-	
Whole yoghurt	6	0.4	0.6	0.1	-	-	0.2	-	-	
Skimmed yoghurt	6	-	-	-	-	-	0.1	-	-	
Whipping cream	6	5.1	5.4	-	-	-	-	-	-	
Margarine	6	93.2	-	-	-	-	-	-	-	
Butter	6	14.9	15.0	-	-	-	-	-	-	
Chocolate	6	6.6	1.5	-	-	-	-	-	-	
Corn oil	6	2.9	-	-	-	-	-	-	-	

Attachment A: Menaquinones in the US diet – for FDA - 210808

Sunflower oil	6	5.7	-	-	-	-	-	-	-
Olive oil	6	53.7	-	-	-	-	-	-	-
Hard cheeses	15	10.4	4.7	1.5	0.8	1.3	16.9	51.1	-
Soft cheeses	15	2.6	3.7	0.3	0.5	1.0	11.4	39.6	-
Curd cheese	12	0.3	0.4	0.1	0.2	0.3	5.1	18.7	0.4
Egg yolk	8	2.1	31.4	-	0.7	-	-	-	-
Egg albumen	8	-	0.9	-	-	-	-	-	-
Prawn	7	0.1	-	-	-	-	-	-	-
Mackerel	7	1.2	0.4	-	-	-	-	-	-
Herring	7	0.1	-	-	-	-	-	-	-
Plaice	7	-	0.2	-	0.3	0.1	1.6	-	-
Eel	7	0.3	1.7	-	0.1	0.4	-	-	-
Salmon	7	0.1	0.5	-	-	-	-	-	-
Kale	3	817	-	-	-	-	-	-	-
Spinach	3	400	-	-	-	-	-	-	-
Broccoli	3	156	-	-	-	-	-	-	-
Green peas	3	36.0	-	-	-	-	-	-	-
Sauerkraut	7	25.1	0.4	0.8	1.5	0.2	0.8	1.1	1.3
Natto	5	34.7	-	7.5	13.8	998	84	-	-
Banana	4	0.3	-	-	-	-	-	-	-
Apple	4	3.0	-	-	-	-	-	-	-
Orange	4	0.1	-	-	-	-	-	-	-
Rue bread	6	0.7	-	-	-	-	-	-	-
Wheaten bread	6	1.1	-	-	-	-	-	-	-
Sourdough bread	6	1.0	-	-	-	-	-	-	-
Buckwheat bread	6	3.0	-	-	-	1.1	-	-	-
Tea	4	0.3	-	-	-	-	-	-	-
Coffee	4	-	-	-	-	-	-	-	-
Orange juice	4	-	-	-	-	-	-	-	-

All samples were assessed in duplicate, n stands for the number of different samples tested, a hyphen for not detectable. Foods were bought from shops in and around Maastricht.

References

[1] *Source: USDA/ERS, Livestock, Dairy, and Poultry Outlook.*

[2] *http://www.ers.usda.gov/Amberwaves/June03/DataFeature/*

[3] *http://www.ers.usda.gov/amberwaves/february05/findings/CheeseConsumption.htm*

[4] *http://www.ers.usda.gov/amberwaves/february05/findings/CheeseConsumption.htm*

[5] *http://future.aae.wisc.edu/data/*

[6] *: http://future.aae.wisc.edu/tab/sales.html*

[7] *: http://www.ers.usda.gov/Data/FoodConsumption/FoodAvailSpreadsheets.htm#eggs and http://www.unitedegg.org/useggindustry_generalstats.aspx*

[8] *Schurgers, LJ: Thesis 2002: Studies on the role of vitamin K1 and K2 in bone and cardiovascular disease. Chapter: 2.1: Determination of phylloquinone and menaquinones in food. Effect of food matrix on circulating vitamin K concentrations. University of Maastricht. Unigraphic, Universiteit Maastricht . ISBN 90-5681-138-X. Also published in: L.S. Schurgers & Cees Vermeer 2000: Determination of phylloquinone and menaquinone in food. Haemostasis, vol 30, p. 298-307*

[9] *Sakano, T et al 1988 : Measurements of K vitamins in food by high-performance liquid chromatography with fluorometric detection. In Vitamins, Japan; vol 62, no 8, p 393-398. Table 1 page 395*

[10] *Source: USDA/FAS, Production, Supply and Distribution Online Database; EuroStat Yearbook; US Census Bureau, International Database; USDA/ERS, Livestock, Dairy and Poultry Outlook.*

[11] *Geleijnse, JM et al: Dietary intake of menaquinone is associated with reduced risk of coronary heart disease: The Rotterdam study. J Nutrition vol 134, p. 3100-3105. 2004).*

[12] *Isobe et al 1995: Natto and vitamin K. in Basic and Clinical Aspects of Japanese Traditional Food Natto. Ed Sumi, Hiroyuki. Page 73-82. Japanese Technology Transfer Association*

[13] *Ikeda, Y et al 2006: Intake of fermented soybeans, Natto, is associated with reduced bone loss in postmenopausal women: Japanese population-based osteoporosis (JPOS) study. J. Nutr Vol 136, p. 1323-1328. 2006.*

ATTACHMENT B

Bioavailability and Safety of MK-7

1

Bioavailability of Menaquinones

Background and summary

FDA is questioning the toxicity of MK-7 and the traces of MK-6 which we can measure in MenaQ7 products from NattoPharma, as well as MK-8 found in certain food products. In this review we have calculated the concentrations of the different MKs in clinical uses and compared the number of molecules available in human bodies. Even if MK-7 has better bioavailability than the safe MK-4, this will not lead to higher numbers of active MK-7 or MK-6 molecules which then may cause any unknown toxic effects.

The effective doses MK-4 given in published clinical studies (45 mg/day- in some studies twice daily) show that MK-4 provides 1460 times as many molecules as MK-7, and 65 000 times as many molecules than MK-6. Even with lower MK-4 bioavailability compared to the other higher menaquinones (MK-6, MK-7, MK-8), such excess numbers of molecules of MK-4 do not give any toxic effects in animals or humans. This is also evident by the daily recommendations of 45 mg MK-4 which supports a continuously high serum level without toxic effects. The lack of toxicity of MK-4 is further supported in several toxicity studies in animals.

Introduction

Molecular weights and concentrations needed for same molar concentration.

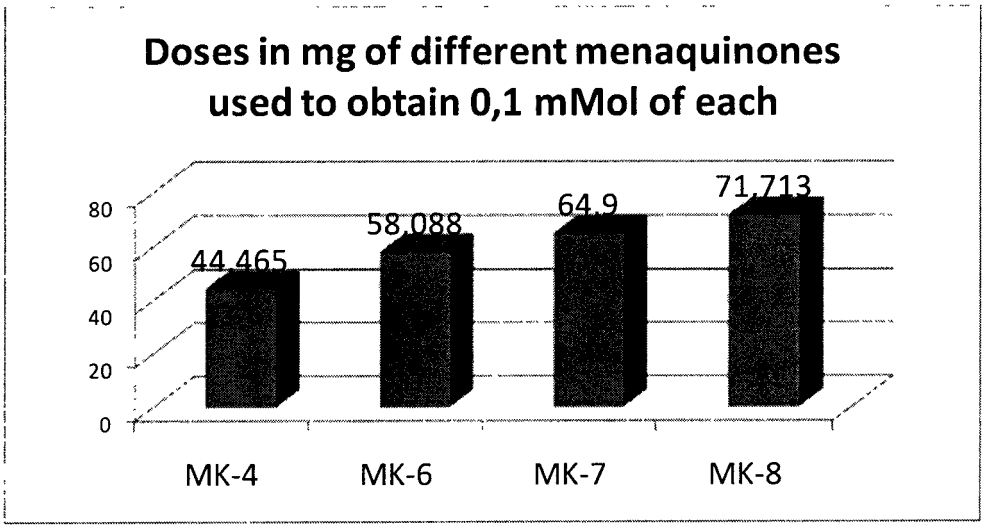

Fig.1

It can be seen that a much lower dose in mg is required for MK-4 to obtain the same number of molecules as the higher menaquinones, especially MK-7 and MK-8. For example we need in 71,7 mg MK-8 to get the same amount of molecules as in 44,4 mg MK-4.

In the following we have used the molar concentrations of the different vitamin K2s. This is a much more relevant concentration measure than milligrams/micrograms when considering biological

2

activities and effects. We know that activation of the vitamin K dependent proteins (coagulation factors, osteocalcin, MGP) is dependent upon the ring structure of the menaquinones molecule, and not the isoprenic units attached to this ring. Thus when considering the biological effects of menaquinones, the number of molecules are the most important as there is a dose-response effect on the carboxylation of the proteins (see figures 5 and 6).

Clinical applications

Molar concentrations of various menaquinones in clinical use

Fig 2 and Table 1 shows the concentrations in micro moles (uMol) used in clinical studies (MK-4) , recommended dose of MK-7 (ref Rotterdam study), and what is found in 100 grams of hard cheese.

Fig 2.

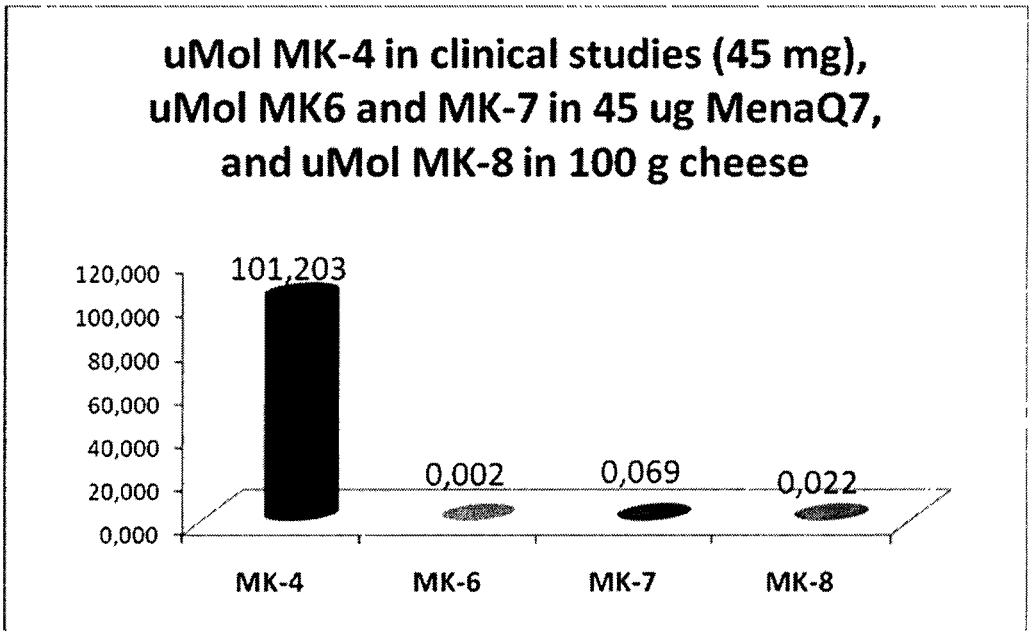

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Table 1

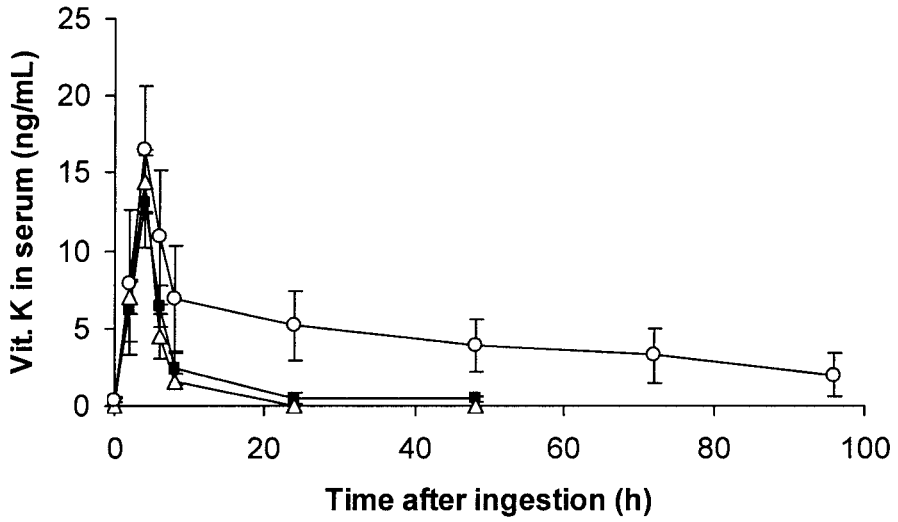
Menaquinone	MW	Conc. in studies/in food grams	Conc in mMol	Comments	Ratio MK4/MKn
MK-4	444,65	0,045	101,2032	Conc used in clinical studies	
MK-6	580,88	0,0000009	0,0015	Content in 45 ug MenaQ7 M1500	65319
MK-7	649	0,000045	0,0693	Recommended dose 45 ug MK-7	1460
MK-8	717,13	0,000016	0,0223	Content in 100 g cheese	4536

3

As can be seen there are 1460 times more molecules MK-4 than MK-7 in the applied clinical dose for MK-4, while the ratios for MK-4/MK-6 is more than 65 000. MK-4 used in clinical studies with no adverse reports published also represents 4500 times higher numbers of molecules than the amount of MK-8 found in 100 grams of cheese.

Considerations enhanced bioavailability of MK-7 compared to MK-4.

When considering bioavailability one must study both absorption and serum half-life. In Fig 3 (ref internal report) it can be seen that at the same ug concentration (500 ug/m l- molar difference: MK-4 1,45 times as many molecules as MK-7) of the three K-vitamins K1 (phylloquinone), MK-4 and MK-7, their initial absorption is the same. However, the serum half-life of MK-7 is about 96 hours, while that of both K1 and MK-4 is 1,5 to 2 hours.



Absorption profiles of K1 (■), MK-4 (Δ) and MK-7 (○). Error bars represent standard deviation.

Design of Absorption Study

An open study among 15 volunteers receiving a mixture of Konakion (Roche, source of K1), Menatetrenone (Eisai, source of MK-4) and MenaQ7 (J-Oil Mills, source of MK-7) in 2 mL of sunflower oil. The final concentration of all vitamins was 500 µg/mL of oil. All participants arrived at our institute in a fasting state and received 2 mL of the K-containing oil together with a standard breakfast consisting of rolls, butter and jam. The total fat content of the breakfast was estimated to be 30 g. Blood samples were taken at time = 0, 2, 4, 6, 8, 24, 48, 72, and 96 h. All samples were analysed for vitamin K by standard HPLC analysis.

Several NattoPharma studies have revealed a 6-10 times better bioavailability of MK-7 compared to MK-4 similar to that found in comparing MK-7 to phylloquinone (Schurgers, L.J., Teunissen, K.J.F., Hamulya, K., Knapen, M.H.J., Vik, H., Vemeer, C., 2007a. Vitamin K- containing dietary supplements: Comparison of synthetic vitamin K1 and natto-derived menaquinone-7. Blood 109, 3279-3283).

The clinical, non-toxic dose of 45mg MK-4 gives a 0,1 mMol concentration while a 45 ug MK-7 dose gives a 0,00007mMol MK-7 concentration. Even if MK-7 has a bioavailability that is approximately 10

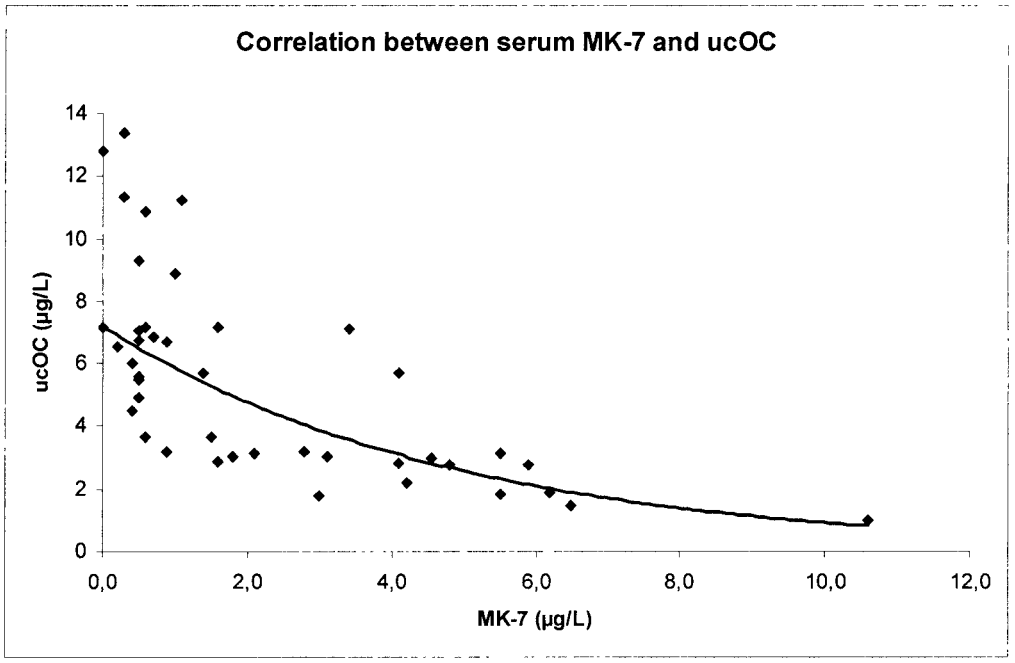
4

times more effective than MK-4, the total concentration of MK-7 still does not reach the levels obtained with MK-4. In addition 45 mg MK-4 is taken daily in published studies giving a constant high supply of MK-4 which will counteract the rapid excretion of MK-4. Again given the abundance of MK-4 molecules in daily 45 mg MK-4 use largely exceeds the number of MK-7 molecules.

Dose-response effects of MK-7

The recommended dose of 45 ug MK-7/day is based upon habitual dietary intake of food products containing MK-7 contribution to favorable health effects (The Rotterdam study). The highest tertile for natural consumption of MK-7 in this study was about 45-50 ug MK-7/day. However, NattoPharma has conducted a MK-7 dose-response study looking at carboxylation of osteocalcin and MGP. This study shows that the higher the MK-7 concentration the more protein is carboxylated. No adverse effect was recorded in this study which included 360 ug MK-7/day as the highest dose. The results shown in Fig 5 and 6 indicate that the body utilizes ingested MK-7 to optimize activation of these important proteins with a clear dose-response way without reaching a steady state level within the dose range applied.

Fig 5 and fig 6 show the correlation of serum level of MK-7 and carboxylation of osteocalcin (A) and matrix Gla protein (B). See report attached for documentation.



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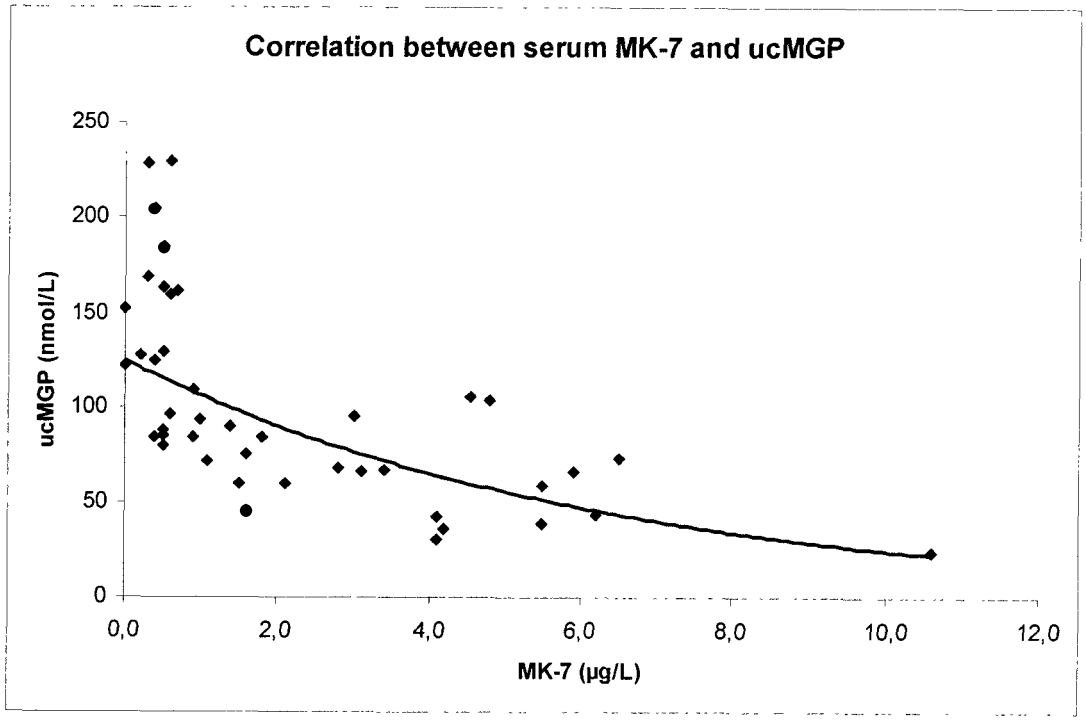

Conclusions:

Circulating MK-7 levels reflect tissue levels, where MK-7 is active in osteocalcin and MGP carboxylation. In this respect MK-7 differs from K_1 and MK-4 both of which show strongly fluctuating serum levels, dependent on recent intake. MK-7 with its long half-life time builds up more stable serum levels, and here we demonstrate the direct correlation between serum MK-7 and tissue vitamin K status.

ATTACHMENT C

Safety of MK-6

Background:

Vitamin K is a fat-soluble vitamin which has a very specific function. It activates so-called vitamin K-dependent proteins in an enzymatic reaction called carboxylation. Scientific evidence shows that this vitamin K-dependent carboxylation exist for centuries, as vitamin K-dependent proteins have been found in all kind of fossils (eg dinosaurs). In all mammalians, vitamin K-dependent proteins are all in an activated (carboxylated) form. However, exceptions are humans. Because sufficient evidence has been provided in the literature vitamin K is safe as stated by the FDA: "vitamin K has no upper tolerance level".

Six proteins involved in the blood coagulation cascade are synthesized in the liver in a vitamin K-dependent way. The step that is catalyzed by vitamin K is a carboxylation reaction which introduces calcium binding sites (Gla-residues) in these clotting factors which render these factors their biological activity. Severe vitamin K-deficiency will thus result in a bleeding tendency; experimental animals on vitamin K-deficient food may even bleed to death. The "western" diet contains a diversity of sources of vitamin K and is adequate to support a normal haemostasis. Dietary vitamin K-deficiency to the level of increased bleeding tendency is not seen in healthy subjects, only in patients with severe fat malabsorption such as in bile obstruction. The liver is capable of extracting vitamin K from the blood stream with very high efficiency and takes what is required for the production of fully carboxylated (i.e.: fully active) clotting factors.

Thus, we do not have problems with the clotting system. All recommendations are made on hepatic requirement only. However, we and others have clearly shown that the extra-hepatic tissues require more vitamin K. During the last two decades it has been clear that vitamin K is also needed for other physiological processes than blood clotting, including correct mineralization of bone and inhibition of vascular calcification. Unfortunately, the vitamin K requirement of bone and the vasculature is not met by the dietary intake, and in the normal "western" diet, vitamin K supplements are required for optimal bone and vascular health. The choice is between vitamin K1 (phylloquinone; synthetic) and K2 (menaquinone). Whereas K1 is primarily targeted to the liver, K2 is equally transported to liver and extra-hepatic tissues such as bone and arteries. So K2 is the product of choice to be used in supplements and functional foods. Within the group of K2 vitamins (menaquinones) we differentiate between the short chain menaquinone-4 (synthetic) and the long chain menaquinones (found at high concentrations in cheese, curds, sauerkraut and natto).

Adverse effects of extra vitamin K on clotting:

Among those who are not familiar with the molecular mechanism of vitamin K action, it is often suggested that increased intake of vitamin K would lead to increased blood clotting, i.e. to an increased thrombosis tendency. That is not true. Incompletely carboxylated clotting factors have never been found in healthy subjects, so the carboxylation of the clotting factors is complete. Extra carboxylation events are not possible (the mechanism is shut off automatically after a well defined number of Gla-residues have been formed); and even if "super-carboxylated" clotting factors could be formed, they would bear extra negative charges leading to distortion and inactivation of the molecules. Only in the case of patients using vitamin K-antagonists (coumarin derivatives, oral anticoagulants) all forms of vitamin K will directly interfere with the therapy.

Since the long chain menaquinones have many advantages over the short chain ones (and K1) products like MenaQ7 (menaquinone-7, the K2 from natto) have recently been introduced in several countries. A number of long- and short-term studies are in progress and some of these studies have been completed at this time. Because of the safety concern expressed by certain regulatory authorities, we have started to monitor the thrombosis tendency in all studies initiated after January 1, 2007. The test we use is the "thrombin generation curve", showing longitudinally the progress of thrombin generation in a plasma sample after activation. Two variables are used to quantify the thrombosis tendency of the donor: the Area Under the Curve known as the Endogenous Thrombin Potential (ETP, i.e. the total amount of thrombin that can be formed during a certain period of time) and the thrombin peak height (the maximal amount of thrombin that can be generated in the peak). These are well-established techniques the basis of which was formed by the group of Hemker at the Maastricht University. The ETP provides the most accurate and sensitive method of assessing the thrombosis risk. In this report we describe the potential affect of MenaQ7 on the thrombosis risk in children and in adults. The protocols for the complete trials has been detailed in the study reports of the VitaKids study (June 6, 2007) and of the Dose-finding study (December 4, 2007).

There is no theoretical basis for the assumption that vitamin K (in whatever form) may increase the risk of thrombosis in healthy subjects. MenaQ7 (menaquinone-7) belongs to the K2 vitamins, which are abundantly found in cheese (mainly menaquinone-7, -8, and -9). Here we have demonstrated that indeed high doses of MenaQ7, rich in menaquinone-7 (and thus including menaquinone-6) do not increase the thrombosis tendency. This has been demonstrated previously for very high doses of vitamin K1 (10 mg/day during 2 years; 220x higher) and menaquinone-4 (45 mg/day during 3 years; 1000x higher).

The reason why it is not to be expected that vitamin K will increase the thrombosis risk (except in patients on oral anticoagulant therapy) is that all the vitamin K-dependent clotting factors are exclusively synthesized in the liver, and the liver is capable of extracting vitamin K from the blood stream with very high efficacy. Therefore, all vitamin K-dependent clotting factors are

fully carboxylated (activated) in all subjects (except in severely sick patients). More carboxylation events (if possible at all) would only lead to the introduction of extra negative charges in the clotting factors and to distortion of their tertiary structure.

The safety issue has been and will be monitored scrutinously in all MenaQ7 trials, but a substantial body of evidence demonstrates that the product is safe for use in the general population. Other studies have demonstrated significant health benefits of long-chain menaquinones; therefore the wide availability of vitamin K2 may have a significant contribution to public health. **As an extra note, we tested MenaQ7 as the product providing menaquinone-7, thus we automatically tested MK-6 (6% of the MenaQ7 product) safety.**

High doses of vitamin K

A number of intervention studies have been published testing the effect of K1 on bone. A general trend is that K1 (in ranges between 250 µg up to 10 mg/day) improves osteocalcin carboxylation. In non of these studies a (negative) side effect was reported. In a large number of Japanese studies, K2 (invariably MK-4; menatetrenone) has been tested in high doses (generally 45 mg/day). With these doses, vitamin K is not used as a nutritional supplement, but as a pharmaceutical drug. Obviously, the extremely high K2 intake resulted in maximal osteocalcin carboxylation. Menatetrenone is now used for over 20 years, and no negative side effects were ever reported. In a randomized placebo-controlled vitamin K2 intervention trial among 340 Caucasian postmenopausal women, conducted by our group, K2 (45 mg/day during 3 years) confirmed these Japanese results: MK-4 induced a significant activation of osteocalcin, improved bone strength and did not have any side effect.

- All K-vitamins are taken up by the food, and absorbed in the intestines. These are all contribute to the vitamin K-status. The bacterial flora in our intestines produce also K-vitamins (menaquinones only, mainly MK10 and higher). The contribution of these to our vitamin K-status is not know, and if any low.

- MK-7 from MenaQ7 is absorbed (in the intestines) equally well as MK-4, but it has a longer half-life time. This result in a longer presence in the circulation, and in a 6-8 fold higher efficacy *in vivo* (measured by the osteocalcin carboxylation). So data on 45 milligrams per day of MK-4 could be translated into about 5.5 **milli**grams per day of MK-7. Here we are not discussing **milli**grams but 20 or 50 **micro**grams, which is 100 fold less.

- The suspected toxicity of MK-6 and MK-8 is not likely. One has to remind that vitamin K2 stands for a whole range of menaquinones, all of which are of bacterial origin. The diverse food items in our diet all contribute to the total intake of about 28 µg of K2 per day (in the Netherlands). Meat and eggs supply MK-4, and a wide variety of other foods supplies the range of menaquinones 6 through 10. MK-8, for instance, is present in cheese at an average amount of 16 µg per 100 grams. It is obvious that this dose is not toxic. The MK-8 content of MenaQ7 (natto-derived MK-7) is about below detection limit; thus no MK-8 will be supplied to people taking MenaQ7.

- The total K2 content of cheese is about 50 µg/100 g

- The discussion about possible toxicity of MK-7 or other forms of menaquinone is not based on our current understanding and scientific output of research. The only point of concern is the potential interference of K-vitamins with oral anticoagulant treatment. In the past we have calculated that vitamin K1 from supplements below 100ug/day is not interfering with OAC. Moreover, a follow-up on this study demonstrated that supplements with MK-7 below 50ug will not be interfering with OAC.

- The comparison of MK-7 and MK-4 is based on the half-life of the two vitamins. MK-4 has a half-life of 1 hour, whereas MK-7 has a half-life of 3 days. In a small study conducted in collaboration with Japanese researchers we found a 6 fold better plasma level of MK-7 compared to MK-4. In terms of absorption, MK-7 is (on a molecular level) 1.5x better absorbed (equally to the 1.5x better absorption compared to K1).

- Consumption of MK6 or MK7 in food. One can easily notice that the major source for MK-6 and MK-7 (besides natto) is cheese and sauerkraut (both fermented food products). One cannot consider these as toxic food items.

- MK-6 is less lipophilic than MK-7, so the half-life is shorter. From my perspective MK-6 will be less "active" compared to MK-7. Therefore the 6% content of MK-6 in the MenaQ7

product will be negligible. Moreover, by testing the MenaQ7 product, the safety of MK-6 is already tested since it is present in the MenaQ7.

ATTACHMENT D

Reproductive Effects of MK-4

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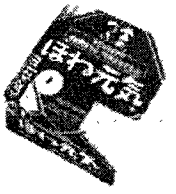
For more products



Food name

Head up.

Format: natto

Net weight: 50 g × 3 Pack

Manufacturing or seller

(Shares) MITSUKAN

Address :475-8585, Nakamura town Handa, Aichi Prefecture, 2-6

Tel :0569-21-3331

Allow the actual area	Allow the Year
The natto, natto bacteria *(Bacillus subtilis OUV23481)* the effect of vitamin K $_2$, including a rich, bone calcium to help the bone protein (osteocalcin) to enhance the work has been devised.	December 27, 2002

Appropriate Use

Per day, per pack to enjoy a measure KUDASAI (per package: 50 g, vitamin K $_2$: 650 μ g or more contained)

Instructions on the use of

None

Component involved in commenting on

[] Involvement in the ingredients and mechanism of action

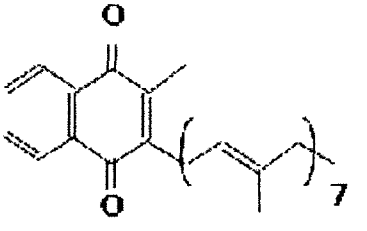
[Characteristics] component

Vitamin K $_2$ (menaquinone 7), vitamin necessary for blood clotting known as natto bacteria, including many bacteria produce electronic communication system as a component. Pale yellow, plate-like crystals, melting point of 54 ℃, soluble organic solvent, methanol fire melting, insoluble in water, light and bases for instability.

[Mechanism] and effect

Osteoblast cells that produce a protein that is osteocalcin, γ-carboxylation (with the Gla) will be activated by the γ-KARUBOKISHIRUOSUTEOKARUSHIN (Gla of osteocalcin) will be converted. Calcium, to join the Gla of osteocalcin, followed by adsorption to hydroxyapatite and bone mineralization is in progress. Vitamin K $_2$, osteocalcin γ-carboxylation of a catalyst, working as a coenzyme of carboxylase, osteocalcin, bone formation and promote the Gla (1) (2).

[] Involvement in the structure of the component



000087.037

From:
(1) Price PA, Vitam Horm vol. 42, 65-108 (1985)
(2) Dictionary second edition of Biochemistry p. 1344 (1990)

[] Involvement in the analysis component
In the extraction of vitamin $K_{2]}$
Natto 12-15 g of distilled water, 200 ml to 30 ℃, 150 rpm, 1 hour thanks to the vibration. [illegible]after the suspension of a 5 ml (no beans), 2 - propanol 5 ml, n-hexane 6 ml, internal standard solution (6.6 ppm vitamin $K_{1)}$ 200 μ l In addition to the traditional 20-minute vibration.
Centrifuge to separate water and oil in the reservoir to take a 4 ml.
Decompression thickener skipped after using a solvent, 2 - propanol dissolved in 500 ml.
In the analysis of vitamin $K_{2]}$
HPLC analysis at the following conditions (1) (2).
Use column: TSKge10DS-120T (φ4.6 × 250mm)
Detection: UV (254nm), column temperature: 50 ℃
Injection volume: 10 μ l, solvents: methanol flow rate: 1.0 ml / min

From:
(1) volume 8 number 62 vitamin 393-398 (1988)
(2) Volume 7, Japan 43 home economics Journal 643-648 (1992)
(3) Monthly Food Chemical 72-74 (1990)

Safety rating

[Human] test
On the test system, 29 to 50-year-old able-bodied adults, 12, the intake, the natto (vitamin $K_{2,}$ 1300 μ g/100g more than) 150 g / day. [Period] seven days of the natto (vitamin $K_{2,}$ 1300 μ g/100g or more). [Result] drug for the treatment of osteoporosis (made by Eisai Co.: GURAKE) has been raised as a side effect of serum-like components of change, the symptoms were not confirmed. Moreover, serum clotting - before and fibrinolytic system of intake has been no significant change.

[Testing] and other animals
Study 1:
In the test series 5-week-old, male, SD-rat, two. [Intake] 2 mg / kg / day. [Period] seven days. [Result] of the natto single oral dose of the rat is weak, LD_{50} is 2000 mg / kg and higher than estimated.

2:
On the test system, Chinese hamster lung[illegible]gland cell line used, one for each dose. On the added amount, up to 5000 μ g / ml. [Result] natto this group of chromosome abnormalities in the process of induction was not clear.

000087.038

Research 3:
On the test system, histidine requirement of Salmonella typhimurium TA, 100, Salmonella typhimurium TA98 to use, two for each dose. On the added amount, up to 5000 μ g / plate. [Result] solvent reversion control group compared to only increase the number of colonies trend was noted. However,

these positive criterion of the increase in solvent control group not to exceed twice the pole only to the test article containing the amino acid release that significant reaction to that is unthinkable.

Study 4:

In the test series, 4-week-old, male female, SD-rat, five each, five female. [Intake] Up to 1 mg / kg / day, period, 13 weeks. [Result] 1. Abnormal animals will not confirm the death of animals observed. 2. Hematological tests, blood chemistry, blood coagulation test, urine test, also was not abnormal. 3. Pathology tests in finding the naked eye is abnormal and pathological findings were not observed. Therefore, this non-toxic amount of natto is the rat 1000 mg / ml / day greater than was thought.

Effectiveness assessment

[Human] test

Study 1:

In the test series] 26 to 50-year-old healthy adult men, each group of eight, the intake of] 50 g / day. [Period] seven days. [Result] 100 g per MK-7, respectively 864 μ g (normal natto), 1295 μ g (the natto) and 1727 μ g natto to contain 50 g / day each week intake and ingestion ago, 1 and 7 days after ingestion of a blood sample from the Serum concentration of MK-7, γ-Gla-OC concentration, un-OC concentration, general biochemical marker of the results of measurements of changes in concentration, 1. Serum concentration of MK-7, all of the group take before intake 1 and 7 days after ingestion compared to a significant rise in the concentration-dependent increase in the concentration of MK-7. (Both p <0.001) and seventh day of the usual intake group compared with natto, the natto intake systems and the MK-7: 1727 μ g-containing natto intake group in the significant increases in (the natto: p <0.05, 1727μ g-containing natto: p <0.001). 2.γ-Gla-OC level, compared to the previous intake natto and MK-7: 1727 μ g-containing natto intake increased significantly after 7, 2007 (both p <0.05). 3.un-OC level of the previous intake than MK-7: 1727 μ g-containing natto, seven days after ingestion of a significant decline (p <0.05). 4.MK-7: 1727 μ g natto intake-containing Ca seven days after the serum concentration than the previous intake increased significantly (p <0.05). 5. MK-7 natto intake seven days after the intake of serum ALP activity increased significantly compared to before. (P <0.05) (1)

000087.039

2:

In the test series] 20 to 40-something-year-old able-bodied adults, three groups, each group of 13 people attribute (gender, age) and equipped. [Intake] 50 g / day. [] 14 days. [Result] 100 g per MK-7, respectively 865 μ g (normal natto), 1295 μ g (the natto) and 1730 μ g natto to contain 50 g / day for 14 days each intake and ingestion ago, 7,10 and 14 days after ingestion of blood Serum collected the MK-7, γ-Gla-OC results of measurements of changes in concentration,

1. Serum concentration of MK-7, natto intake of any group in the previous intake increased significantly compared to (Student's t-test, Paired t-test: both p <0.001). In addition, three significant difference between groups was observed (one-way ANOVA p <0.001). Usually natto and a comparison of the natto and 1730 μ g containing significant rise in natto (usually natto: Student's t-test p <0.05 ~ 0.001 (All Time), one-way ANOVA p <0.05 (10,14 days), 1730μ g-containing natto: Student's t-test p <0.001 (All Time), one way-ANOVA p <0.05 (All Time)). 2. Serum concentration γ-Gla-OC, 14 days after ingestion of three significant difference between groups was observed (one-way ANOVA Tukey: p <0.05). Also, between the two groups in the ingestion natto are generally 14 days, as compared to the natto and 1730 μ g containing significant rise in natto has been confirmed (both co-natto, Student's t-test p <0.05, Paired t-test p <0.05). 3. Intake and non-γ-Gla-OC serum concentration in comparison, are usually natto intake 7,10 and 14, before and after they ingested significant than the rise was not

admitted to the natto And 1730 μ g containing natto, the intake of 7,10, and 14 days before the intake of all significant rise compared to confirm that (the natto: Student's t-test p <0.01 (7 days), p <0.001 (10, 14 days) Paired t-test: p <0.01 (7 days), p <0.001 (10,14 days), 1730 μ g-containing natto: Student's t-test p <0.05 (7,10 days) , P <0.001 (14 days) Paired t-testp <0.01 (7,10 days), p <0.001 (14 days)) (2).

From:
(1) Tsukamoto Y., et al., J Health Sci, Vol. 46, 317-321 (2000).
(2) Tsukamoto Y., et al., J Bone Miner Metab, Vol. 18, 216-222 (2000).

[Testing] and other animals

Study 1:

In the test series] 6-week-old, Wistar female rat system, each group of six to seven, the intake of] 10 g / day refinery standard diet (MK-4, MK-7 Zone is the addition of a prescribed amount of powder-containing) Free intake. [] 24 days. [Result] rat rat using sham surgery (Sham), spayed rat (OVX), MK-4 equivalent to the weight used to treat osteoporosis if the same amount to be ingested to the OVX (OVX + MK - 4), MK-7 and MK-4 equimolar to be ingested and the OVX (OVX + MK-7) after raising serum MK-4, MK-7 level, the thigh bone of MK-4, MK-7 content, serum γ-carboxylated osteocalcin (γ-Gla-OC) concentration, the thigh bone of γ-carboxylated osteocalcin (γ-Gla-OC) content, dry weight of thigh bone, thigh bone measurements of changes in content Ca Results: 1. Serum MK-4, MK-7 is about concentration, Sham, OVX than OVX + MK-4, OVX + MK-7 in each MK-4, MK-7 level is significantly increased. And MK-7 addition Sham, OVX MK-4 compared to a significantly increased level (both p <0.01). Two. Bone-MK-4, MK-7 is about content, Sham, OVX than OVX + MK-4, OVX + MK-7 in each MK-4, MK-7 content is significantly increased. And MK-7 addition Sham, OVX TOBE MK-4 with a significantly increased content (both p <0.01). 3. Γ-Gla-OC serum concentration is about, Sham, OVX than OVX + MK-4, OVX + MK-7 is the average value rose significantly. 4. Dry weight of the femur, OVX than OVX + MK-4, MK-7 in the recovery trend. 5. Femur bone Ca content of the cortex, OVX than OVX + MK-7 in the bone Ca content increased significantly (p <0.01). 6. Femur bone Ca content of the cavernous, OVX compared, OVX + MK-4, MK-7 in the bone Ca content increased significantly (both p <0.01) (1).

000087.040

2:

In the test series] 6-week-old, Wistar female rat system, each group of six to seven, the intake of] 10 g / day refinery standard diet (natto-containing freeze-dried powder) intake of freedom. [] 77 days. [Result] rat rat using sham surgery (Sham), rat ovariectomy (OVX), OVX Usually natto (MK-7 content: 864 μ g/100g) to 50 g / weight 60 kg / day and be ingested the OVX (OVX + MK-7 × 1), MK-7, including a 1727 μ g/100g natto intake to his OVX (OVX + MK-7 × 2), MK-7, including a 4318 μ g/100g natto intake to his OVX (OVX + MK-7 × 5) raised the long-term serum after MK-4, MK-7 level, γ-Gla-OC concentration, the thigh bone of MK-4, MK-7 content, γ-Gla-OC content, thigh Dry weight of bone, the femur bone mineral content and density of the femur bone, thigh bone Ca results of measurements of changes in content, 1. Serum concentration of MK-7, Sham compared with natto, including MK-7 plus all OVX groups increased significantly (p <0.01). Also, OVX + MK-7 × 5, sham, OVX MK-4 compared to a significantly increased level of (anti-Sham p <0.05, versus OVX p <0.01). 2. Bones in the MK-4 content, MK-7 plus all, including Natto OVX groups Sham, OVX MK-4 content compared to a significantly increased (both p <0.01). 3. Γ-Gla-OC serum concentrations, OVX compared, OVX + MK-7 × 5 in significantly increased (p <0.01). 4. Thigh bone dry weight, OVX than natto, including MK-7 plus all increased significantly in OVX group (p <0.01). 5. Femur Ca content in the spongy bone cortical

bone(VX compared with MK-7 plus natto, including('l OVX groups significantly increased. (P <0.( )
(1)

Research 3:
In the test series] 6-week-old, Wistar system, female rats, each group of six to eight. [Intake] 10 g / day to measure the standard purified diet, MK-7 Zone is natto addition to the prescribed amount of powder-containing freeze-dried) free food. [] 5 months. [Result] rat rat using sham surgery (Sham), spayed rat (OVX), usually natto (MK-7 content: 864 μ g/100g) to 50 g / weight 60 kg / day and be ingested the OVX (OVX + MK-7 × 1), MK-7, including a 1295 μ g/100g natto intake to his OVX (OVX + MK-7 × 1.5), MK-7, including a 1727 μ g/100g natto intake to his OVX (OVX + MK -7 × 2) raised the long-term serum after MK-4, MK-7 level, γ-Gla-OC concentration, the thigh bone of MK-4, MK-7 content, dry weight of thigh bone, the femur bone mineral content , The femur bone density, thigh bone Ca results of measurements of changes in content, 1. Γ-Gla-OC serum concentrations, OVX than OVX + MK-7 × 1.5, OVX + MK-7 × 2, significantly Increased (both p <0.01). 2. Natto of MK-7 except to the natto-containing dietary intake of OVX his case could not curb the decline. 3. Thigh bone dry weight, OVX than MK-7, including OVX natto gave all groups increased significantly (both p <0.01). 4. Femur bone density, OVX than OVX + MK-7 × 2 bones significant recovery in the overall increase was observed (p <0.05). 5. Ca content of the femur bone cortex, MK-7, including natto OVX groups all gave a significant increase of OVX + MK-7 × 1, as compared to OVX + MK-7 × 1.5 or more of Recovery is high (MK-7 × 1: p <0.05, MK-7 × 1.5, MK-7 × 2: p <0.01) (2).

From:
(1) Yamaguchi M., et al., J Bone Miner Metab, Vol. 17, 23-29 (1999).
(2) Yamaguchi M., et al., J Bone Miner Metab, Vol. 18, 71-76 (2000).

ATTACHMENT E

Concern About Patients on Anti-coagulant Therapy

18 Aug. 2008

To whom it may concern;

We hereby report the acute toxicological test of vitamin K2 (menaquinone-7) on mice, which was conducted by Institute of Food Hygiene, Japan Food Hygiene Association in 1996.

Attached file 1 is a translated report.
Attached file 2 is an original report.

(b)(6)

Toshiro SATO, Ph.D.
General Manager,
Fine Chemical Laboratory
J-OIL MILLS, INC.

J-OIL MILLS

000087.043

Attached file 1

Acute toxicological test conducted in 1996

Experimental report

1. Aim of the test

Determination of acute toxicity (LD50)

2. Test sample

Extract of *Bacillus subtilis natto* (vitamin K 3500ppm): raw material of vitamin K2 (menaquinone-7)

3. Animal

Four-week-old male ddy mice

Divided into three groups (ten mice per group)

4. Experimental method

Oral administration: Healthy mice were selected. After 18-hour-fast, test sample which kept at 40 °C is orally administered by stomach catheter.

Dose of test sample : Based on preliminary test, 50 ml/kg body weight (physically maximum dose), 37 5ml/kg body weight and 25 ml/kg body weight is administered.

Conditions : Mice were kept in an stable temperature and humidity condition. After administration (day0), body weight was measured and general symptoms and life or death were observed for 7 days.

5. Result

In every group, general symptoms were not abnormal for 7 days. Body weight increased well. No mice was dead.

6: Conclusion

It is estimated that LD50 is over 50 ml/kg of this extract of *Bacillus subtilis natto.*

J-OIL MILLS

000087.044

第S8－2315号別紙

試験報告書

1．試験の目的

急性毒性（LD_{50}）の測定

2．被検物質

納豆菌発酵抽出物（ビタミンK原液3500ppm）

3．使用動物

マウス　ddy系統　4週令　雄　3群（1群10匹）を用いた。

4．試験方法

経口投与：健康なマウスを選択し18時間絶食させた後、各群10匹づつ被検物を40度の恒温水槽に保持しながら、マーゲンゾンデを用いてを強制経口投与した。

投与量：予備試験において把握した結果から、被検物原液が個体体重に対し物理的最大投与量50ml/kg.及び37.5ml/kg.25.0ml/kg.の3群を編成し投与した。

飼育条件：恒温恒湿の条件で飼育し、投与日を0として各個体の体重を測定しつつ、7日間一般症状及び生死の状態を観察した。この間水と餌は自由に与えた。

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5．試験結果

各群とも投与後7日間一般状態に何ら異常を認めず、体重も順調に増加して死亡例は認められなかった。

6．考察

上記試験結果から本被検物納豆菌発酵抽出物のLD_{50}は、50 mℓ/kg 以上と推定する。

000087.045

Attached file 2

試験検査成績書

第S８－２３１５号

平成８年１１月２１日

依頼者　株式会社　ホーネンコーポレーション　殿

平成　８年１０月　９日当協会食品衛生研究所に依頼された供試品について試験検査した結果は下記のとおりです。

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000087.046

第Ｓ８－２３１５号別紙

試験報告書

1．試験の目的

急性毒性（LD_{50}）の測定

2．被検物質

納豆菌発酵抽出物（ビタミンＫ原液３５００ｐｐｍ）

3．使用動物

マウス　ｄｄｙ系統　４週令　雄　３群（１群１０匹）を用いた。

4．試験方法

経口投与：健康なマウスを選択し１８時間絶食させた後、各群１０匹づつ被検物を40度の恒温水槽に保持しながら、マーゲンゾンデを用いてを強制経口投与した。

投与量：予備試験において把握した結果から、被検物原液が個体体重に対し物理的最大投与量50ml/kg.及び37.5ml/kg.25.0ml/kg.の３群を編成し投与した。

飼育条件：恒温恒湿の条件で飼育し、投与日を０として各個体の体重を測定しつつ、７日間一般症状及び生死の状態を観察した。この間水と餌は自由に与えた。

000087.047

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5．試験結果

各群とも投与後７日間一般状態に何ら異常を認めず、体重も順調に増加して死亡例は認められなかった。

6．考察

上記試験結果から本被検物納豆菌発酵抽出物のＬＤ$_{50}$は、50 mℓ/kg 以上と推定する。

Attached file 2

試験検査成績書

第Ｓ８－３３１５号

平成８年１１月２１日

依頼者　株式会社　ホーネンコーポレーション　殿

平成　８年１０月　９日当協会食品衛生研究所に依頼された供試品について

試験検査した結果は下記のとおりです。

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Pages 000087.049 - 000087.169 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

SU

Anderson, Blondell

From: Richard Kraska [kraska@gras-associates.com]
Sent: Friday, September 26, 2008 9:24 AM
To: Anderson, Blondell
Subject: NattoPharma

Blondell

Any progress with a meeting date? Also please note that the reference to Minaki on page 2 of my letter is incorrect; it should be Mikami. The citation for the Mikami reference in Attachment E is correct.

Have a good weekend.

Richard C. Kraska Ph.D., DABT
COO and Co-Founder
GRAS Associates, LLC
216-470-7280
Fax: 239-514-0606
kraska@gras-associates.com

000087.170

SU

Anderson, Blondell

From: Richard Kraska [kraska@gras-associates.com]
Sent: Thursday, November 06, 2008 11:56 AM
To: Anderson, Blondell
Cc: 'Bob McQuate'
Subject: *Additional Information for Meeting on MK-7*
Attachments: Omaye MK.pdf; Tyl Review Final.doc

Blondell

Here are two more resources for the toxicology review for the meeting scheduled for November 17.

1. A review of the toxicology data on MK-4 by Dr. Rochelle Tyl. Dr. Tyl is especially knowledgeable in reproductive toxicology and has some useful insights into the Mikami study which was of concern to FDA reviewers in our conference call back in May.
2. A short summary for Dr Stanley Omaye, University of Nevada, one of the original expert panel members summarizing the basic reasons that MK-7 is GRAS.

Please distribute these to the toxicology reviewers. Let me know if you have any questions. Thanks again for your help.

Richard C. Kraska Ph.D., DABT
COO and Co-Founder
GRAS Associates, LLC
216-470-7280
Fax: 239-514-0606
kraska@gras-associates.com

SU

Anderson, Blondell

From: Richard Kraska [rich@kraskaconsultants.com]
Sent: Tuesday, November 11, 2008 9:20 AM
To: Anderson, Blondell
Subject: Meeting Monday

Blondell

Four questions for Monday:

1. I w ould like to manage the discussion with a PowerPoint presentation that summarizes the major points for discussion. My recollection is that your conference rooms are all set up with computers and all I will need to do is bring the presentation on a memory stick. Please let me know if my assumptions are incorrect.
2. W hat do you recommend as far as handouts? I can make copies of the PowerPoint presentation for all to take notes on. Do I need to have copies of the submission requesting the prenotice conference or will the major reviewers already have those?
3. Are y ou expecting any additional FDA staff at the meeting?
4. W hich of the two buildings do we need to go to?

Thanks for your help.

Richard C. Kraska Ph.D., DABT
Kraska Consultants Inc.
216-470-7280
Fax: 239-514-0606
rich@kraskaconsultants.com

GRAS Assessment of MK-7

NattoPharma ASA

Vita K

GRAS Associates, LLC

GRAS Status

- GRAS Associates Expert Panel concluded that 50 µg/person/day MK-7 (DC = 17 ppb) is GRAS based on the following evidence:
  - High doses (>45 mg/day) of the structurally similar menaquinone MK-4 are used safety in clinical applications
  - Adequate toxicology data available on MK-4
  - Significant levels of menaquinones are naturally present in foods in US & in Europe
  - High levels of MK-7 consumed in Japanese diets
  - Clinical studies using MK-7 document absence of harm at doses up to
    - 1730 µg/day for two weeks
    - 75 µg/day as long as 1 year

Toxicology Studies on MK-4

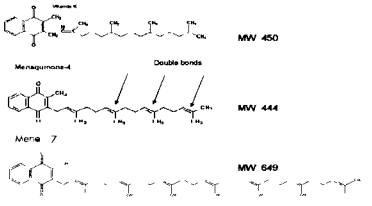
Study	NOAEL	Reference
Subchronic rat	30 mg/kg	Doi et al., 1995
1 Year rat	20 mg/kg	Hosokawa et al., 1995
1 Year dog	200 mg/kg	Vanatta et al., 1995
Rat repro/developmental	1000 mg/kg	Mikami et al., 1981
Rat repro/developmental	1000 mg/kg	Goto et al., 1986
Mice reproduction	100 mg/kg	Suzuki et al., 1971
Rabbit deviopmental	100 mg/kg	Ohsumi et al., 1986

FDA Feedback on MK-7 Review

- FDA questions natural levels of MK-7 in the diet as adequately supporting safety determination
- FDA questions the use of MK-4 data to judge the safety of MK-7 due to differences in bioavailability of MK-4 & MK-7
- Question raised about level of MK-6 in the product
- Clarification needed regarding MK-4 reproductive studies, especially Mikami et. al., 1981
- Clarification needed regarding consumption of MK-7 by subjects on anti-coagulant therapy
- FDA acknowledged that MK-7 has vitamin K activity

Structure of K Vitamins

Molecular structures K1, MK-4 and MK-7



Natural Levels of MK-7 in Foods

- Relevant information was summarized in attachment A to our letter
- High levels of MK-8 (5-20 µg/100g) in cheese
- High levels of MK-4 (5-20 µg/100g) in eggs
- High levels of MK-7 (>800 µg/ 100g) in natto
- Rotterdam study estimated daily consumption at 20 µg/day MKn
- Consumers using natto as a diet staple typically consume 100-500 µg/day MK-7
- Discussion?

Toxicology & Bioavailability of MK-4, MK-6 & MK-7

- Discussed in Attachment B and C
- Typical Level of MK-6 (1-3%) in NattoPharma product discussed in Attachment A
  - Steady state blood levels of MK-7 about 6-fold higher (3-fold higher on a molar concentration) than phylloquinone when equal doses are given
  - MK-7 is expected to be more bioavailable than MK-6.
  - 1000-fold margin of safety between NOEL of MK-4 of 1 mg/kg and desired use of MK-7 of 1 µg/kg
  - Adequate margin of safety even if an additional uncertainty factor is used to account for increased bioavailability

MK-4 Effects on Reproductive Performance & on Neonate

- The Mikami et al. study does not exhibit dose related adverse effects
- 9 relevant studies reviewed by Dr. R. Tyl, RTI
  - 2 rat reproductive studies, teratology studies on mice & rats
  - Various other studies on pregnancy, lactation & neonates
  - Conclusion: No safety concerns
- Discussion

Mikami et al.,1991- Table 8

Dose Group	Number of Neonatal Deaths Observed Within 4 days of birth	Percentage of Total Neonates
Control	9	2.9%
10 mg/kg	30	10.1%
100 mg/kg	7	2.3%
1000 mg/kg	21	6.6%

Patients on Anti-coagulant Therapy

- Information Included in Attachment C
- Discussion will be led by Dr. Schurgers

Summary

- **There is adequate evidence to show that 50 µg/day (DC = 17 ppb) of MK-7 is GRAS for human consumption**
  - High doses of structurally related MK-4 are used successfully in Japan
  - High no effect levels for MK-4 in various animal studies; ample margin of safety to judge the safety of MK-7 to account for higher bioavailability
  - MK-7 is a proven natural form and becoming recognized as an important form of vitamin K
- We plan to submit an updated GRN for FDA review

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SU

Anderson, Blondell

From: Richard Kraska [kraska@gras-associates.com]
Sent: Tuesday, November 18, 2008 1:50 PM
To: Anderson, Blondell
Cc: 'Bob McQuate'
Subject: FW: NattoPharma
Attachments: EFSA nda_op_ej822_vit_k2_en.pdf

Blondell

Thanks for orchestrating a great meeting. I will be forwarding the translation of the Mikami study again but it is a big file so I will do by separate email. Here is also the recent EFSA opinion. Please pass these along to the reviewers. We would be interested in a conference call on the reproductive studies but it will need to be after Dec. 1 because will be out of the office from Nov. 21 to Dec. 1. I will not be in phone contact but will be responding to emails during this period if you want to schedule the call.

Have a good Thanksgiving if I do not talk to you.

Richard C. Kraska Ph.D., DABT
COO and Co-Founder
GRAS Associates, LLC
216-470-7280
Fax: 239-514-0606
kraska@gras-associates.com

From: Richard Kraska [mailto:kraska@gras-associates.com]
Sent: Thursday, October 09, 2008 10:49 AM
To: 'Anderson, Blondell'
Subject: RE: NattoPharma

We also have a full translation of the Mikami study that you can provide to the toxicologist.

Richard C. Kraska Ph.D., DABT
COO and Co-Founder
GRAS Associates, LLC
216-470-7280
Fax: 239-514-0606
kraska@gras-associates.com

From: Anderson, Blondell [mailto:blondell.anderson@fda.hhs.gov]
Sent: Thursday, October 09, 2008 10:11 AM
To: Richard Kraska
Subject: RE: NattoPharma

I'm glad this made your day!!

Blondell Anderson

Consumer Safety Officer

DPR/OFAS/CFSAN

301-436-1304

From: Richard Kraska [mailto:kraska@gras-associates.com]
Sent: Thursday, October 09, 2008 9:32 AM
To: Anderson, Blondell
Subject: RE: NattoPharma

Blondell

I was so excited I forgot to say thank you very much!!

Richard C. Kraska Ph.D., DABT
COO and Co-Founder
GRAS Associates, LLC
216-470-7280
Fax: 239-514-0606
kraska@gras-associates.com

From: Anderson, Blondell [mailto:blondell.anderson@fda.hhs.gov]
Sent: Thursday, October 09, 2008 9:11 AM
To: Richard Kraska
Subject: RE: NattoPharma

Hello Rich:
I was able to change time to 11 AM.

Blondell Anderson

Consumer Safety Officer

DPR/OFAS/CFSAN

301-436-1304

From: Richard Kraska [mailto:kraska@gras-associates.com]
Sent: Thursday, October 09, 2008 7:51 AM
To: Anderson, Blondell
Subject: RE: NattoPharma

Blondell

My clients are having difficulty getting flights out of Dulles on Monday to get back to Europe. There is a flight at 5:20 PM but I don't think that they can through security and all in time especially if there is traffic on the beltway. Would it be possible to move up the meeting to 11 AM?

Richard C. Kraska Ph.D., DABT
COO and Co-Founder
GRAS Associates, LLC
216-470-7280
Fax: 239-514-0606
kraska@gras-associates.com

From: Anderson, Blondell [mailto:blondell.anderson@fda.hhs.gov]
Sent: Monday, September 29, 2008 11:45 AM
To: Richard Kraska
Subject: RE: NattoPharma

Dear Rich:
I have scheduled the meeting for Nov. 17th from 1:30 - 2:30 in room 2013 at the University Station building in College Park, MD. Do you all plan to attend in person or by telephone? If in person, I need to arrange for parking if needed. If any changes, will let you know immediately.

Blondell Anderson

Consumer Safety Officer

DPR/OFAS/CFSAN

301-436-1304

From: Richard Kraska [mailto:kraska@gras-associates.com]
Sent: Friday, September 26, 2008 9:24 AM
To: Anderson, Blondell
Subject: NattoPharma

Blondell

Any progress with a meeting date? Also please note that the reference to Minaki on page 2 of my letter is incorrect; it should be Mikami. The citation for the Mikami reference in Attachment E is correct.

Have a good weekend.

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Pages 000096- 000126 have been removed in accordance with copyright laws.
Please see appended bibliography list of the references that have been removed from this request.

SU

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May 13, 2009

Ms. Blondell Anderson
Division of Petition Review (HFS-265)
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Prenotice Communication (GRN)

Dear Ms. Anderson:

Attached is information from our client NattoPharma, which addresses FDA concerns expressed in our last meeting on November 17, 2008. This will all be addressed in a presentation our client is preparing for the scheduled May 27, 2009 meeting. Please distribute this information to your colleagues that will be attending the meeting. All information should be treated as a confidential prenotice communication.

Sincerely yours,

(b)(6)

Richard Kraska, Ph.D., DABT
Chief Operating Officer and Co-Founder
GRAS Associates, LLC

To FDA

From NattoPharma

Ref: Request for a FDA "No Comment" letter to the GRAS application for Vitamin K2 as Menaquinone-7 (MK7) from NattoPharma ASA, Norway. Answers to FDA questions of March 2009.

FDA has raised several questions to the self-affirmed GRAS for vitamin K as MK7 – branded as MenaQ7 products. In short, the points raised by FDA can be grouped as follows:

a. MK7 is not substantially equivalent to MK4
b. Questions about toxicity of MK4 in reproduction studies
c. No toxicity data on MK7 – using MK4 toxicity data not relevant (ref point 1)
d. No animal safety data on MK7 available
e. A NOAEL could not be established for MK4 from the available data – FDA disagrees with EFSA
f. Lack of long term MK7 consumption safety data
g. Not seen VitaK safety data
h. Effects of MK7 on glucose-6-phosphate deficient (G6PD) patients were not discussed
i. Problems with patients on anticoagulant therapy
j. Reference to some studies missing

In our meeting with FDA scheduled for May 27th, 2009 we will structure the answers to the questions in a presentation as follows:

1. Relevance of using MK4 toxicity data to reveal safety of MK7, and comments to MK4 toxicity data (points a-d) as well as establishment of NOAEL (point e)
2. Safety of long term consumption of MK7 and the VitaKids study data (points f and g)
3. Effects of MK7 on G6DP patients (point h)
4. Effects of MK7 on coagulation system and for patients on anticoagulant therapy (point i)
5. Missing study references (point j)

This letter is to outline the major answers to the questions while the details will be presented in the meeting. We hope for a fruitful discussion during our meeting in May.

A. Relevance of using MK4 toxicity data to reveal safety of MK7.

MK4 and MK7 are two in a series of homologous molecules called "vitamin K2", which differ only in the length of the isoprenic chain attached to the common ring structure.

The following points will be discussed during the meeting:

- While it is true that there are benefits to supplementation with vitamin K2 in the form of MK7 rather than MK4, the function, metabolism and excretion of breakdown products of MK4 and MK7 progress by identical mechanism in humans; differences between both vitamers originate from a longer half life, a better absorption and more equal tissue distribution of MK7. From a toxicological perspective, MK7 and MK4 are similar in that they both contain the naphthoquinone ring and are substituted at the 3 position with an aliphatic chain containing isoprenoid units. The only difference in the molecules is the length and saturation degree of the aliphatic side chain. Long chain meaquinones like MK7, MK8, and MK9 are incorporated into low density lipoproteins, whereas the less lipophylic phylloquinone and short-chain menaquinones (like MK4) are mainly found in the triglyceride fraction. The association with different lipoprotein particles explains the difference in half life in the circulation and the different tissue distribution of MK4 and MK7.
- Tissue conversion of vitamin K1 to MK4 via K3; of MK4 to MK4 via K3 and conversion of MK7 to MK4 via MK3 has been demonstrated indicating a bio-equivalence of K-vitamins *in situ.*[I],[II],[III],[IV],[V]. We note, however, that the conversion of MK7 to K3 has not been detected as a result of nutritional levels of intake of MK7.
- The toxicity of K3 (menadione) at high dosages is well known. Toxicity of K vitamins is due to the a free 3-position in the common naphthoquinone ring structure (=K3) - not the isoprenic side chain[VI]. The reactive 3-postion in K3 is known to bind to thiols of larger molecules causing hemolysis of red blood cells, for example. The active site for such reactions is not available in menaquinones because the 3-position is occupied by the isoprenoid chain. Thus MK4, MK7 etc. are not toxic by this well known mechanism. We note, specifically, that the adverse effects of MK4 observed when animals are fed very high doses may be a result of the transient increase in the concentration of MK3 resulting from the interconversions of menaquinones cited above. In this regard, we note that the toxic effects of K3 are observed only at very high dosage levels and these levels are highly unlikely to occur as the result of ingestion of MK7 at the envisioned usage levels.
- A longer isoprenic tail connected to vitamin K-like ring structure as such *does not* contribute to increased susceptibility of toxic effects. Coenzyme Q10 has a functional ring structure different from menaquinones, but a comparable aliphatic side chain. Q10 has 10 isoprenoid units in the aliphatic side chain compared to 7 in the MK7 molecule. Risk assessment[VII] and recent toxicology data[VIII],[IX] for Q10 reveal no toxicity. Absence of a well- defined critical effect precludes the selection of a NOAEL; the use of the observed safe level (OSL) or highest observed intake (HOI) approach established by FAO/WHO is therefore require to conduct risk assessment. Clinical human data indicate an upper level for supplements of Coenzyme Q10 of 1200 milligrams/day. We note, specifically, that the usage level

envisioned for MK7 is 100ug; 12,000 times lower than the supplement upper level for Coenzyme Q10.

B. Questions about toxicity of MK4 in reproduction studies

We appreciated the FDA comments relative to the mortality of the F2 neonatal rats in the Mikami study. While one could argue about whether the Goto study negates the Mikami result, we believe it is more important to focus on the fact that the lower number of neonates in the F2 generation in the Mikami study was not due to dose dependent treatment as the lowest number of neonates in F2 was observed at the lowest dose while the high doses were not significantly different than the control group. Thus, the Goto study can be viewed as corroborative in that no adverse treatment-related effects were observed.

C. No toxicity data on MK7 – using MK4 toxicity data not relevant (ref point 1)

a. MK4 has been chosen as the reference K vitamin derivative for conducting toxicology studies. This is highly relevant due to the similarities in structure and other properties to MK7 [see1 above]. Moreover, high doses are required to run toxicology studies; such doses can only be realized with synthetic (pure) MK4. In addition, MK4 is more hydrophilic than MK7, which makes it easier to handle when mixing it in animal food.

b. No animal toxicity data: We agree that there are no animal toxicity data on the purified MK7 product. We do not agree however that there is no relevant animal toxicity data which can be used for assessing the safety of MK7 consumption. We have previously shown that the absorption of MK7 from natto food and from capsules containing MK7 have the same pharmacokinetics. Therefore, we believe that

   i. the toxicity data presented below on the natto product performed by the Japanese National Institute of Health is relevant to the safety determination of MK7 (see below);

   Because of these results plus

   ii. the similarity of the MK4 and MK7 molecules noted above;
   iii. the known mechanism for the toxicity of K3 which is not operational in MK4 and higher menaquinones;
   iv. and the absence of toxicity of long-isoprenoid chain containing molecules like Q10,

   we believe that additional studies are not warranted. Such studies do not seem to contribute to any new information, and would constitute unnecessary use of laboratory animals.

Safety Evaluation of Natto by Japanese Authorities

The National Institute of Health in Japan has evaluated the safety of the Natto product "Kinnotsubu", containing 650 µg MK-7/50 g (http://hfnet.nih.go.jp/contents/detail405.html).

Unofficial summary translation:

"[Human test]
Twelve (12) healthy adults (age 29 to 50) took 150 g portion of the Natto (equivalent to 1.95 mg/kg body weight/day MK-7) for 7 days. Serum parameters and coagulating system parameters did not change compared to the values before starting this test.

[Animal test]
(Acute toxicological study)
LD_{50} of Natto is over 2000 mg/kg. (which is equivalent to 26 µg MK-7) in 5-week-old male rats.
(Subchronic toxicological study)
Four-week-old male and female rats were used. Each group was divided into groups (n=5/group) tested for 13 weeks. Result: No animal died and behaved abnormally. Examinations of haematology and biochemistry values in blood and urine revealed no abnormality. Also, any histopathological abnormality has not been observed. Thus, none toxicological dose of this Natto was concluded to be over 1000 mg/kg body weight/day (which is equivalent to 13 µg/kg body weight/day MK-7)."

c. We have determined MK7 to be GRAS for the described uses based on scientific principles and we consider the demonstrated consumption of MK7 in natto products and in fermented foods in Europe and the US such as hard cheeses to contribute to the body of knowledge related to the safety of the molecule. Estimates of consumption of MK-7 in Natto food in Japan were based on the analysis of MK-7 content in 26 commercial Natto products sold in Japan, MK-7 content in various Natto products is provided in Table 1.

Table 1: MK-7 Content in Commercial Natto Products Sold in Japan

Reference	Natto product	µg MK-7/100g Natto
Schurgers, LJ Thesis, 2002, p 37	Unknown, product from Asian supermarket Maastricht	998
Sumi, H, 1999 Food Sci Tech Res vol 5	B subtilis Miyagino strain	1230
Tsukamoto et al 2000, J Health Sci 46	Natto from Asahi Freshia Co Ltd	865
Tsukamoto et al 2000 J Bone Min Meta	Natto from Asahi Freshia Co Ltd	775
Kanki et al 2001.Nutrition vol 17	Natto from Shoujikimura Ltd	1398
Katsuyama et al 2004 J Nutr Sci Vitam 50	Natto - unknown source	900
Kamao en al 2007 J Nutr Sci Vitaminol	Natto - unknown source	939
Isobe, et al Natto and vitamin K in Basic and Clinical Aspects of Japanese Traditional Food Natto Ed Sumi, Hiroyuki Page 73-82. 1995 Japanese Technology Transfer Association.	19 different commercial Natto products (average MK-7 value)	1186

The average MK-7 content is approximately 1100 µg/100 gram Natto. Intake of MK-7 from one serving of Natto (which is approximately 40 to 50 g[x],[xi]) was estimated for different body weights. Body weights were estimated from several published studies, including persons in the Japanese Population-Based Osteoporosis (JPOS) study (Ikeda *et al.*, 2006). Low body weight men (65 kg) are comparable to high body weight women (65 kg), while average body weight for Japanese women in several studies (see also Ikeda *et al.*, 2006) is set to be 55 kg. No adverse effects have been reported from daily Natto consumption in hundreds of years. Furthermore, the safety of Natto has been evaluated by the National Institute of Health in Japan.

D, E and F. Long term consumption of MK7. We have previously shown that the absorption of MK7 from natto food and from capsules containing MK7 have the same pharmacokinetics. Several studies using high dose MK7 from natto food as well as studies using capsules with MK7 extracted from natto food will be presented. Such studies reveal 3 years continued use of MK7 without any reported adverse effects. In addition we have collected data from published studies as well as NattoPharma studies using MK7 over both short terms with high doses as well as longer terms with several dose levels. Table 2 gives an overview over such studies not tabulated in the first GRAS submission to FDA.

Table 2

Type of study	Duration	Dose range	No of person-treatment days
Short-term MK-7 from Natto food	< 1 year	123- 1230 µg/day	1 440
Short-term MK-7 (MenaQ7 ™)	< 1 year	45 – 360 µg/day	7 228
Long-term MK-7 from Natto food	≥ 1 year	38,6 - > 200 µg/day	586 190
Long-term MK-7 (MenaQ7™)	≥ 1 year	360 µg/day	54 750

G. Not seen VitaKids safety data. This study has now been accepted for publication [citation] and further unpublished data are now available. All participants in the study were evaluated for potential effects on the coagulation system using the ETP (Endogenous Thrombin Potential) test. Study data showed no effects on the coagulation system of adults or children. No adverse effects have been reported in these studies. The results will be presented.

H. Effects of MK7 on glucoe-6-phosphate dehydrogenase deficient (G6PD) patients were not discussed.

We appreciate the concern raised by the FDA related to the effects of K3 on patients deficient in glucose-6-phosphate and will present information which will – according to our understanding – put the concerns in the right perspective.

The adverse effect on these patients when exposed to K3 (menadione) is not surprising given the physiopathology of G6PD deficiency related to hemolytic anemia and the action of the known toxicity of K3 to red blood cells. In effect, the two adverse effects are additive. We note that this effect is not solely the result of K3 ingestion, but can be caused by numerous other drugs including analgesics, as well as, illness, e.g. severe infections and other conditions.

We, of course, agree that people with G6PD should avoid high dosage exposure to substances that lead to lower red blood cell count. Having said that, the generation of menadione from any form of vitamin K is known (as described above), but has never been shown at nutritional intake levels, only at much higher levels such as experienced in therapeutic use. We note that newborns are routinely given 1 mg of vitamine K1, which is also converted in part to K3. Also, we noted in the GRAS notification that green leafy vegetables are sources of vitamin K and thus of K3 production by bio-conversion, but limitation of consumption of these foods to protect G6PD patients is not advised nor are warnings given to such patients to our knowledge. In this context, we also note that menadione sodium bisulfite (Hykinone) is listed as a low risk drug "that probably can be safely given in normal therapeutic doses to G6PD-deficient patients without nonspherocytic hemolytic anemia and can be given safely to most patients with G6PD deficiency" [Blood, 1 January 2008, Vol. 111, No. 1, pp. 16-24; http://www.g6pd.org/favism/english/index.mv?pgid=safe.]

Considering the dose of vitamin K1 given to newborns and the common exposure of people to sources of vitamin K in their diet all of which is converted to K3 at some level, it is extremely unlikely that the consumption of 100 ug of MK7 will increase K3 in any detectable, significant, clinically relevant amount, especially in light of the fact that experts in the field feel that even therapeutic doses of K3 may be taken if the G6PD deficient patient is without nonspherocytic hemolytic anemia. These issues will be discussed.

I. Problems with patients on anticoagulant therapy. We agree with FDA that this is an area of concern. We know that Vitamin K2 as MK7 will not affect the coagulation process in people not on anticoagulant treatment, as their coagulation factors will be fully carboxylated at any time with normal consumption of vitamin K containing foods. No more glutamic acid residues are free to become activated under normal condition, thus "over" carboxylation is not biochemically possible. This is well known and recent studies with MK7 support this. Thus, supplementation of food with 100ug of MK7 is not a concern for this population. In addition, studies have shown that lack of effect on the coagulation system of healthy children who are not taking anti-coagulant drugs [see 5 above]. It is interesting that low vitamin K status may

lead to the under carboxylation of the only extrahepatic protein taking part in the clotting cascade: coagulation-inhibitor protein S; the poor protein S carboxylation results in loss of biological activity and in an increased thrombosis tendency. Under these conditions supplemental MK7 intake was shown to induce a decrease of the thrombosis risk.

We agree that there is a concern for consumption of vitamin K supplementation for patients taking anti-coagulant drugs which interfere with vitamin K. Studies have been conducted to check the influence of supplementing healthy individuals on anticoagulant therapy with increasing doses of vitamin K2 as MK7. The effects on the coagulation system are monitored by measuring the INR values for the persons on anticoagulant therapy. It is clear from these studies that vitamin K has an effect on INR values as any other K-vitamin would have. This can be in the form of foods rich in vitamin K1 such as green vegetables and vegetable oils, foods rich in vitamin K2 such as curds and cheeses, or K vitamins present in supplements. The risk is the same. Studies have shown that 10% of patients ingesting a dose of 50 ug/day of MK7 show a slight decrease in the INR value, but that it remains within the therapeutic target window. Lower levels of MK7 did not decrease the INR values in test subjects. [Schurgers, Blood 2004, 2007]. We note that INR values are actively managed by frequent monitoring of INR values in these patients in Thrombosis Clinics, routinely every 2-3 weeks, to balance the system either by adjusting the diet or medication. We have conducted more research in this area which we will present at the FDA meeting.

It is interesting that the lower the vitamin K intake is on a daily basis, the larger will be the effect on the INR when high vitamin K foods are eaten. This leads to fluctuation in the INR values in the patient and the net result is an unstable anticoagulation condition. To counter this problem, a high intake of vitamin K combined with a high dose of anticoagulant is rapidly gaining interest of clinicians because it is a preferred method to improve the stability of the anticoagulation effect. MK7 is the ideal vehicle for supplying this stable dose of vitamin K as the uptake and halflife are enhanced making it easier to maintain a stable *in situ* concentration.

Having said that, NattoPharma suggests that all foods fortified with vitamin MK7, should be labeled: ***Contains vitamin K2 or fortified with vitamin K2*** to insure that patients on anticoagulant therapy and their physicians have the information they need to manage the patient's vitamin K intake, either higher or lower depending on the need. More important in our view is that such labeling would assist consumers in finding foods that provide additional vitamin K2 as two published population surveys (Rotterdam with 4,500 subjects[XII] and Prospect with 16,000 subjects[XIII] as well as the recent study by Beulens et al[XIV] with 564 post menopausal women) indicate that long chain menaquinones were important nutrients to decrease cardiovascular mortality. The Prospect study had sufficient power to calculate that each extra daily intake of 10 ug of K2 will decrease the cardiovascular mortality by 9%.

J. Reference to some studies missing. These will be commented upon during the meeting

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REVIEW OF THE LITERATURE ON THE EFFICACY AND TOXICITY OF MENATETRENONE (VITAMIN K_2; MK-4)

For

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1.0 BACKGROUND

Several reports (see below) indicate an important role for vitamin K in bone health, as well as in blood coagulation (e.g., in cases of newborns with evidence of hypoprothombinemia). Dietary vitamin K intake in young women living in eastern Japan was estimated (from HPLC analysis of phylloquinone [PK] and menaquinones [MKs]) as PK, MK-4, and MK-7 contents in food and from standard tables of food composition in Japan) (Kamao et al., 2007). The mean total vitamin K intake for all subjects was about 230 μg/day; 94% of the study participants met the adequate intake (AI) standards for Japanese women (aged 19-29 years) of 60 μg/day. The relative concentrations of PK, MK-4, and MK-7 to total vitamin intake was 67.7%, 7.3%, and 24.9%, respectively. The authors voiced concern that the current AIs may not be sufficient for bone or blood health, or during pregnancy (Kamao et al., 2007).

In support of the role of vitamin K in bone and blood health (and the recognition that additional vitamin K may be prophylactic), two studies were performed. In the first study (Suzuki et al., 1984), a double-blind study of oral vitamin K_2 syrup was performed on Japanese newborn children. They concluded that general vitamin K prophylaxis for newborns is not necessary (or recommended), but that vitamin K (one dose at 6 mg orally) is helpful for infants with thrombotest results below 20% (indicating low coagulatory ability), with efficacy of vitamin K-2 at 6 mg better than vitamin K-2 at 2 mg, which was better than vitamin K-1 at 2 mg, which was better than placebo. They also recommended vitamin K therapy for "infants at risk and in all premature, dystrophic or otherwise underweight infants."

In the second study, Shiraki et al. (2000) reported that vitamin K_2 (menaquinone-4; menatetrenone) was given orally at 45 mg/day to 120 osteoporotic patients for 24 months (with 121 osteoporotic patients as controls). The patients were followed by lumbar bone mineral density measurements and for occurrence of new clinical fractures. Serum levels of glucose OC (osteocalcin) and menaquinone-4, and urinary excretion of deoxypyridinoline (DPD), were also periodically measured. The two groups were identified prior to study start. The incidence of clinical fractures was lower in the vitamin K group, and the loss in lumbar bone mineral density was less in the vitamin K group. The serum glucose OC and OC were higher in the vitamin K group. There was no significant change in urinary DPD excretion. The authors concluded that vitamin K treatments effectively prevented the occurrence of new fractures, although the vitamin K_2 group did not have an increase in lumbar bone mineral density; however, there was a slower

decline over the 24-month duration of the study. Furthermore, vitamin K treatment enhanced gamma-carboxylation of the OC molecule.

Since there is strong evidence for vitamin K efficacy in newborns, a series of studies examined *in vivo* maternal levels of vitamin K_2 (MK-4) during pregnancy, *in vitro* placental transport, vitamin K_2 in mothers after delivery, and in their newborns.

In a preliminary study, Iioka et al. (1991) examined the pharmacokinetics of vitamin K_2 after it was injected intravenously into human mothers just before pregnancy termination or delivery. The vitamin K_2 was followed from maternal plasma to placental tissues and into the fetuses. They also tracked the vitamin K_2 into maternal milk (after delivery) over time. The results indicated that incorporation of vitamin K_2 into the placental tissue was relatively active, while transfer of vitamin K_2 to fetal blood (cord plasma) was small. This was interpreted to mean that vitamin K_2 from maternal blood is initially stored in the placenta and then gradually released into the fetal blood over time. The release of vitamin K_2 from maternal plasma (from iv injection) into milk increased over time, even after the maternal plasma levels of vitamin K_2 had decreased. This suggested that there is a concentrating mechanism for vitamin K_2 in mammary tissue.

Iioka et al. (1992) also drew systemic venous and umbilical vein blood from 12 women "in the 10th month of pregnancy," and vitamin K_2 was measured at delivery at 0.32±0.07 ng/ml in maternal venous blood, undetected in the umbilical venous blood, and 1.01±0.37 ng/g wet tissue in the placental villous tissue. When the 12 women (scheduled for caesarean section) were given vitamin K_2 (MK-4) at 20 mg/day orally for seven days and then c-sectioned at full term, the vitamin K_2 values were 0.89±0.21 ng/ml in maternal venous blood (up almost three-fold), 0.18±0.06 ng/ml in the umbilical venous blood (up 18-fold), and 5.38 ng/g wet tissue in the placental villous tissue (over five-fold). Villous tissue specimens were obtained from placentae from therapeutic abortions at the end of the first trimester, as well as immediately after normal full-term parturition, and cultured in serum-free medium with different concentrations of vitamin K_2 at 37°C for various times to determine transport of vitamin K_2 into the villous tissue *in vitro*. The results indicated that the transport into placental villous tissue increased with increasing concentrations in the medium and with increasing time in culture, with final uptake of vitamin K_2 at ~2.2 μg/g tissue after two hours in medium with 20 μg/ml vitamin K_2, at ~1.3 μg/g after two hours at 10 μg/ml, and at ~0.25 μg/g after two hours at 2 μg/ml (based on Figure 1 of paper).

Transport of vitamin K_2 into villous tissue was somewhat higher in full-term tissue samples than in tissue samples at the end of the first trimester. The authors suggested that vitamin K_2 transport into villous tissue was not active, but that there was a active transport system highly specific for vitamin K_2 into the placenta. Transport of the vitamin K_2 was higher than that of vitamin K_1 . They concluded that while transport into the fetus is not "especially pronounced," transport into the placental villous tissue is "comparatively good."

Motohara et al. (1990) examined the effects of vitamin K supplementation for 33 pregnant Japanese women on plasma vitamin K and PIVKA-II (protein-induced vitamin K absence-II) in their neonates (also n=33). Randomly selected mothers were orally given 20 mg of VK_1 (n=11) or VK_2 (n=12) seven to ten days prior to delivery. Maternal blood and cord blood (plasma) at delivery was analyzed for VK_1 and VK_2. On the fifth postnatal day, breast milk was analyzed for VK_1 and VK_2, and their infants were evaluated for the presence of PIVKA-II. At delivery, maternal plasma concentrations of VK_1 and VK_2 were significantly higher in treated mothers (both $p<0.01$) than in control mothers. Similarly, cord plasma VK_1 and VK_2 concentrations were also significantly higher in the treated groups than in the control group ($p<0.05$ for both). Cord plasma contained less than one-tenth the vitamin K concentrations than did maternal plasma for every mother in both groups, but a significant positive correlation ($p<0.01$) was found in VK concentration between maternal and cord plasma. Also, the proportion of PIVKA-II infants was significantly lower in the VK-treated groups than in the control group ($p<0.05$). Breast milk on postnatal day (pnd) 5 contained significantly higher concentrations of VK_1 and VK_2 (both $p<0.01$) in the VK-treated mothers. In the control group, nine of ten infants were positive for PIVKA-II, but no infant in either treatment group was positive (difference significant at $p<0.01$ for both). The authors concluded that administration of either VK_1 or VK_2 to mothers prior to delivery may prevent hypoprothonbinemia in their infants at birth and during their neonatal stages.

2.0 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY ASSESSMENTS

Embryofetal toxicity was assessed in rats, mice and rabbits, and postnatal offspring toxicity was assessed in rats and mice by various authors from oral and intraperitoneal (ip) injected doses of vitamin K_2 (menaquinous-4, MK-4) over a broad dose range by a number of authors.

2.1 Suzuki et al. (1971)

2.1.1 Prenatal Oral Dosing

Suzuki et al. (1971) administered MK-4 in cottonseed oil by oral gavage once daily for six days to Wistar rats on gestational day (gd) 9-14 (17-20 dams/groups) and to ICR-JCL mice (16-18 dams/group) on gd 7-12 (gd 0 was defined as the day of confirmed copulation by vaginal plug or sperm for both species). The oral doses in both mice and rats were 0, 10, 500, and 1000 mg/kg/day. In the mice, there was no effect at any dose on maternal body weights, on pregnancy rate, number of uterine implants, number of live or resorbed and/or dead implants, fetal body weight at term (gd 18 for mice), sex ratio, or incidence or type of malformation. There was a statistically significant increase in "non-ossified mid of forelimbs" at 500 mg/kg/day (19.8%) and at 1000 mg/kg/day (16.6%), but no effects on the extent of ossification for sternebrae, hindlimbs, or tail and no dose-related effects on fetal skeletal variations. For rats, there was also no effect at any dose on maternal body weight, pregnancy rate, number of uterine implants, number of live or resorbed and/or dead implants, fetal body weight at term (gd 20 for rats), sex ratio, or incidence or type of malformation. There were statistically significant increases in the incidence of undeveloped metatarsus of the foot and "non-ossified prox. of the hindlimb" at 1000 mg/kg/day, and a significant reduction in the incidence of lumbar ribs at 1000 mg/kg/day (there should only be ribs on the thoracic vertebrae; presence of lumbar ribs is usually designated a variation).

2.1.2 Postnatal Oral Dosing

In the postnatal component of the oral mouse study by Suzuki et al. (1971), seven to eight additional dams each at 0, 10, and 1000 mg/kg/day were allowed to deliver their litters. There was no effect at either dose on viability, index at birth or weaning (pnd 21), number of implants/dam, number offspring/dam, and no effect on pup weights/litter on pnd 0, 7, 14, or 21. There were significant delays in pinna detachment and emergence of abdominal hair in the pups at 1000 mg/kg/day, but no effects on incisor eruption or eye opening. One pup/sex/litter was retained after weaning, and there was no effect on age at testis descent or vaginal opening.

In the postnatal component of the oral rat study, seven to eight additional dams each at 0, 10, and 1000 mg/kg/day were allowed to deliver their litters. There was no effect at either dose on viability index at birth or at weaning on the number of implants/dam, number of

offspring/dam, or on pup body weights/litter on pnd 0, 7, 14, or 21. There were no effects at either dose on acquisition of any of the developmental landmarks tested, including, for one pup/sex/litter retained after weaning, vaginal patency (opening) and testes descent.

2.1.3 Prenatal Intraperitoneal Administration

The authors (Suzuki et al., 1971) repeated the developmental toxicity studies using ip injection of MK-4 at 0, 5, 50, or 100 mg/kg/day (on gd 7-12 for mice and gd 9-14 for rats). They also used a nondosed control.

In the mice, with 15-19 dams/groups, there were no effects at any dose on total implants/dam, live or dead and/or resorbed implants/dam, mean fetal body weight/litter at term (gd 18), sex ratio, incidence of fetal malformations, and no dose-related incidences of reduced ossifications. There were increased incidences at all doses for cervical ribs versus the solvent control but not the untreated control,) and increased incidences of lumbar ribs at 5 and 100 mg/kg/day (but not at 50 mg/kg/day) versus the solvent control but not the untreated control.

For rats (with 15-21 dams/group), there were no effects at any dose on the number of implants/dam, number of live, dead, or resorbed implants/litter, fetal body weight/litter, sex ratio, or incidence of malformations. As with the mice, there were significantly increased incidences of reduced ossification for sternebrae, metacarpals of the forelimb, and "non-ossified prox. of forelimb" relative to the solvent control, but not relative to the untreated control. There were no effects of MK-4 on incidences of lumbar or cervical ribs or of abnormal sternebrae.

2.1.4 Postnatal Intraperitoneal Administration

Of the seven to ten mouse dams at 0, 5, or 50 mg/kg/day allowed to deliver their litters (Suzuki et al., 1971), there were no effects on the number of implants/dam, viability index at birth (pnd 0) or weaning (pnd 21), or on litter size or pup body weights/litter on pnd 0, 7, 14, or 21. There were also no effects of treatment or on dose acquisition of any developmental landmarks evaluated during lactation or for the pups/sex/litter retained after weaning, and no effects on acquisition of vaginal patency or testes descent.

The authors concluded that there is no effect of MK-4 on prenatal developmental toxicity, including teratogenicity in rats or mice by oral gavage (0, 10, 500, or 1000 mg/kg/day) or by ip injection (0, 5, 50, or 100 mg/kg/day). There is also no effect of MK-4 on postnatal survival,

growth, or development, including no effects on acquisition of developmental landmarks (including vaginal patency and testes descent) when administered by oral gavage (0, 10, or 1000 mg/kg/day) or by ip injection (0, 5, or 50 mg/kg/day) to rats or mice.

2.2 Takehara et al. (1981)

2.2.1 Acute Toxicity Studies in Rats

Takehara et al. (1981) examined the postnatal survival, growth, and development of SLC-SD rat pups (from Shizuoka Prefecture Agricultural Cooperative) administered a single dose of "Kaytwo syrup" (vitamin K_2 syrup containing 6 mg/ml of menatetrenone/menaquinone-4) orally on pnd 4 (4 males and 4 females/maternal rat, and 4 dams/group) at 0 (physiological saline), 0 (solvent), 60, 360, or 500 mg/kg (dosing volume 0, 90 [solvent], 10, 60, or 90 ml/kg, respectively).

In a companion study, rat pups from the same strain and supplier were administered a single dose of "Kaytwo syrup" by "intraperitoneal injection after penetrating the left medial femoral muscle" once on pnd 4 (4/sex/dam and 4 dams/group) at 0 (physiological saline), 0 (solvent), 60, 360, or 720 mg/kg (dosing volume 0, 120 [solvent], 60, 360, or 120 ml/kg, respectively). The rat pups were raised by their dams until pnd 21-22 and transferred to group housing by sex/dam until pnd 35 (31 days postadministration).

The authors reported that there was transient cyanosis with abdominal distension immediately after gavage administration at 540 mg/kg (120 ml/kg) and transient cyanosis with abdominal distention immediately after ip administration of 720 mg/kg (120 ml/kg). Three rats (1 male – solvent, 1 male and 1 female – ip 360 mg/kg) died due to abdominal bleeding, likely due to ip administration. There were no differences among gavage groups for periodic body weights for the orally dosed groups. There were reductions in male body weights on days 7 and 14 at 720 mg/kg and in female body weights on day 7 at 720 mg/kg. In contrast, the male body weights were higher on day 35 at 720 mg/kg by ip administration. There were no effects on acquisition of incisor eruption, eye opening, testes descent, and vaginal patency, and no differences between the saline and solvent group or between either control group and any of the "Kaytwo syrup" doses by either oral or ip administration. There was also no evidence of treatment or dose effects at the "megascopical" (gross) examination at necropsy by either route.

2.2.2 Subacute Toxicity Studies in Rats

Takehara et al. (1981) also evaluated "Kaytwo syrup" in a subacute study. The rat strain was CRJ-CD rats (from Charles River, Japan), with neonates adjusted to five/sex/maternal rat. A total of 240 neonates/sex were used. These pups were weaned on pnd 21-22 and then housed by sex/litter until termination. There were five groups (45 male and 50 female neonates/group) at 0 (physiological saline), 0 (solvent), 6, 30, and 60 mg/kg vitamin K_2 (MK-4) at a dosing volume of 10 ml/kg. The pups were dosed daily by oral gavage, starting on pnd 4. Ten pups/sex/group each were terminated and necropsied after one and three months of administration, and the remaining rats were sacrificed after the one-month recovery period. In order to adjust group numbers, some ("non-selected") rats were also sacrificed in week 7 after initiation of administration and once during the recovery period on week 14 after initiation of administration.

During the study, some rats died due to gavage misadministration, but the remaining rats survived with no clinical symptoms. There were no effects of MK-4 on feed or water consumption, body weight change, or food efficiency. There were also no effects on the eye examinations, urinalysis, hematological parameters, or serum biochemical parameters. At the necropsies, there were also no effects on organ weights, necropsy gross findings, or from histopathological examination at any MK-4 dose, according to the authors (Takehara et al., 1981).

3.0 FERTILITY AND PERI- AND POSTNATAL STUDIES OF MENATETRENONE (VITAMIN K_2; MK-4)

3.1 <u>Mikami et al. (1981)</u>

3.1.1 Fertility Study

Mikami et al. (1981) administered vitamin K_2 to SLC®-SD rats from the Shizuoka Prefecture Agricultural Cooperative (22-24 males and 22-24 females per dose group) once per day (between 9:00 and 10:00 a.m.) by oral gavage for 13 weeks (males) or 14 days (females) at 0 (vehicle; 5% gum arabic), 10, 100, or 1000 mg/kg/day, with a dosing volume of 1 ml/100 g body weight. (F0 estrous cyclicity was monitored for 14 days prior to the start of drug administration, during the prebreed dosing period, and during the mating period.) The rats were then paired within groups, for a maximum of 14 days, with daily dosing continuing. Paired females were examined each morning for evidence of copulation (vaginal sperm or vaginal copulation plus). The date of copulation was designated gd 0. Daily dosing continued through the mating period

for both sexes until copulation. The F0 males were sacrificed once "fertility" was confirmed by placental signs, on gd 12-16 (approximately 120 days of dosing total), and necropsy performed. The sperm/plug positive (presumed pregnant) females were dosed daily until gd 7. Pregnant dams were terminated on gd 20, and maternal reproductive information (pregnancy rate, ovarian corpora lutea, uterine implantation sites) and embryofetal information (total, resorbed, dead/live implants, litter size, fetal parameters [sex, body weight, placental weight, external, visceral and skeletal findings]) were collected and reported. One-third of the fetuses/litter were fixed in Bouin's (and refixed in 80% ethanol) for visceral examination (by Wilson sectioning techniques). Two-thirds of the fetuses/litter were fixed in ethanol, their internal organs examined, and then double stained with Alizarin red S (for ossified structures) and alcian blue (for cartilaginous structures).

3.1.2 Peri- and Postnatal Study

Mikami et al. (1981) also distributed timed-mated SLC®-SD rats into four groups (24 presumed pregnant females/group) and administered vitamin K_2 by oral gavage once daily (between 9:00 and 10:00 a.m.) on gd 17 until pnd 21 (at weaning). The F0 dams were allowed to deliver, and F1 litters were standardized to eight pups (four males and four females) on pnd 4. The F1 litters were weaned on pnd 21, and the F0 dams were terminated. During lactation, the F1 pups were evaluated for morphological and functional development by assessing them for pinna detachment, eye opening, incisor eruption, palmar grasp, "walking," auditory startle, and free-fall righting reflex. At weaning on pnd 21, approximately one-half of each F1 litter was terminated for examination of the F1 pup organs and skeletons, and blood were taken for clinical and serum biochemistry tests. The remaining F1 pups per litter (approximately one-half) were retained for: open-field evaluation at five and seven weeks of age, rotarod performance, and the learning test (pole climbing method), each with 1 F1 pup/sex/litter. In addition, nine to ten F1 males/group and ten F1 females/group were evaluated for respiratory rate, heart rate, and blood pressure, and age at testes descent (males) or vaginal patency (females) was recorded. At 11 weeks of age, one F1 offspring/sex/litter (20-23 mating pairs/group) was mated 1:1 within groups, excluding sibling pairing (for up to 14 days). Pregnant F1 females were allowed to deliver their F2 offspring. The F2 pups remained with their F1 mothers until pnd 4. On pnd 4,

the F1 dams and their F2 offspring were terminated, and maternal and offspring parameters assessed.

3.1.3 Fertility Study Results

For the F0 parental animals, there were no effects of treatment or dose on weekly body weights or weekly feed or water consumption (expressed as g or ml/day/rat) over the 13 weeks for the males, or body weights or feed or water consumption for days -14, -7, 0, 2, 4, 6, 8, 10, 12, and 14 for the females. There were no male or female mortalities and no reported clinical observations. Estrous cycle length and frequency of estrus were also equivalent across all groups. The percentages of F0 mating pairs which copulated were 95.8% at 0 and 10 mg/kg/day and 100.0% at 100 and 1000 mg/kg/day. The percentages of copulated females which were pregnant were 91.3% at 0 and 10 mg/kg/day, 95.5% at 100 mg/kg/day, and 95.8% at 1000 mg/kg/day.

For the pregnant F0 dams, 20, 21, 21, and 23 at 0, 10, 100, and 1000 mg/kg/day, respectively, there were no differences among groups for the numbers of ovarian corpora lutea/dam, total uterine implantations/dam, or ratio of implantations to corpora lutea. The number of resorptions was significantly increased, and the number of live fetuses was therefore significantly decreased at 10 mg/kg/day (but still "well within the range of background data"), and not affected at 100 or 1000 mg/kg/day. F1 fetal sex ratios were equivalent across all groups, as were fetal body weights (sexes combined) and placental weights. There were no external malformations in any fetus in any group. There were no differences among groups on the incidence or type of internal fetal "anomalies" (malformations) expressed as percentage/fetus or as percentage average/dam. There were three fetuses at 0 mg/kg/day, two at 10 mg/kg/day, three at 100 mg/kg/day, and two at 1000 mg/kg/day with malformations. The evaluations of the fetal skeletons (two-thirds/litter) indicated no effects of treatment or dose on the mean number of ossified sacral and caudal vertebrae or in the incidence of non-ossified hyoid body or sternebrae V and VI (the last to ossify). There were significant <u>reductions</u> in the incidences of non-ossified odontoid process (for fetuses at 100 mg/kg/day), cervical body (for fetuses at 10, 100, and 1000 mg/kg/day and for litters at 100 mg/kg/day), and for undeveloped metacarpals (forelimb) for fetuses at 1000 mg/kg/day). Therefore, these effects represented "significant progress in ossification" (i.e., accelerated ossification with no dose-response pattern). The type and

incidence of fetal skeletal "anomalies" were unaffected by treatment or dose (Mikami et al., 1981).

Based on the conclusions of the authors, it is this reviewer's considered opinion that the no observable adverse effect level (NOAEL) for both reproductive and developmental toxicity was at or above 1000 mg/kg/day.

3.1.4 Peri- and Postnatal Study Results

Reproductive parameters for F0 dams were equivalent across all groups, including duration of pregnancy, number of implantation sites, number of total and live neonates at birth, sex ratio, and percentage of live births (one F0 dam at 10 mg/kg/day did not deliver a live litter; she had one uterine implantation site at necropsy). There was only one neonatal external malformation observed (proboscis at 1000 mg/kg/day). F0 maternal body weights were equivalent across all groups for gd 0, 17, and 20 and for pnd 0, 4, 7, 14, and 21. Maternal weight gains were also equivalent across all groups for gd 0-17, 17-20, and 0-20 (after delivery) and for pnd 0-7, 7-14, and 14-21, except that for pnd 7-14, there was a significant reduction in maternal weight gain ($p<0.01$) at 100 mg/kg/day, but not at 1000 mg/kg/day. The 24 pregnant F0 dams/group delivered 24, 23, 24, and 24 litters, and there were 23, 22, 23, and 24 live litters during lactation, all at 0, 10, 100, and 1000 mg/kg/day, respectively. At termination on pnd 21, the F0 dams exhibited no differences among groups for hematology or clinical chemistry. Mean spleen wet weight was significantly increased at 100 and 1000 mg/kg/day, and mean wet adrenal gland weight was significantly increased at 1000 mg/kg/day.

F1 litter sizes (number of pups/litter) were equivalent across all groups on pnd 0, 4 (precull), 4 (postcull), and 21. The mean pup body weights/litter by sex were also equivalent across all groups on pnd 0, 4 (precull) 4 (postcull), 14, and 21 (pnd 7 weights were not provided). The F1 litters were standardized to eight pups/litter (four males and four females) on pnd 4. The brains of the culled pups were normal at all doses. Notably, the number of dead neonates postpartum (from pnd 0-4) were significantly increased at 10 mg/kg/day (30 pups, 10.1%, $p<0.01$) and at 1000 mg/kg/day (21 pups, 6.6%, $p<0.01$), but not at 100 mg/kg/day (seven pups, 2.3%), versus the vehicle control value of nine dead pups (2.9%). There were no effects on F1 pup age of acquisition of pinna detachment, eye opening, or incisor eruption in any group for either sex. The mean F1 age at palmar grasp was slightly, but significantly, delayed in F1 males

at 10 and 100 mg/kg/day and for F1 females at 100 mg/kg/day. The F1 ages at "walking" and auditory startle were unaffected at any dose for either sex. Free-fall righting reflex was slightly but statistically delayed at 10 mg/kg/day ($p<0.05$) and at 1000 mg/kg/day ($p<0.01$) for F1 males, and only at 1000 mg/kg/day ($p<0.01$) for F1 females. At F1 weaning, the absolute (but not relative) liver weights were reduced at 1000 mg/kg/day. The absolute weights of the F1 male spleens at 10 and 100 mg/kg/day and the relative F1 male spleen weights at 10, 100, and 1000 mg/kg/day were significantly increased. All other organ weights (absolute and relative) were equivalent for both sexes across all groups. For the open field test at five and seven weeks of age, there were no effects on ambulation, rearing, or defecation for F1 males at any dose at either age. For F1 females, there were no effects on ambulation or rearing at any dose at either age. Defecation was significantly increased only for females, only at five week of age, and only at 10 mg/kg/day. Rotarod performance was equivalent across all groups for both sexes. The learning test (pole climbing method) reported frequencies of conditioned response, unconditioned response, or no response, over five sessions for the F1 male and female pups separately. For F1 males, there were significant reductions in unconditioned response ($p<0.05$) and a significant increase in no response ($p<0.01$) at 1000 mg/kg/day for Session 1, and only a significant reduction in conditioned response ($p<0.05$) at 1000 mg/kg/day for Session 2. The values were equivalent across all groups for all three parameters for Sessions 3-5. For F1 females, the values were equivalent across all groups for all parameters for Sessions 1 and 5. For Session 2, unconditioned response was significantly reduced at 100 and 1000 mg/kg/day, both at $p<0.05$. For Session 3, conditioned response was significantly reduced ($p<0.05$), and no response was significantly increased ($p<0.05$) at 1000 mg/kg/day. For Session 4, only conditioned response was significantly reduced ($p<0.05$) and only at 1000 mg/kg/day. There were no differences in respiratory rate, heart rate, or blood pressure for either sex in any group. Ages at acquisition of testes descent or vaginal patency were equivalent for both sexes across all groups.

The F1 males and females (one/sex/group, 20-23 mating pairs/group) were paired 1:1 with groups, excluding sibling matings. Copulation ratio (number copulated/number paired) was equivalent across all groups for both males and females (90-100%). A total of 20, 14, 21, and 19 females were pregnant at 0, 10, 100, and 1000 mg/kg/day, respectively. All of the pups from one dam each at 100 and 1000 mg/kg/day died. The pregnancy ratio (number pregnant/ number copulated) was therefore 90.9, 77.8, 100.0, and 90.5% at 0, 10, 100, and 1000 mg/kg/day,

respectively. The precoital interval (time to mating) was also equivalent across all groups. All pregnant F1 females delivered. Gestational length was slightly, but significantly, shorter at 10 mg/kg/day ($p<0.05$) and equivalent at 0, 100, and 1000 mg/kg/day.

Total implantation sites/F1 dam were equivalent across all groups. The number of total and live F2 neonates/dam on pnd 0 was significantly reduced ($p<0.01$) at 10 mg/kg/day and equivalent at 0, 100, and 1000 mg/kg/day. F2 pup sex ratio was equivalent across all groups, and there were no external malformations ("anomalies") in any F2 pups in any group. The number of F2 live neonates/litter was also significantly reduced at 10 mg/kg/day on pnd 4. F2 pup body weights/litter by sex were equivalent across all groups on pnd 0 and 4. The number and percentage of dead F2 neonates on pnd 0 were significantly increased at 100 mg/kg/day (to 20; 6.4%, $p<0.01$), but not at 1000 mg/kg/day (eight, 2.7%). For pnd 0-4, the values were: control (four, 1.3%), 10 mg/kg/day (ten, 5.3%, $p<0.05$), 100 mg/kg/day (40, 12.8%, $p<0.01$), and 1000 mg/kg/day (36, 12.2%, $p<0.01$). The percentage of F1 dams with one or more dead F2 neonates was equivalent across all groups (25.0, 35.7, 33.3, and 26.3% at 0, 10, 100, and 1000 mg/kg/day, respectively). The F1 dams and their F2 pups were terminated on pnd 4.

The two offspring findings of concern were: (1) the increased incidence (number and percentage) of dead F1 neonates on pnd 0-4 at 10 and 1000 mg/kg/day (but not at 100 mg/kg/day), with the values at 10 mg/kg/day higher (30 F1 pups, 10.1%) than at 1000 mg/kg/day (21 pups, 6.6%); and (2) the increased number and percentage of dead F2 neonates on pnd 0-4 at 10 (10 pups, 5.3%, $p<0.05$), 100 (40 pups, 12.8%, $p<0.01$), and 1000 mg/kg/day (36, 12.2%, $p<0.01$). There were no other indications of offspring toxicity in the F1 or F2 offspring generations. It is interesting that the F1 pup mortality rate was higher at 10 mg/kg/day than at 1000 mg/kg/day and was equivalent for the vehicle control rate and that at 100 mg/kg/day. The F2 pup mortality rate at 1000 mg/kg/day was slightly lower than that at 100 mg/kg/day, at ten-fold the dose (100 to 1000 mg/kg/day). In the F1 and F2 offspring generations, there were no differences in live litter size on pnd 0 or 4 at 100 or 1000 mg/kg/day. For F2 pups, there was a significant reduction in live litter size on pnd 4 at 10 (but not at 100 or 1000) mg/kg/day (Mikami et al., 1981). The effects on the F1 and F2 pups at 10 mg/kg/day were clearly not dose related, since in most cases they were not observed at 100 mg/kg/day (e.g., reduced F1 maternal gestational duration; F1 and F2 litter size at birth). The animal room temperature was maintained at 20-24°C (68-75.2°F), with lights on from 7:00 to 19:00 hours. An animal room at

the low end of that temperature range with neonates is rather cool and may have contributed to perinatal pup loss.

The authors' conclusions were that there were "no observable effects of K_2" on any parameter in either study. In this reviewer's considered opinion, the most conservative NOAEL from the data in this study is 100 mg/kg/day (due to neonatal losses at 1000 mg/kg/day) for offspring toxicity, and at or above 1000 mg/kg/day for maternal toxicity.

3.2 Goto et al. (1986)

Goto et al. (1986) used the same rat strain (and source); female Slc-SD (SPF) rats from Shizuoka Prefecture Agricultural Cooperative. The F0 females were bred 1:1, and the morning a vaginal plug was detected was designated gd 0. There were four groups with 36 plug positive (presumed pregnant) females per group. The doses of menatetrenone (vitamin K_2) in 5% gum anabic were 0 (vehicle), 10, 100, and 1000 mg/kg/day (equivalent to approximately five fold, 50 fold, and 200 fold the usual human dose), administered once daily by oral gavage on gd 7-17. A total of 24 dams/group were terminated on gd 20, and the dams and conceptuses were examined for maternal and/or developmental toxicity, including teratogenicity. The fetuses were subjected to external (all/litter), visceral (approximately one-third/litter), and skeletal examination (approximately two-thirds/litter after double staining with alizarin red S for ossified structures and alcian blue for cartilaginous structures). The remaining 12 dams/group were allowed to deliver their F1 litters, with the date of delivery designated pnd 0. The pups were examined, weighed, and sexed throughout lactation. On pnd 4, each litter was adjusted to eight pups, four/sex/litter. During lactation, the F1 pups were examined for physical growth and functional development (including pinna detachment, incisor eruption, eye opening, palmar grasp reflex, auditory startle, and righting reflex). At weaning, F0 dams and 1 F1 pup/sex/litter were terminated and necropsied, with organs weighed, examined grossly, and then examined histopathologically. In addition, one additional F1 pup/sex/litter was terminated at weaning, double stained with alizarin red S and alcian blue, and their skeletons examined. The remaining F1 pups/litter, retained after weaning on pnd 21, were evaluated in a series of tests: one-minute motion test, struggle response, surface righting reflex, withdrawal reflex, pinna reflex, Preyer's reflex (to noise), visual placing, and tail flick. In addition, five-week-old F1 pups of both sexes were observed in an open field test. Six-week-old F1 pups of both sexes were evaluated in the

rotarod test, a pain test, and a pole climbing (learning) test. At eight to nine weeks of age, pups of both sexes were evaluated for heart rate, respiration, and blood pressure. Postwean retained F1 pups were also evaluated for acquisition of testes descent (starting on pnd 21) and vaginal patency (starting on pnd 30).

One F1 male and one F1 female/litter were designated for breeding at 13 weeks of age, with 1:1 pairing within dose groups, avoiding sibling mating. Pairing was for a maximum of 14 days, with the date of vaginal sperm or a copulation plug designated gd 0. The pregnant F1 females were allowed to deliver their F2 litters. The F1 dams and their F2 pups were maintained until pnd 7, with pup counts, sexes, body weights, gross abnormalities, and mortalities recorded on pnd 1, 4, and 7. The F2 pups were terminated on pnd 7, and the F1 dams were terminated on pnd 7-10. F1 females which never mated and all F1 males were terminated after pregnancies were counted in each group.

3.2.1 Prenatal Developmental Toxicity Results

At the gd 20 necropsy of F0 dams and F1 fetuses, there were no effects of treatment or dose on maternal survival, pregnancy rate, or maternal body weights on gd 0 and daily on gd 7-20. Maternal body weight gain exhibited a rebound (significantly increased body weight gain) for gd 17-20 at all doses in the postdosing gestational period. Feed and water intakes (g or ml/rat) were also unaffected. At the gd 20 maternal necropsy, terminal body weights were equivalent, and there were no effects on absolute or relative weights of the maternal liver, kidneys, or spleen across all groups. The maternal adrenal gland absolute and relative weights were equivalent at 0 through 100 mg/kg/day and slightly (but statistically significantly) reduced at 1000 mg/kg/day (mean absolute weight 89.47% of control, mean relative weight 90.35% of control). The mean numbers of ovarian corpora lutea/dam were equivalent across all groups, as were the mean numbers of implantation sites/dam. There were no effects of treatment or dose on incidence of resorptions (or fetal deaths, with only one at 10 mg/kg/day). The percentage of live fetuses was significantly <u>increased</u> at 1000 mg/kg/day. The number of live fetuses/litter, sex ratio, mean fetal body weight, and placental weight/litter were all equivalent across all groups. There were no effects of treatment or dose on incidence of fetal malformation or variations, including no effects on ossification patterns, skeletal variants, or on the number of ossified sacral plus caudal (tail) bodies (vertebrae).

3.2.2 Postnatal Developmental Toxicity Results

All 12 F0 females in every group were pregnant, and 12 at 0, 100, and 1000 mg/kg/day (and 11 at 10 mg/kg/day) delivered live litters. Maternal body weights and weight gain during gestation and lactation were equivalent across all groups. Maternal feed and water consumption were also equivalent across all groups during gestation and lactation. There were no effects on duration of gestation, number of implantation sites, total or live F1 pups/litter, or sex ratio. All F0 dams exhibited appropriate nursing behavior (i.e., eating placentae, retrieving pups, nursing, lack of cannibalism). Survival ratios pre- and poststandardization (to eight pups, four/sex/litter on pnd 4) were also high and equivalent across groups. F1 pup male and female body weights/litter were also equivalent across all groups throughout lactation. There were no effects of dose or treatment on acquisition of lactational developmental landmarks or reflex behavior in F1 pups. For the F1 pups necropsied at weaning (pnd 21), there were no differences among groups for absolute or relative weights of the liver, kidney, spleen, brain, adrenal gland, testes for males, or ovaries for females. There were also no effects on histopathological findings of organs from weanlings of either sex across all groups. The skeletal evaluations of the weanlings were also equivalent across all groups. Age at acquisition of vaginal patency (F1 females) or testes descent (F1 males) was also equivalent across all groups. There were no effects of treatment or dose on any of the open field evaluations, reflex evaluations, time for tail flick, or for body weights for F1 males and females. Parameters for the rotarod test, pain test, and pole climbing test were also all equivalent for across all groups. Respiration, heart rate, and blood pressure were equivalent across all groups for F1 males. Respiration and blood pressure were equivalent across all groups for F1 females; heart rate was slightly (but significantly) increased at 1000 mg/kg/day ($p<0.05$), but the mean value for 10 mg/kg/day exceeded that at 1000 mg/kg/day. Body weights of the retained F1 animals (males and females), starting at weaning (three weeks of age) through week 10 were equivalent across all groups. Body weight gain for F1 males for weeks 3-5 and 5-7 were also equivalent across all groups. F1 male weight gain for weeks 7-10 was significantly lower at 1000 mg/kg/day ($p<0.05$) but similar to the value at 10 mg/kg/day. F1 female body weight gains were equivalent across all groups for all intervals examined.

3.2.3 Postnatal Reproductive Toxicology Results

When the 11-12 F1 males and females/group were paired at approximately 13 weeks after birth, there were no differences among groups for percentage of pairs copulating, precoital interval (days to copulation), percent pregnancies, deliveries, or nursing. There were no dead F1 dams. Body weights during gestation, gestational weight gain (gd 0-20), lactational body weights (pnd 1, 4, and 7), and lactational weight gain (gd 1-7) were also all equivalent across all groups. Duration of gestation, number of implantation sites/litter, and live F2 neonates/litter were all equivalent across groups. The percentage of dead F2 newborns was significantly reduced (and the live birth ratio therefore significantly increased) at 10, 100, and 1000 mg/kg/day. F2 sex ratio was equivalent across all groups. F1 maternal behavior was equivalent across all groups. F2 litter sizes on pnd 0, 1, 4, and 7 were equivalent across all groups. The percentage of dead F2 neonates was significantly reduced at 100 mg/kg/day. F2 pup body weights were equivalent across all groups for pnd 1, 4, and 7, except for significantly reduced F2 male and female body weights at 10 (but not 100 or 1000) mg/kg/day on pnd 4.

The authors (Goto et al., 1986) did not confirm the findings of Mikami et al. (1981) and concluded that the no-effect dosage of vitamin K_2 is 1000 mg/kg/day for adults, parents, and offspring.

3.3 Ohsumi et al. (1986)

3.3.1 Rabbit Developmental Toxicity Study

Ohsumi et al. (1986) administered menatetrenone (vitamin K_2) in gum arabic vehicle to timed-pregnant, six- to seven-month-old JW-N1BS female rabbits (from the Test Animal Center of Eisai Co., Ltd.), which had been bred in-house 1:1 with syngenic males. Copulation was confirmed by visual observation or by a sperm-positive vaginal smear. The does were dosed by oral gavage once daily in the morning on gd 6-18 (13 days), with the dosing volume 10 ml/kg, calculated on each dosing day. The doses were 0 (vehicle), 10, 100, and 1000 mg/kg/day. Clinical signs, body weights, and feed and water consumption were recorded frequently throughout gestation. Blood was collected from an ear vein (into heparin-containing tubes) from seven does/group on gd 19 (the day after the last dose), and hematology and clinical chemistry parameters were assessed. All does were terminated on gd 28. In seven does/group, maternal liver, kidney, spleen, heart, and adrenal gland were weighed, fixed, and evaluated histopathologically. Does exhibiting premature labor or spontaneous abortion were terminated,

and the organs plus "womb" (uterus) and ovary were removed and examined. At scheduled term necropsy, the ovarian corpora lutea were counted, the uterus removed, total implantations, resorptions, dead fetuses, and live fetuses counted, and placentas weighed. Live fetuses were weighed, crown-rump length and tail length measured, and the fetuses individually identified. The live fetuses were then incubated at 30-35°C and 70-80% humidity and observed for any clinical signs or mortality at 1, 3, 6, and 24 hours. For dead fetuses, the skeletal examination was done by the soft x-ray method and the carcass fixed in Bouin's fixative for internal examination. The live fetuses were euthanized and the abdominal wall incised to determine sex and status of abdominal organs. Two fetuses/doe were sacrificed under pentobarbital anesthesia, and the liver and kidney were removed, weighed, fixed, and examined histopathologically. Fifty percent of the live fetuses/litter were fixed in ethanol, double stained for cartilage and bone (alcian blue and alizarin red S, respectively), and examined for ossification patterns and skeletal variations and malformations. The other 50% of the live fetuses/litter were sacrificed by CO_2 asphyxiation, and the abdominal organs examined after opening the abdominal cavity. The diaphragms were then cut and the bodies fixed in Bouin's fixative and refixed in 80% alcohol. The internal morphology of the head and chest was then examined by Wilson's serial sectioning method.

3.3.2 Maternal Results

There were two does at 100 mg/kg/day and three does at 1000 mg/kg/day which aborted or suffered from premature labor on gd 25-28. These findings did not exhibit statistically significant differences in incidence across groups. Therefore, there were 14, 13, 9, and 9 does (and litters) at 0, 10, 100, and 1000 mg/kg/day, respectively. Maternal body weights on gd 0 and daily on gd 6-28 were equivalent across all groups, as was maternal body weight gain for gd 0-6, 6-18 (dosing period), and 18-28. Maternal feed consumption was equivalent at 0 through 100 mg/kg/day for all intervals and significantly reduced ($p<0.05$ for all) at 1000 mg/kg/day for days 7, 8, 9, 12, 14, 16 (during the dosing period), and 20 (in the postdosing period). Water consumption was significantly reduced only at 1000 mg/kg/day for days 7, 9, and 12 (during the dosing period). There were no differences in any hematologic parameters on gd 19 across groups for seven does/group. Biochemical parameters were similarly unaffected across all groups (on gd 19) except for significantly reduced globulin (in g/dl) at 1000 mg/kg/day ($p<0.05$). Maternal organ weights at necropsy were equivalent across all groups.

3.3.3 Embryofetal Results

The numbers of ovarian corpora lutea and uterine implantation sites were equivalent across all groups. The implant ratio (no. live/total implants) was significantly increased at 100 mg/kg/day. The number and percentage of resorptions, dead fetuses, live fetuses, live fetuses/litter, sex ratio, mean fetal body weight, mean placental weight, mean crown rump length or tail length, and fetal survivors after 24 hours of incubation were all equivalent across all groups. There were no fetal malformations in any fetuses in any group. For completeness, one fetus at 10 mg/kg/day exhibited a flexion contraction of the wrist joint. There were no differences in fetal ossification patterns in any group, including the number of sacral and caudal vertebrae, nonossified 5th sternebra (the last of the six to ossify), nonossified "oleclanon," or underdeveloped phalanges in the fore- or hindlimbs. There were also no differences among groups in the incidence of fetal variations, such as the 13th rib (rabbits usually have 12 pairs), eight lumbar vertebrae (rabbits usually have seven), asymmetrical sternebrae, etc. There were no differences among groups for the two fetuses examined/doe, totaling 27, 28, 18, and 18 fetuses at 0, 10, 100, and 1000 mg/kg/day, respectively, for body weight or absolute and relative liver or kidney weights.

Based on the above results, Ohsumi et al. concluded that the NOAEL of K_2 was 100 mg/kg/day for the does and 1000 mg/kg/day for embryofetal development in rabbits after maternal oral exposure during major organogenesis.

4.0 CONCLUSIONS

The oral LD_{50} for menatetrenone (vitamin K_2; MK-4) is 5000 mg/kg (Mikami et al., 1981), and most of the studies reviewed in this document resulted in no adult, parental, or developmental toxicity up through 1000 mg/kg, the highest oral dose tested ("ip" dosing also resulted in no toxicity up through 500 mg/kg, the highest dose tested). Acute, subchronic, and chronic studies in rodents have been negative. Rat, mouse, and rabbit Segment II (developmental toxicity) studies have also been uniformly negative for offspring, up to and including oral doses of 1000 mg/kg. Ohsumi et al. (1986) in their Segment II rabbit study indicated a maternal NOAEL of 100 mg/kg/day and an embryofetal NOAEL of 1000 mg/kg/day. Goto et al. (1986), with daily dosing during prebreed, mating, and early gestation (to gd 7), or

from late gestation to weaning (sd 17 to pnd 21) at the same doses, found no effects on dams, fetuses, or postnatal offspring rats, and determined the NOAEL for adult maternal, fetal, and offspring rats to be 1000 mg/kg. The only study with developmental toxicity (F1 and F2 neonatal deaths) is the Mikami et al. (1981) pre- and postnatal study at 1000 mg/kg/day, with reviewer concerns that the animal room was kept rather cool for rodent newborns and the lack of clear dose-response or generation-to-generation patterns.

In the absence of any effects on prebreed, early gestation (to gd 7), or late gestation (gd 17 to pnd 21) in the study by Goto et al. (1986), and in the absence of a dose-response pattern in the effects observed by Mikami et al. (1981), it is this reviewer's opinion that the **weight of evidence** of the human and animal studies indicates that menatetrenone is not likely to present any hazard to reproductive processes in humans at doses of up to 1 mg/kg . A minimum safety margin can be calculated of **100** (lowest animal developmental toxicity NOAEL of 100 mg/kg/day by Mikami et al. (1981) versus highest human dose of 1 mg/kg/day) to **1000** (based on the developmental toxicity NOAEL of 1000 mg/kg/day established by Goto et al., 1986, and Ohsumi et al., 1986, for developmental toxicity in rats and rabbits, respectively).

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September [illegible], 2008

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Food and Drug Administration
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College Park, MD 20740-3835

Reference: GRAS Notification [illegible] MK

Dear Sir or Madam:

Thank you for the opportunity to comment on Estee MK [illegible] as GRAS. Citing several well respected peer-reviewed references [illegible] vitamin K and vitamin K [illegible] has been [illegible] and [illegible] the [illegible] 2000 [illegible] NAS [illegible] administered [illegible] to have negligible toxicity [illegible]

There is little evidence [illegible] MK-7 [illegible] from MK4. Furthermore, note that the DRIs [illegible] tolerable intake level for vitamin K.

Information [illegible] MK-7 [illegible] GRAS [illegible]

1) Historical use of foods containing MK-7. Asian populations consume MK-7 as various fermented soybean products, [illegible] ingredient of [illegible]. In the Japanese market fermented soybean products [illegible] MK-7. In addition, other common foods, mainly cheeses, [illegible] amount of long chain MKs (50-70 mcg/100g) are foods for other populations.
2) The biopotencies of MK-7 is similar to phylloquinone for clotting activity. Based on biopotencies comparisons, MK-7 is ranked at 7[illegible] using the quick prothrombin/clotting time bioassay, when compared to MK-4 and MK-6 [illegible] ranked at 100%. The research has generally shown that MK homologs with [illegible] isoprenoid groups have greater activities than those with longer side chains.
3) There is evidence indicating that MK-7 [illegible] than other MKs. Absorption is passive for MK homologs and dependent on the lipid [illegible]. Data indicates that the absorption [illegible] MKs is substantially [illegible] than the

absorption of phylloquinones from green vegetables. There is no evidence of any adverse reaction attributed to an increased intake of MK-7.

4. Transport and metabolism of MK-7 is similar to other isoprenoids. Transport is via Chylomicrons and lipoproteins. Isoprenoid side chain conversion can occur and subsequent metabolites become part of the circulation. In rats, cattle and animals ingesting plant material, normally contain menaquinones with 6-, 7- or 10-prenyl units in their side chains.

The additional information provided by GRAS Associates LLC, and cited elements on the justification for MK-7 to be GRAS as GRAS.

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Respectively,

(b)(6)

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Reference: GRAS Association LLC [illegible] MK

Dear Sir or Madam,

Thank you for the opportunity to comment on the MK [illegible] GRAS. Our [illegible] with respected colleagues [illegible] Vitamin K has been [illegible] with [illegible] Dr. Card 2006, [illegible] NAS [illegible] administered [illegible] to have negligible toxicity [illegible] 2006.

There is little evidence [illegible] MK-7 [illegible] from MK-4. Furthermore, [illegible] the GRAS [illegible] levels to vitamin K.

Information [illegible] MK-7 [illegible] GRAS [illegible]

1) Historical use of foods containing MK-7: Asian populations consume MK-7 as various fermented soy bean products, [illegible] In the Japanese market fermented soy bean product [illegible] 100g of MK-7. In addition, other fermented foods, mainly cheeses, contain significant amount of long chain MKs (50-70 mcg/100g) are foods for other populations.
2) The bioprotencies of MK-7 is similar to phylloquinone for clotting activity. Based on biopotencies comparisons, MK-7 is ranked at 70% using the prothrombin clotting time bioassay, when compared to MK-4 and MK-6, which ranked at 100%. The research has generally shown that MK homologs with more isoprenoid groups have greater activities than those with longer side chains.
3) There is evidence indicating that MK-7 is absorbed more [illegible] than MK-4. Absorption is passive for MK homologs and independent of the lipid nature of the diet. Data indicates that the absorption of [illegible] MKs is [illegible] than the

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absorption of phylloquinone from green vegetables. There is no evidence of any adverse action attributed to ingestion of natural vitamin K1 or MK-7.

4. Transport and metabolism of MK-7 is similar to vitamin K1. Transport is via Chylomicrons and lipoproteins. Improved side chain conversion can occur and subsequent metabolites become part of the enterohepatic circulation. Issues of acute ingesting vitamin materials normally contain menaquinones with 6-9 isoprenoid units (short side chains).

The additional information provided by GRAS Associates, LLC, further elaborates on the justification for MK-7 to be considered GRAS.

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