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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of vitamin K_3 (menadione sodium bisulphite and menadione nicotinamide bisulphite) as a feed additive for all animal species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

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ABSTRACT

Vitamin K describes a group of lipophilic vitamins that exist naturally in two forms: vitamin K_1 (phylloquinone, found in green plants) and vitamin K_2 (a group of menaquinones synthesised by bacteria in the intestine). Vitamin K_3 (or menadione) is a synthetic form of vitamin K without a side chain. To become active, menadione needs to undergo prenylation. Vitamins K_1, K_2 and K_3 are metabolically activated in the liver to become co-factors in the activation of vitamin K-dependent proteins, which are important for normal blood coagulation, and normality of bones and arteries (Gla proteins). Acute toxicity of menadione or its derivatives is reached at levels exceeding the requirements by a factor of at least 1 000. Menadione sodium bisulphite (MSB) and menadione nicotinamide bisulphite (MNB) are safe for all animal species at practical use levels in feed. The use of MSB in water for drinking is likely to increase the exposure of target animals to chromium(VI). Therefore, the FEEDAP Panel has concerns about the safety of MSB when administered by this route. The use of MSB and MNB in animal nutrition does not give rise to safety concerns for consumers. MSB is an eye irritant; in the absence of adequate data, the additive should be considered as a skin sensitiser. In the absence of data, MNB should be considered as irritant to skin and eyes and as a skin sensitiser. Considering the high dusting potential of MSB and MNB, the absence of data on inhalation toxicity and the chromium(VI) content of dust, inhalation exposure resulting from handling of MSB and MNB could be hazardous. The use of MSB and MNB in animal nutrition does not pose a risk to the environment. MSB and MNB are regarded as effective sources of vitamin K in animal nutrition.

KEY WORDS

nutritional additive, vitamins and pro-vitamins, vitamin K_3, menadione sodium bisulphite, menadione nicotinamide bisulphite, safety

[1] On request from the European Commission, Question No EFSA-Q-2012-00111, adopted on 5 December 2013.

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SUMMARY

Following a request from European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of vitamin K_3 in the form of menadione sodium bisulphite and menadione nicotinamide bisulphite as a nutritional additive for all animal species. Menadione sodium bisulphite (MSB) and menadione nicotinamide bisulphite (MNB) are intended for addition to feed. MSB is also intended for use in water for drinking.

Vitamin K describes a group of lipophilic vitamins that exist naturally in two forms: vitamin K_1 (phylloquinone, found in green plants) and vitamin K_2 (a group of menaquinones synthesised by bacteria in the intestine). Vitamin K_3 (or menadione) is a synthetic form of vitamin K without a side chain. To become active, menadione needs to undergo prenylation. Vitamins K_1, K_2 and K_3 are metabolically activated in the liver to become co-factors in the activation of vitamin K-dependent proteins, which are important for normal blood coagulation, and normality of bones and arteries (Gla proteins).

Acute toxicity of menadione or its derivatives is reached at levels exceeding the requirements by a factor of at least 1 000. MSB and MNB are safe for all animal species at practical use levels in feed.

The use of MSB in water for drinking is likely to increase the exposure of target animals to chromium(VI). Therefore, the FEEDAP Panel has concerns about the safety of MSB when administered by this route.

The use of MSB and MNB in animal nutrition does not give rise to safety concerns for consumers.

MSB is an eye irritant; in the absence of adequate data, the additive should be considered as a skin sensitiser. In the absence of data, MNB should be considered as irritant to skin and eyes and as a skin sensitiser. Considering the high dusting potential of MSB and MNB, the absence of data on inhalation toxicity and the chromium(VI) content of dust, inhalation exposure from handling of MSB and MNB could be hazardous.

The use of MSB and MNB in animal nutrition does not pose a risk to the environment.

MSB and MNB are regarded as effective sources of vitamin K in animal nutrition.

The FEEDAP Panel made some recommendations on (i) the labelling of the additives, (ii) the reduction of the content of hexavalent chromium, (iii) the storage of vitamin mineral premixtures containing MSB, and (iv) the restricted distribution of the pure substances on the market.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7; in addition, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from VITAC EEIG Vitamins Authorisation Consortium[5] for authorisation of a new use (i.e. use in water for drinking) of vitamin K_3 in the form of menadione sodium bisulphite and re-evaluation of authorisation of vitamin K_3 in the form of menadione sodium bisulphite and menadione nicotinamide bisulphite when used as a feed additive for target species (nutritional additive; functional group: vitamins, pro-vitamins and chemically well-defined substances having similar effect) under the conditions mentioned in Tables 1 and 2.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[6] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 22 February 2012.[7]

Vitamin K has been authorised without time limit under Council Directive 70/524/EEC[8] for its use for all animal species as a nutritional additive.

The Scientific Committee on Food expressed an opinion on the tolerable upper intake level of vitamin K (EC, 2003). The Scientific Committee for Veterinary Medicinal Products expressed an opinion on phytomenadione (vitamin K_1) and menadione (vitamin K_3) (EMEA, 1998). The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) issued an opinion on the inability to assess the safety of vitamin K-enriched yeast for nutritional purposes as a source of vitamin K in food supplements and the bioavailability of vitamin K from this source, based on the supporting dossier (EFSA, 2009a). The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) expressed one opinion on the safety of vitamin K_2 (EFSA, 2008) and three opinions on the substantiation of several health claims pursuant to Article 13(1) of Regulation (EC) No 1924/2006 (EFSA NDA Panel, 2009, 2011, 2012).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the

[4] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[5] On 13/03/2013, EFSA was informed by the applicant that VITAC EEIG was liquidated on 19/12/2012 and their rights as applicant were transferred to FEFANA asbl (EU Association of Specialty Feed Ingredients and their Mixtures, representing notably the following companies: Lohmann Animal Health GmbH & Co KG, Cuxhaven, Germany; Trouw Nutrition International BV, Putten, the Netherlands). Avenue Louise, 130A, Box 1, 1050 Brussels, Belgium.

[6] EFSA Dossier reference: FAD-2010-0099.

[7] A new mandate was received in EFSA in January 2012.

[8] Commission List of the authorised additives in feedingstuffs published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs (2004/C 50/01). OJ C 50, 25.2.2004, p. 1.

safety for the target animals, consumer, user and the environment and the efficacy of vitamin K3 in the form of menadione sodium bisulphite and menadione nicotinamide bisulphite, when used under the conditions described in Tables 1 and 2.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Vitamin K_3 – Menadione Sodium Bisulphite (MSB)
Registration number/EC No/No	Not appropriate
Category of additive	3. Nutritional Additives
Functional group(s) of additive	(a) Vitamins, pro-vitamins and chemically well-defined substances having similar effect

Description			
Composition, description	Chemical formula	Purity criteria	Method of analysis
Menadione Sodium Bisulphite	1,2,3,4-Tetrahydro-2-methyl-1,4-dioxo-2-naphthalenesulfonic acid, sodium salt, trihydrate CAS No.: 6147-37-1	Minimum 50 % Menadione, which represents at least 96% Menadione sodium bisulphite trihydrate complex	Photometric method (VDLUFA Bd. III, 13.7.1)

Trade name	Not appropriate
Name of the holder of authorisation	Not appropriate

Conditions of use				
Species or category of animal	Maximum age	Minimum content	Maximum content	Withdrawal period
		mg/kg of complete feedingstuffs, supplementary feed (based on end feed) and in water*		
All animal species and categories	No restrictions - during all life cycle	-	-	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use	Additive has to be incorporated in premixture. Directly dosage to mixed feed is not recommended.
Specific conditions or restrictions for handling	Handle in accordance with good industrial hygiene and safety practice.
Post market monitoring	Not appropriate
Specific conditions for use in complementary feedingstuffs or water	Not appropriate

Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Marker residue	Species or category of animal
Not appropriate	Not appropriate	Not appropriate	Not appropriate

Table 2: Description and conditions of use of the additive as proposed by the applicant

Additive	Vitamin K_3 – Menadione Nicotinamide Bisulphite (MNB)
Registration number/EC No/No	Not appropriate
Category of additive	3. Nutritional Additives
Functional group(s) of additive	(a) Vitamins, pro-vitamins and chemically well-defined substances having similar effect

Description			
Composition, description	Chemical formula	Purity criteria	Method of analysis
Menadione Nicotinamide Bisulphite	1,2,3,4-Tetrahydro-2-methyl-1,4-dioxonapthalen-2-naphthalenesulfonic acid compound with 3-Pyridinecarboxamide CAS No.: 73581-79-0	Minimum 44% Menadione, which represents at least 96% Menadione nicotinamide bisulphite complex	Photometric method (VDLUFA Bd. III, 13.7.1)

Trade name	Not appropriate
Name of the holder of authorisation	Not appropriate

Conditions of use				
Species or category of animal	Maximum age	Minimum content	Maximum content	Withdrawal period
		mg/kg complete feedingstuffs, supplementary feed (based on end feed) and in water*		
All animal species and categories	No restrictions - during all life cycle	-	-	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use	Additive has to be incorporated in premixture. Directly dosage to mixed feed is not recommended.
Specific conditions or restrictions for handling	Handle in accordance with good industrial hygiene and safety practice.
Post market monitoring	Not appropriate
Specific conditions for use in complementary feedingstuffs or water	Not appropriate

Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Marker residue	Species or category of animal
Not appropriate	Not appropriate	Not appropriate	Not appropriate

ASSESSMENT

This opinion is based in part on data provided by a consortium of companies involved in the production/distribution of vitamin K_3 in the form of menadione sodium bisulphite (MSB) and menadione nicotinamide bisulphite (MNB). It should be recognised that these data cover only a fraction of existing additives containing vitamin K_3 in the form of MSB and MNB. The application is for the active substance and the composition of the additive formulations is not the subject of the application. The Panel has sought to use the data provided together with data from other sources to deliver an opinion.

The application contains data from two sources each of MSB and MNB, both obtained by chemical synthesis.

1. Introduction

Vitamin K describes a group of lipophilic vitamins that exist naturally in two forms: vitamin K_1 is phylloquinone (also called phytomenadione or phytonadione), which is found in green plants, and vitamin K_2 is a group of menaquinones which are synthesised by bacteria in the intestine. These compounds contain a common 2-methyl-1,4-naphthoquinone ring structure but differ from each other in the length and saturation degree of the polyisopropenoid side chain attached at position 3. Phylloquinone has a phytyl substituent (four isoprenic units, one of which is unsaturated). Menaquinones (MK) have side chains composed of a variable number (*n*) of prenyl units, designated as MK-*n*. Vitamin K_3 (or menadione) is a synthetic form without side chain. To become active, menadione must undergo prenylation. Menadione release occurs in the intestine, its prenylation into MK-4 occurring in the intestine and tissues. Lipophilicity varies according to the number of isoprenic units, impacting on the different functions of intestinal absorption, transport, bioavailability and tissue distribution.

Vitamins K_1, K_2 and K_3 are metabolically activated in the liver to become co-factors in the activation of vitamin K-dependent proteins. In the first step, they are reduced to their semiquinone form by a quinone reductase (NADH dehydrogenase). This reduced form acts as a co-factor to the gamma-carboxylase which carboxylates glutamyl residues to gamma-carboxy-glutamic acid residues on certain proteins (Gla proteins). Gla proteins are important for normal blood coagulation, known for more than 60 years, and normality of bones and arteries (for details see Vermeer and Schurgers, 2000). Each converted glutamyl residue produces a molecule of vitamin K epoxide which is returned to vitamin K via the vitamin K epoxide reductase.

Vitamin K is included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 and foreseen for re-evaluation. It is authorised as a nutritional additive for use in all animal species without time limit and maximum content.

The applicant asks for the re-evaluation of the use of vitamin K_3 (nutritional additive, functional group vitamins, pro-vitamins and chemically well-defined substances having similar effects) in the form of menadione sodium bisulphite (MSB) and menadione nicotinamide bisulphite (MNB) for use in feed for all animal species and categories and for a new use of vitamin K_3 in the form of MSB in water for drinking.

Menadione is described in the European Pharmacopeia (PhEur, 2010) as Monograph (MG) 0507.

Menadione (and phytomenadione) is listed as a pharmacologically active substance in veterinary medicinal products[9] and not subject to maximum residue levels when used in food-producing animals.

[9] Commission Regulation (EU) 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1.

Menadione (and phytomenadione) is listed as an ingredient in cosmetic products as a masking agent and skin conditioning agent.[10]

Vitamin K in the form of phylloquinone (phytomenadione) and menaquinone is authorised for use in food[11] and food supplements,[12] and for addition to foods for particular nutritional uses.[13] Vitamin K in the form of phylloquinone (phytomenadione) is authorised for addition to processed cereal-based foods and baby foods for infants and young children[14] and to infant formulae and follow-on formulae when reconstituted as instructed by the manufacturer.[15]

2. Characterisation

2.1. Menadione sodium bisulphite

2.1.1. Characterisation

Menadione sodium bisulphite (IUPAC name: 1,2,3,4-tetrahydro-2-methyl-1,4-dioxo-2-naphthalenesulfonic acid, sodium salt trihydrate; synonyms: vitamin K_3, MSB) is identified by Chemical Abstracts Service (CAS) number 6147-37-1 and the European Inventory of Existing Chemical Substances (EINECS) number 204-987-0 (anhydrous). The structural formula of MSB trihydrate is shown in Figure 1.

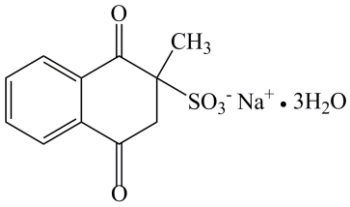

Figure 1: Structural formula of menadione sodium bisulphite trihydrate

The molecular formula of MSB is $C_{11}H_9NaO_5S \cdot 3H_2O$ and its molecular weight is 330.29. It has a melting point of 121–124 °C, a bulk density of approximately 700–800 kg/m^3 and is freely soluble in water (0.5 g/mL), slightly soluble in alcohol and almost insoluble in benzene and diethyl ether.

MSB is a free-flowing white to yellowish crystalline, practically odourless, powder. The additive contains by specification ≥ 50 % menadione, ≤ 13 % water (as hydrate), ≤ 5 % free sodium bisulphite and ≤ 100 mg Cr/kg. The minimum content of 50 % menadione would correspond to 96 % MSB trihydrate complex. Analysis of five batches (from two different producers) showed an average content of 51.0 % menadione (range: 50.3–51.5 %).[16] Free sodium bisulphite was between 3.1 and 4.4 % and water between 10.8 and 12.0 %.

[10] Commission Decision 2006/257/EC of 9 February 2009 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97 5.04.2006, p. 1.
[11] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404 30.12.2006, p. 26. Last amended by Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. OJ L 314 1.12.2009, p. 36.
[12] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183 12.7.2002, p. 51.
[13] Commission Regulation (EC) 953/2009 of 13 October 2009 on substances that may added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269, 14.10.2009, p. 9.
[14] Commission Directive 2006/125 EC of 5 December 2006 on processed cereal-based foods and baby-foods for infants and young children. OJ L 339 6.12.2006, p. 16.
[15] Commission Directive 2006/141 EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ L 401 30.12.2006, p. 1.
[16] Technical dossier/Section II.

Chromium was in the range 30–45 mg/kg and heavy metals (expressed as lead) were below 20 mg/kg.[16] The concentrations of cadmium, mercury, lead and arsenic determined in three additional batches were ≤ 0.3 mg/kg, < 0.01 mg/kg, ≤ 0.2 mg/kg and ≤ 0.1 mg/kg, respectively.[17] The content of residual solvents (ethanol determined in two batches 176–208 mg/kg or *n*-butanol determined in three batches 17.5–37.4 mg/kg) was below the thresholds proposed by International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) (EMA, 2010).

Two batches of MSB (one from each producer) were analysed for particle size distribution by laser diffraction. The particle fraction below 50 µm was 14.3 % and 19.6 % (v/v).[18] Another two batches of the substance showed a dusting potential of 15.4 g/m^3 (average of three repeated measurements)[19] and 3.1 g/m^3 (average of five repeated measurements).[20] The content of chromium in the dust determined in the latter batch was 182 mg/kg.[21] The respirable fraction (< 10 µm) of the dust amounted to 54 %.[22]

Menadione is obtained by oxidation of 2-methylnaphthalene using sodium dichromate ($Na_2Cr_2O_7$) as catalyst. MSB is then produced by reaction of menadione with sodium bisulphite in ethanol or *n*-butanol. After decolorisation on active carbon or diatomaceous earth, the crude product is crystallised, centrifuged, washed with ethanol or *n*-butanol, and dried. The applicant provided a flow chart of the synthetic process.

2.1.2. Stability and homogeneity

MSB is stable when exposed to air but is sensitive to light and heat. In alkaline media (pH > 11), MSB is converted to menadione (Demirkaya-Miloglu et al., 2013).

MSB (three batches, stored in sealed plastic bags) was demonstrated to have a shelf life of 18 months, when stored at 25 ± 5 °C. No data from tests under accelerated conditions were submitted, but at higher temperature the risk of discoloration, indicating loss of activity, increased after 12 months.[23]

The stability of MSB was tested when incorporated in three different premixtures—(i) without trace elements (at 2 000 mg MSB/kg), (ii) with trace elements (at 400 mg MSB/kg) and (iii) with trace elements and choline (at 400 mg MSB/kg)—and stored in sealed plastic bags at 30 °C. Recovery of MSB was 84.2 %, 82.3 % and 72.7 %, respectively, after one month, and 71.1 %, 67.2 % and 49.6 % after three months.[24] The data indicate that stability of MSB will be strongly affected by choline and to a lesser extent by trace elements. This is in accordance with the literature. Stability data published by BASF (1994, cited by Whitehead, 2002) showed a recovery of MSB in premixtures containing choline chloride and trace minerals of 64 % after one month and of 0 % after six months' storage.

A new stability study to demonstrate stability for six months was performed upon request. MSB was incorporated at the level of about 30 mg/kg in a mineral/amino acid premixture without trace elements and choline. Owing to inconsistent results (decline of MSB until three months and a subsequent increase after six months' storage) the data were not considered further.[24]

The stability of MSB (one batch) was tested during processing of maize–soya bean meal-based feed (pelleting at 90 °C) and storage (30 °C, 70–80 % humidity, sealed plastic bags). Pelleting reduced MSB to about 53 % of the initial content (6 mg MSB/kg feed). Storage of the pelleted samples for two months (30 ± 5 °C) reduced the MSB content further, to about one-third of the initial level.[24]

[17] Technical dossier/Supplementary Information January 2013.
[18] Technical dossier/Section II/Annex II.02 and Annex II.03.
[19] Technical dossier/Supplementary Information October 2012.
[20] Technical dossier/Supplementary Information June 2013/Annex C.
[21] Technical dossier/Supplementary Information June 2013/Annex E.
[22] Technical dossier/Supplementary Information June 2013/Annex D.
[23] Technical dossier/Section II/Annex II.16.
[24] Technical dossier/Supplementary Information October 2012/Annex Qi.

Data published by BASF (1994, cited by Whitehead, 2002) suggest an inverse correlation between MSB stability in compound feed and the temperature used during expansion. At 93 °C for 30 seconds (or 110 °C for 5 seconds) recovery was 80 %, and at 149 °C for 30 seconds (or 165 °C for 5 seconds) recovery was 30 %.

In commercial fish feed (Marchetti et al., 1999) supplemented with 60 mg MSB/kg, the initial concentration was reduced by 50 % after pelleting and by 66 % after extrusion. Storage of pelleted and extruded feed, for three months resulted in a further loss of 31 % and 38 %, respectively.

The stability of MSB (one batch) in water for drinking at a concentration of 2.5 g/L was shown when stored for up to 24 hours at 20 °C.[24] In a second submission, a solution containing 1.1 g MSB/L water was shown to be stable for up to 48 hours, at both 15 °C and 25 °C.[25] Although the data apparently demonstrate stability, it is questionable if they can be extrapolated to practical concentrations of MSB in water for drinking, which are about 100 to 1 000 times lower.

To assess the capacity of the additive to homogeneously distribute in feed, the applicant performed a statistical calculation (Jansen, 1992). The coefficient of variation of the MSB concentration in poultry feed was calculated to be approximately 8 %. However, this method has been developed to test the working accuracy of mixing equipment and is not accepted by the FEEDAP Panel as a valid method for assessing the capacity of the additive to distribute homogeneously in feedingstuffs.

As MSB is highly soluble in water, homogeneity in water for drinking does not need to be demonstrated.

2.2. Menadione nicotinamide bisulphite

2.2.1. Characterisation

Menadione nicotinamide bisulphite (IUPAC name: 1,2,3,4-tetrahydro-2-methyl-1,4-dioxo-2-naphthalenesulfonic acid compound with 3-pyridinecarboxamide; synonyms: 2-methoxysulfonyltetralin-1,4-dione with pyridine 3-carboxamide, MNB, vitamin K_3) is identified by the CAS number 73581-79-0 and the EINECS number 277-543-7. The structural formula of MNB is shown in Figure 2.

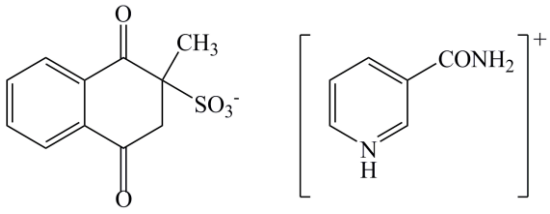

Figure 2: Structural formula of menadione nicotinamide bisulphite

The molecular formula of MNB is $C_{11}H_9O_5S{\cdot}C_6H_7N_2O$ and its molecular weight is 376.42. It has a boiling point of 193–198 °C, shows a bulk density of 700–800 kg/m^3 and is sparingly soluble in water (19 g/L at 20°C).

MNB is a free-flowing yellowish odourless powder. The additive contains by specification ≥ 44 % menadione, ≥ 31 % nicotinamide, ≤ 1 % water and ≤ 150 mg Cr/kg. The minimum content of 44 % menadione would correspond to 96 % MNB complex. Analysis of five batches (from two different producers) showed an average content of 44.1 % menadione (range 43.9–44.2 %).[26] Nicotinamide was between 31.2 and 32.4 % and water between 0.2 and 0.5 %.

[25] Technical dossier/Supplementary information October 2012/Annex Qii.
[26] Technical dossier/Section II.

Chromium was in the range 77–142 mg/kg and heavy metals (expressed as lead) were below 20 mg/kg.[26] The concentrations of cadmium, mercury, lead and arsenic determined in three additional batches were < 0.01 mg/kg, < 0.01 mg/kg, ≤ 0.2 mg/kg and < 0.1 mg/kg respectively. The concentration of chromium in the same batches ranged from 90 to 117 mg/kg.[27] The content of residual solvents (ethanol determined in three batches 4.1–13.3 mg/kg or *n*-butanol determined in two batches 19.5–44.7 mg/kg) was below the thresholds proposed by VICH (EMA, 2010).

Two batches of MNB (one from each producer) were analysed for particle size distribution by laser diffraction. The particle fraction below 50 µm was 7.9 % and 1.6 %.[28] Another two batches of the additive showed a dusting potential of 0.39 g/m^3 (average of three repeated measurements)[29] and 0.46 g/m^3 (average of four repeated measurements).[30] The content of chromium in the dust determined in the latter batch was 71 mg/kg.[31] The respirable fraction (below 10 µm) in the dust was 34 %.[32]

Menadione is obtained by oxidation of 2-methylnaphthalene using sodium dichromate ($Na_2Cr_2O_7$) as catalyst. MNB is prepared by reaction of menadione with nicotinamide in aqueous sulphuric acid. The crude product is then centrifuged, washed with ethanol or *n*-butanol, and dried. The applicant provided a flow chart of the synthetic process.

2.2.2. Stability and homogeneity

MNB is stable when exposed to air, but is sensitive to light and heat.

MNB (three batches, stored in sealed plastic bags) showed a shelf life of 30 months when stored at 25 ± 5 °C. No data from tests under accelerated conditions were submitted, but at higher temperature the risk of discoloration, indicating loss of activity, increases after 24 months.[33]

The stability of MNB was tested when incorporated in three different premixtures—(i) without trace elements (at 2 273 mg MNB/kg), (ii) with trace elements (at 455 mg MNB/kg) and (iii) with trace elements and choline (at 455 mg MNB/kg)—and stored in sealed plastic bags at 30 °C. Recovery of MNB was 93.1 %, 91.3 % and 87.2 %, respectively, after one month and 82.3 %, 80.9 % and 73.5 % after three months.[34] The data indicate that stability of MNB will be affected by choline.

A new stability study to demonstrate stability for six months was performed upon request. MNB was incorporated at the level of about 20 mg/kg in a mineral/amino acid premixture without trace elements and choline. Owing to inconsistent results (e.g. increase of MNB after nine weeks by 73 %) the data were not considered further.[34]

The stability of MNB (two batches) was tested during pig feed processing (pelleting at 70 °C) and storage (30 ± 5 °C). Pelleting reduced MNB by about 52 % of the initial content (4.5 mg MNB/kg feed). Storage of pelleted feed for three months did not further reduce MNB content in one batch, whereas in another batch MNB content was reduced to about one-third of the initial level.[34]

However, Graff et al. (2010) added increasing amounts of MNB to salmon diets (10, 100 and 1 000 mg menadione/kg feed), which was produced according to commercial manufacturing procedures, and found recovery of only 3–5 % two months after production.

To assess the capacity of the additive to homogeneously distribute in feed, the applicant performed a statistical calculation (Jansen, 1992). The coefficient of variation of the MSB concentration in poultry

[27] Technical dossier/Supplementary Information January 2013.
[28] Technical dossier/Section II/Annex II.04 and Annex II.05.
[29] Technical dossier/Supplementary Information October 2012.
[30] Technical dossier/Supplementary Information June 2013/Annex C.
[31] Technical dossier/Supplementary Information June 2013/Annex E.
[32] Technical dossier/Supplementary Information June 2013/Annex D.
[33] Technical dossier/Section II.
[34] Technical dossier/Supplementary Information October 2012/Annex Qi.

feed was calculated to be approximately 11 %. However, this method has been developed to test the working accuracy of mixing equipment and is not accepted by the FEEDAP Panel as a valid method for assessing the capacity of the additive to distribute homogeneously in feedingstuffs.

2.3. Physico-chemical incompatibilities in feed

No physico-chemical incompatibilities or interactions have been reported between MSB or MNB and feed materials, carriers or feed additives (except choline chloride) when the additive was added to premixtures and feed. No such incompatibilities or interactions are expected.

2.4. Conditions of use

Vitamin K_3 in the form of MSB and MNB is intended for use in all animal species and categories without maximum limit. MSB and MNB are intended for use in feed (premixtures, complete or complementary feed) and it is recommended that they be incorporated in compound feed only via premixtures. MSB is intended also for use in water for drinking.

2.5. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of vitamin K_3 (menadione) in animal feed. The Executive Summary of the EURL report can be found in the Appendix.

3. Safety

According to Regulation (EC) No 429/2008, tolerance, metabolism and residue, and toxicological (concerning consumer safety) studies are not required for vitamins, pro-vitamins and chemically defined substances having similar effects which are already authorised as feed additives under Directive 70/524/EEC and which do not have the potential to accumulate, which the FEEDAP Panel considers is the case for vitamin K_3 in the form of MSB and MNB.

The FEEDAP Panel notes that MNB is also a source of nicotinamide. The safety of nicotinamide as feed additive for all animal species has been recently assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2012).

3.1. Absorption, distribution, metabolism and excretion

A detailed description of the absorption and metabolism of vitamin K can be found in the IARC monograph (2000) and Scientific Committee on Food (SCF) assessment (EC, 2003).

Phylloquinone administered orally to mammals and birds is absorbed and partly converted into MK-4 via integral side-chain removal and replacement, which implies that menadione is an intermediary metabolite (Martius, 1967; Hirota et al., 2013; Dialameh et al., 1970). Menadione release occurs in the intestine, its prenylation into MK-4 occurring in the intestine and tissues (Okano et al., 2008). Caco-2 cells exposed to menadione produced MK-4 (Al Rajabi et al., 2012). Part of the MK-4 in tissues results from uptake and alkylation (prenylation) of circulating menadione (Thijssen et al., 1996). Menadione urinary excretion in humans increases greatly after oral intake of phylloquinone (amounting to 5 to 25 % of the administered dose) but also after intake of MK-4 or MK-7 (Thijssen et al., 2006). In isolated rat hepatocytes, cellular glutathione (GSH) reacted directly with menadione to produce a GSH–menadione conjugate (Di Monte et al., 1984). The same conjugate was identified in the Hep G2 cell line (Mouzaroll et al., 2004). The side chains of phylloquinone and MK-4 are shortened by the rat to seven carbon atoms (ω-oxidation followed by β-oxidation), yielding a carboxylic acid group at the end that cyclises to form a γ-lactone which is excreted in the urine, presumably as a glucuronic acid conjugate. Recently, glutathione– and N-acetylcysteine–menadione conjugates were identified in the rat plasma after intraperitoneal administration of menadione (Elgawish et al., 2013).

Vitamins K_1, K_2 and K_3 are metabolically activated in the liver to become co-factors in the activation of vitamin K-dependent proteins. In the first step, they are reduced to their semiquinone form by a quinone reductase (NADH dehydrogenase). This reduced form acts as a co-factor to the membrane enzyme vitamin K-dependent gamma-carboxylase, which carboxylates glutamyl residues to gamma-carboxy-glutamic acid residues on certain proteins, activating them. Each converted glutamyl residue produces a molecule of vitamin K epoxide, which is returned to vitamin K via the vitamin K epoxide reductase enzyme, also an integral membrane protein.

Phylloquinone is the major type (> 90 %) of dietary vitamin K. Its concentration in animal tissues is remarkably low compared with menaquinones, the major forms (> 90 %) of vitamin K in tissues. MK-7 to MK-10 have been found as the major compounds in pig liver, and MK-10 to MK-12 have been found in beef. MK-4 and saturated MK-4 (H_4) were found also in the liver of these species and rabbit and chicken (review from Duello and Matschiner, 1972). Generally, as shown in the rat, MK-4 concentrations are low in liver and plasma, the greatest accumulations being observed in non-hepatic organs (pancreas, salivary gland and brain) (Thijssen et al., 1996). The conversion of dietary menadione into MK-4 has been shown to occur in the liver of fish (Grahl-Madsen and Lie, 1997; Ostermeyer and Schmidt, 2001; Graff et al., 2002) and chicks (Will et al., 1992).

No direct evidence of release of menadione from its complexes sodium bisulphate and nicotinamide is available. The bioavailability of menadione and nicotinamide, measured as plasma concentration of both vitamins, was shown to be similar in pigs administered either MNB or an equivalent quantity of menadione and nicotinamide given separately (Marchetti et al., 2000). In pre-ruminant calves, MSB was converted to MK-4, which was found at high concentration in the faeces, implying the release of menadione and isoprenylation by the intestinal flora; only MK-4 was found as a metabolite in intestine tissue, liver and spleen (Nestor and Conrad, 1990).

Thus, it is expected that residues of vitamin K_3 in animal products, if any, will be low and common to metabolites of phylloquinone, the predominant vitamin K in feedingstuffs.

3.2. Toxicological profile

Toxic effects of the vitamin K family are manifested mainly as haematological disorders. However, marked differences are observed among the toxicity potentials of the various vitamin K compounds.

3.2.1. Vitamin K_3

The applicant, citing studies but without providing the full report, provided data on acute oral toxicity in rats in a limit test on MSB and MNB which showed no mortality at doses of 2 000 and 5 000 mg/kg body weight (bw), respectively.[35]

In a single-dose oral toxicity study in chicks (eight days of age), 3 630 mg MNB/kg bw caused 25 % mortality and growth depression in a 14-day post-treatment observation period. In a 14-day feeding study in chicks (days 8 to 22), 681 mg MNB/kg diet did not affect growth rate, feed intake, feed to gain ratio or blood haemoglobin when compared with an unsupplemented control diet (Oduho et al., 1993).

Acute oral doses of menadione were slightly toxic in chicks and mice, with a median lethal dose (LD_{50}) of about 800 and 600 mg/kg bw, respectively (Molitor and Robinson, 1940; Ansbacher et al., 1942). The main toxicity symptoms were haemolysis and anaemia associated with Heinz bodies in the erythrocytes and spleen enlargement (Munday et al., 1991). In rats given oral gavage doses of menadione for 30 days, 500 mg/kg bw per day was lethal and 350 mg/kg bw per day caused marked reductions in erythrocyte count and haemoglobin (Molitor and Robinson, 1940).

[35] Technical dossier/Section III/Ref. 3.3.02 and Ref. 3.3.03.

3.2.2. Vitamin K_1 and K_2

The natural forms of vitamin K, phylloquinone and menaquinone, are non-toxic at very high dosage levels. Acute oral doses of phylloquinone up to 25 000 mg/kg bw caused no fatalities in mice (Molitor and Robinson, 1940).

Vitamin K_2, MK-7, was tested for acute oral toxicity in mice using a limit test in accordance with OECD Guideline 425. Five female mice (Bontac: NMRI strain) were given a single oral dose of 2 000 mg MK-7/kg bw in corn oil and observed for 14 days. Two untreated mice were kept as controls. Mortality and body weight gain were unaffected by the treatment (Pucaj et al., 2011).

The repeated-dose oral toxicity of MK-7 was tested in a 90-day study in groups of 15 Sprague–Dawley rats of each sex given daily gavage doses of 0, 2.5, 5.0 or 10.0 mg MK-7/kg bw in corn oil. Ten rats of each sex were killed on day 90 and the remainder were retained for an untreated recovery period of 30 days. Blood samples were collected on day 44 from five males and five females from each group, and a limited range of haematological and clinical parameters were examined. Urine was collected for analysis during the final week of treatment and the final week of recovery. No adverse effects were seen on mortality, body weight gain, clinical appearance, ophthalmology or in a battery of functional observation tests. The only statistically significant haematological effect was an increase in activated partial thromboplastin time (without affecting prothrombin time) in the mid- and top-dose females. The effect was due to unusually high partial thromboplastin time in three of the rats and was probably not treatment related. There were no effects on clinical biochemistry or urinalysis parameters. There were also no treatment-related effects on organ weight, gross pathology or histopathology. In summary, no treatment-related effects of MK-7 were seen in this study (Pucaj et al., 2011).

Repeat-dose oral toxicity studies were conducted with rats and dogs receiving MK-4 doses for a period of one year (Hosokawa et al., 1995; Vanatta et al., 1995).

Hosokawa et al. (1995) administered menatetrenone (synthetic MK-4) to Fisher 344 rats (20/sex/group) at dietary concentrations of 0.04, 0.2 or 1.0 % (approximately 20, 100 and 500 mg/kg bw per day, respectively). Two control groups were used: one was called ‘control’ and the other ‘naïve’. There were no treatment-related effects on food intake, body weight, mortality, clinical signs of toxicity. Haematology of females showed dose-related and significant (*t*-test; $P < 0.05$) reductions, compared with ‘controls’, in erythrocyte counts in all treated groups, and in packed cell volume and haemoglobin in the mid- and high-dose groups, while significant elevations were seen in mean corpuscular volume and platelet count in all treated groups and in the lymphocyte to neutrophil ratio in the high-dose group of females. In males, there were significant decreases in haemoglobin and prothrombin time in all dose groups, although not dose-related, in activated partial thrombin time in the mid- and high-dose groups and in erythrocyte count and mean corpuscular volume only in the high-dose group, along with an elevated platelet count in the high-dose group. The changes in haematological parameters were either less marked or did not occur when the results for treated groups were compared with the ‘naïve’ rather than the ‘control’ group.

Whilst there were some statistically significant changes in some blood biochemistry parameters, none was considered to be treatment related as there was inconsistency between sexes, a lack of dose–response relationships and/or an absence of associated lesions seen on gross pathology and histopathology. Urine analyses were unremarkable. Dose-dependent increases in absolute and relative spleen weights in the mid- and high-dose groups of both sexes were accompanied by histopathological changes that indicated increased extramedullary haematopoiesis and increased haemosiderin deposition. Other significant organ weight differences, which were not associated with any histopathology, included a small but dose-dependent increase in relative kidney weights in both sexes (statistically significant in the mid- and high-dose groups). Increased relative liver and adrenal and sub-maxillary gland weights were noted in all female dose groups, and elevated absolute liver weight, and relative brain and thyroid gland weights in the mid- and high-dose female groups. The FEEDAP

Panel regarded the no observed adverse effect level (NOAEL) for this study to be 0.04 % (equivalent to 20 mg/kg bw per day) based on haematological changes and changes in the spleen, which were seen in both sexes at 0.2 % or greater.

Menatetrenone (synthetic MK-4) was provided orally in capsules to groups of six male and six female dogs at doses of 0, 20, 200 or 2 000 mg/kg bw per day for a period of one year followed by a three-month observation period (Vanatta et al., 1995). Blood was collected after 3, 6 and 12 months of treatment and at the end of the recovery period. Some of the dogs (unspecified number) were killed for autopsy at the end of the 12-month treatment period and the remainder were killed at the end of the recovery period. There were no treatment-related effects on mortality, body weight gain or food and water consumption, and no ophthalmoscopic changes. Yellow faeces and yellow coat stains were observed in animals in the high-dose (2 000 mg/kg bw per day) group, but no other clinical signs were reported. In high-dose female dogs, significant increases ($P < 0.05$) in reticulocyte count were seen at 6 and 12 months, whereas in males the platelet count was elevated in the high-dose group at 12 months. In females there was increased serum alkaline phosphatase activity in the high-dose group that was significant at 6 and 12 months. Significantly reduced serum sodium levels were noted in all treated males at 12 months, while decreases in serum triglycerides were seen in high-dose males at 6 and 12 months and serum alanine aminotransferase was elevated in males in the high-dose group at 6 months. The haematological and clinical chemistry changes observed appeared to be reversible once MK-4 treatment was ceased. No effects on absolute organ weight were noted in either sex, but a dose-dependent increase in relative liver weights was observed in high-dose males that were killed at the end of the treatment period. At necropsy conducted immediately following the treatment period, gross examination revealed yellow body fat in two high-dose males. A NOAEL of 200 mg/kg bw per day was established for MK-4 administered orally to dogs, based on yellow coloration of faeces and various changes in blood parameters which were seen at 2 000 mg/kg bw per day.

3.3. Safety for the target species

The vitamin K requirement of target animals is met by a combination of dietary intake and microbial biosynthesis in the gut (which could become orally available by coprophagia), which involves intestinal microorganisms (such as *Escherichia coli*) as well as ruminal microbes. Ruminal microorganisms in particular synthesise large amounts of vitamin K, explaining why ruminants do not appear to need a dietary source of the vitamin. Because of microbial synthesis and high content of forages, a precise expression of vitamin K requirements for ruminants, horses and lagomorphs is not feasible (NRC, 1996, 2001, 2007a, b; GfE, 1995, 2001, 2003, 2014 in press).

Requirements for menadione are in the range 0.5–1.8 mg/kg feed for poultry (NRC, 1994), 0.5 mg/kg for pigs (NRC, 2012), 0.2–10 mg/kg for fish (NRC, 2011) and 1.0–1.3 mg/kg for pets (NRC, 2006). The German Society of Nutrition Physiology (Gesellschaft für Ernährungsphysiologie, GfE) proposed the following ranges for vitamin K requirements (as vitamin K_3): 0.6–1.5 mg/kg feed for poultry (GfE, 1999, 2004) and 0.1–0.15 mg/kg for pigs (GfE, 2008).

It should be noted that the requirement data may have a high degree of uncertainty since they refer to intended supplementation values. Modern feed-producing techniques (e.g. extrusion) may result in considerable losses. The real requirements may therefore be considerably lower. They require at least a new assessment (Graff et al., 2010).

Vitamin K_3 supplementation of commercial compound feed is mainly oriented towards meeting recommended allowances (probably also considering the reduced stability), which are in the range of 2–4 mg menadione/kg feed for pigs, 1–4 mg/kg for poultry, 4–8 mg/kg for fish and 1 mg/kg for pets (AWT, 2002). A survey of vitamin supplementation of commercial feeds for pigs and poultry in Europe (Belgium, Denmark, Germany, Italy, Netherlands, Portugal, Spain and the United Kingdom) identified a range of 0–6.3 mg vitamin K_3/kg as commercial use levels (Gropp, 1994). A range for piglets and pigs of 1–5 mg menadione/kg feed was reported by Whittemore et al. (2002).

No data on the tolerance of animal species including fish were provided by the applicant. Only three studies, one in lovebirds, one in laying hens and another one in Atlantic salmon, which could be considered as indicative of tolerance to vitamin K were found.

The tolerance of lovebirds (*Agapornis* spp.) to MSB has been studied using three groups of birds fed pelleted diets containing 0, 20 or 200 mg MSB/kg (analytically confirmed) for 10 months (Wolf et al., 2005). No adverse effects of vitamin K_3 were seen on feed and water intake, quality of excreta, growth, reproduction status, haemotogram, clinical blood biochemistry, pathology (four birds each after 6 and 10 months). The authors concluded from liver vitamin K levels that MSB is rapidly metabolised. The data show that MSB up to 200 mg/kg diet is tolerated by lovebirds. Consequently, 20 mg MSB/kg is considered safe for all other ornamental birds.

Suzuki and Okomoto (1997) fed laying hens with 1 000 mg MSB/kg diet for 31 days. No adverse effects on feed intake and laying performance were found.

Graff et al. (2010) supplemented salmon diets with MNB to provide 0, 10, 100 or 1 000 mg menadione/kg. The recovery in feed was only 3–5 % of the intended values. Since requirement data are in the range 1–10 mg menadione/kg, the study provides a rather low margin of safety for MNB for fish.

3.3.1. Interactions

3.3.1.1. Interactions with fat-soluble vitamins

Interactions between the fat-soluble vitamins retinol, cholecalciferol, tocopherol and phylloquinone/menaquinone have been widely described in the literature (e.g. Matschiner et al., 1967; March et al., 1973; Abawi and Sullivan, 1989; Grisdale-Helland et al., 1991; Traber, 2008; Farley et al., 2012). In short, overdoses of one fat-soluble vitamin seem to require comparable overdosing of other fat-soluble vitamins to compensate for adverse effects. These interactions are of scientific interest but of little or no relevance for common feeding practice, since they are expected to occur only at concentrations above the legal thresholds for retinol and cholecalciferol.

3.3.1.2. Interactions with antagonists

Dicoumarol and coumarin derivates (e.g. warfarin) are well known anticoagulants acting as vitamin K antagonists, increasing the vitamin K requirement of the animals. Actinomycin D antagonises the prothrombin formation induced by vitamin K_3 in chicks (Olson, 1964). Scott et al. (1982) concluded that coccidiosis causes an increased requirement for vitamin K.

Some broad-spectrum antibiotics may interact with microbial synthesis of vitamin K_2 in the intestine (Conly and Stein, 1994; Shearer, 1995; Hendler and Rorvik, 2001), reducing the supply to the animal. Similar effects have been described with sulphonamides in the diet. Nelson and Norris (1961) showed that the inclusion of 0.1 % sulphoquinoxaline increased the chick's need for supplemental vitamin K by four- to seven-fold.

3.3.2. Chromium

Since sodium dichromate ($Na_2Cr_2O_7$) is used as catalyst in the manufacture of the additive (see section 2.1.1), it is assumed that chromium in MSB (30–45 mg/kg) and MNB (77–142 mg/kg) is hexavalent chromium (Cr(VI)); speciation was not provided despite being requested. Cr(VI) is an established human and animal carcinogen, including when ingested (Witt et al., 2013). Evidence indicates also a potential for endocrine disruption (EFSA, 2009b). A conservative estimate of the contribution of vitamin K_3 sources to the chromium content of complete feed is made assuming that (i) MNB is used, (ii) the dose in complete feed is 10 mg menadione/kg, equivalent to 20 mg MNB/kg, (iii) the chromium content of MNB is 150 mg/kg (by specification) and (iv) chromium in MNB is Cr(VI). Based on these assumptions, 3 µg chromium would be added to 1 kg complete feed. Background concentrations of chromium in feed would be between 0.2 and 7 mg/kg and it is expected that about

10 % would exist as Cr(VI) (EFSA, 2009b; Soares et al., 2010; Mandiwana et al., 2011). Thus, a highly conservative estimate is that the sources of vitamin K_3 under assessment would add 0.4 to 15 % of the background of Cr(VI) to feed. It should be noted that soluble Cr(VI) compounds are unstable and readily reduced to Cr(III) in the presence of electron donors such as organic matter or reducing inorganic compounds. It is therefore likely that the contribution of the additive to the total chromium content in feed would mostly be as Cr(III), which has low oral toxicity (NOAEL (rat) = 286 mg/kg bw per day; NTP, 2010). Although a full assessment of the risk arising from the intake of chromium from vitamin K_3 sources for the target animals is not possible, owing to the lack of data on chromium speciation, the FEEDAP Panel considers the risk from added chromium as negligible.

Making the same assumptions as those above for the addition of the MSB in feed, water for drinking would contain 3 µg/L of added chromium when MSB is added to water for drinking. Natural concentrations in surface waters range from 0.3 to 6 µg/L (Rakhunde et al., 2012). The carcinogenic Cr(VI) is a major chromium species in water, and the addition of MSB to water for drinking would likely increase the exposure of target animals to Cr(VI). The added risk may be marginal but cannot be ignored.

3.3.3. Conclusions on the safety of MSB and MNB for target species

Acute toxicity to menadione or its derivatives is reached at levels exceeding the requirements by a factor of at least 1 000. Few and incomplete data on lovebirds, laying hens and salmon indicate that tolerated vitamin K concentrations in feed exceed the requirements by factors of 5 to 1 000. It is concluded that MSB and MNB are safe for all animal species at practical use levels. Setting a maximum content for MSB and MNB in feed is not considered necessary.

The addition of chromium as a contaminant in MSB and MNB is not a concern for its use in feed, but would likely add to Cr(VI) exposure if used in water for drinking. The FEEDAP Panel has therefore some concerns regarding the safety of the latter application.

3.4. Safety for the consumer

3.4.1. Tolerable upper intake level (UL)

There are insufficient data to establish a safe upper level for vitamin K in humans. The SCF identified an adequate intake of 1 µg vitamin K/kg bw per day in adults, which would be provided by a normal diet (EC, 2003). According to the UK Expert Group on Vitamins and Minerals, a daily supplementary intake of 1 mg/day (equivalent to 17 µg/kg bw in a 60-kg adult) would be unlikely to result in adverse effects (EVM, 2003).

3.4.2. Consumer exposure

According to the SCF (EC, 2003), the mean intake of phylloquinone in the USA ranges between 60 and 110 µg phylloquinone/day in young adults (< 45 years), and between 80 and 210 µg phylloquinone/day in older adults (> 45 years) because of their greater vegetable consumption. In a longitudinal study performed in the UK in 1985, the mean estimate for men and women aged 40–59 years was 67 and 69 µg phylloquinone/day, respectively. At follow-up 10 years later the intakes had fallen to 54 and 56 µg phylloquinone/day, respectively, for men and women then aged 50–60 years. For people aged 65 years and over, mean intakes for men and women were 66 and 57 µg/person/day, respectively. The EVM reported a mean intake of 68 µg/day, and an estimated maximum intake of 270 µg/day including supplements (EVM, 2003). In The Netherlands, mean daily per capita intake was estimated to be up to 250 µg as a consequence of the relatively high intake of green vegetables. For menaquinone intake there are no population-based data available except for The Netherlands, where menaquinones are estimated to account for about 10 % of total vitamin K intake (Schurgers et al., 1999). Based on the average per capita food consumption in Finland (Ministry of Agriculture and Forestry, 2000; Statistics Finland, 2000, cited by EC, 2003), the average vitamin K intake from different foods was estimated to be 120 µg/day (Koivu-Tikkanen, 2001). The more recent German food surveys (Nationale Verzehrsstudie, 2008 and 2013) do not include data on the vitamin K intake.

The vitamin K content of food of animal and plant origin compiled from European data (Souci et al., 2008), based on phylloquinone measurement, confirmed the results of earlier studies (Schurgers and Vermeer, 2000; Damon et al., 2005) that vegetables, and especially green leafy vegetables, contain much higher quantities of phylloquinone than animal products (by a factor 5 to 100). Among foods of animal origin, very low levels were found in cow milk (3.0–4.4 µg/L) and in increasing order in fish flesh (26–400 µg/kg), whole egg (19–500 µg/kg), meat (130–180 µg/kg) and liver (560–890 µg/kg). A study of the vitamin K contents of food in The Netherlands considered separately phylloquinone, MK-4 and higher MK-*n*, using a high-performance liquid chromatography (HPLC method) (Schurgers and Vermeer, 2000). It appeared that MK-4 was present in all animal tissues tested (meat, dairy products and eggs), but the lack of substantial differences between wild (hare, deer), free-range and intensively raised animals suggests that the conversion and subsequent retention of menadione from supplemented feed does not contribute substantially to MK-4 concentration in tissues. The authors concluded that the major part of MK-4 in animal tissues is likely to originate from the conversion of phylloquinone, as already shown to occur in the rat (Thijssen et al., 1996, 2006); on the basis of food questionnaires and data collected, these authors calculated that phylloquinone represents almost 90 % of the total dietary vitamin K intake in the Dutch population, whereas menaquinones account for less than 12 %.

3.4.3. Conclusions on safety for the consumer

Since vitamin K supplementation of feedingstuffs as MSB and MNB is in widespread routine use, the FEEDAP Panel considers that above-mentioned population exposure figures already include the contribution of edible tissues and products of animals fed vitamin K-supplemented diets. Moreover, MSB and MNB administration to farmed animals generates MK-4 residues which represent only a small fraction of total vitamin K active compounds in animal tissues. Therefore, the FEEDAP Panel considers that the use of MSB and MNB in animal nutrition does not give rise to safety concerns for consumers.

3.5. Safety for the user

3.5.1. Effects on respiratory system

The moderate to high dusting potential and the high proportion of particles of diameter < 50 µm indicate a potential for inhalation of MSB and MNB. Since no data on inhalation toxicity of MSB and MNB have been provided, inhalation of dust is considered as potentially hazardous.

The FEEDAP Panel assessed the user exposure resulting from the residual content of chromium in MSB. Since sodium dichromate ($Na_2Cr_2O_7$) is used as catalyst in the manufacture of the additive (see section 2.1.1), and in the absence of more detailed information on the chromium species in MSB, it is plausible that all residual chromium would be Cr(VI), i.e. the most toxic species and a recognised human carcinogen.

Using the highest values as a worst-case assumption (dusting potential up to 15.4 g/m^3, chromium content in the dust up to 182 mg/kg), the chromium concentration in the air would be up to 2 801 µg Cr/m^3 for MSB. As for MNB, a worst-case calculation based on the highest values (dusting potential up to 0.46 g/m^3, chromium content in the dust up to 71 mg/kg) provides a chromium concentration in the air of 32.7 µg/m^3.

The calculated values for both MSB and MNB are above the threshold limit value set by ACGIH (2004; 10 µg/m^3 threshold limit value (TLV) time-weighted average (TWA), insoluble Cr(VI) compounds), the action level set by OSHA (2009; 2.5 µg Cr(VI)/m^3 for an eight-hour TWA exposure) and the more recent NIOSH (2013) recommended exposure limit (0.2 µg Cr(VI)/m^3 for an eight-hour TWA exposure, 40-hour working week). In particular, the worst-case calculation for MSB would greatly exceed the ACGIH, OSHA and NIOSH parameters by factors of 280, 1 120 and 14 000 respectively, whereas that for MNB would exceed these parameters by factors of 3, 13 and 164, respectively.

The FEEDAP Panel recognises that the scenarios used for setting occupational exposure limits depict a more prolonged and continuous exposure than expected from occupational handling of feed additives. However, considering the magnitude by which these thresholds are exceeded, the FEEDAP concludes that the content of chromium in both MSB and MNB gives rise to a concern for users' safety.

3.5.2. Effects on the eyes and skin

Skin and eye irritation tests with MSB were performed in accordance with the appropriate OECD guidelines.[36]

In the skin irritation test, 500 mg MSB was applied in single dose to intact skin of three albino rabbits for a period of four hours. No significant signs of irritation were detected.

In the eye irritation test, the conjunctival sac of three albino male rabbits was instilled with 100 mg MSB. There were no effects on the cornea and the iris, but the conjunctivae showed severe congestion and chemosis. The congestion regressed in all treated animals seven days after the treatment and chemosis regressed 72 hours after the treatment. MSB should be considered as an eye irritant.

Since no data on skin or eye irritation of MNB are available, it would be prudent to consider MNB as an irritant to skin and eye.

No skin sensitisation tests are available on either MSB or MNB. A single case of contact dermatitis upon occupational exposure to airborne MSB when mixing additives in feeds has been reported.[36] Both substances should be regarded as potential skin sensitisers.

Cr(VI) is recognised as dermatologically toxic, inducing sensitisation and allergic contact dermatitis after repeated exposures to levels lower than those present in the additives (Shelnutt et al., 2007; Geier et al., 2011). Potassium dichromate was found to be the most frequent allergen among 20 common allergens (Geier et al., 2011). Exposure by inhalation to Cr(VI) dust exacerbates allergic asthma (Schneider et al., 2012). Owing to the presence of Cr(VI), the possibility of allergic reactions cannot be ruled out.

3.5.3. Conclusions on user safety

MSB is as an eye irritant; in the absence of adequate data, the additive should be considered as a skin sensitiser.

In the absence of data, MNB should be considered as irritant to skin and eyes and as a skin sensitiser.

Considering the high dusting potential of MSB and MNB, the absence of data on inhalation toxicity and chromium(VI) content of the dust, the FEEDAP Panel concludes that inhalation exposure from handling of MSB and MNB could be hazardous.

3.6. Safety for the environment

Vitamin K occurs widely in nature as phylloquinone in plants. Phylloquinone from feed materials, as well as MSB and MNB used as feed additives, is excreted mainly as menaquinones in faeces and as menadione in small amounts in urine. Thus, the natural plant vitamin K and the chemically synthesised K_3 compound add to the same pool of substances in the environment. Considering the huge amount of phylloquinone in nature, the use of MSB and MNB in animal nutrition is not expected to substantially increase the concentration of vitamin K metabolites in the environment. Therefore, no risk to the environment resulting from the use of MSB and MNB in animal nutrition is expected.

[36] Technical dossier/Section III/Reference 3.3.02.

4. Efficacy

According to Regulation (EC) No 429/2008, efficacy studies are not required for vitamins, pro-vitamins and chemically well-defined substances having similar effects which are already authorised as feed additives. Owing to the long history of use and its established nutritional role in domestic animals, MSB and MNB are regarded as effective sources of the vitamin K. Vitamin K has been globally used in animal nutrition for decades. Data on requirement, allowances and recommendations for feed supplementation are easily accessible as standard literature for animal nutrition.

One milligram of vitamin K_3 is defined as 1 mg menadione. Equivalent doses of MSB and MNB would be 2.00 and 2.27 mg, respectively.

The FEEDAP Panel notes that MNB is also a source of nicotinamide. The efficacy of nicotinamide as a feed additive for all animal species has recently been assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2012).

5. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[37] and by good manufacturing practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Acute toxicity of menadione or its derivatives is reached at levels exceeding the requirements by a factor of at least 1 000. MSB and MNB are safe for all animal species at practical use levels in feed.

The use of MSB in water for drinking is likely to increase the exposure of target animals to Cr(VI). Therefore, the FEEDAP Panel has concerns about the safety of MSN when administered by this route.

The use of MSB and MNB in animal nutrition does not give rise to concerns for consumers.

MSB is as an eye irritant; in the absence of adequate data, the additive should be considered as a skin sensitiser. In the absence of data, MNB should be considered as irritant to skin and eyes and as a skin sensitiser. Considering the high dusting potential of MSB and MNB, the absence of data on inhalation toxicity and the chromium (VI) content of dust, inhalation exposure from handling of MSB and MNB could be hazardous.

The use of MSB and MNB in animal nutrition does not pose a risk to the environment.

MSB and MNB are regarded as effective sources of vitamin K in animal nutrition.

RECOMMENDATIONS

The substances under application should be listed as vitamin K_3 (not as vitamin K). Vitamin K_3 is equivalent to menadione; 1 mg vitamin K_3 would be equivalent to 2.00 mg MSB and 2.27 mg MNB. When labelled, the active substances should be labelled as MSB or MNB.

Considering the content of non-speciated chromium in MSB and MNB, the FEEDAP Panel recommends that the chromium content of all production batches be monitored and that the content of hexavalent chromium be reduced as much as possible.

Vitamin mineral premixtures containing MSB should not be stored for longer than one month.

[37] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

Considering the potential hazard to users by inhalation, the FEEDAP Panel strongly recommends that the use of the additives MSB and MNB be restricted to premixture manufacturers.

DOCUMENTATION PROVIDED TO EFSA

1. Vitamin K_3 in the form of menadione sodium bisulphite and menadione nicotinamide bisulphite as a feed additive for all animal species. October 2010. Submitted by VITAC EEIG Vitamins Authorisation Consortium.

2. Vitamin K_3 in the form of menadione sodium bisulphite and menadione nicotinamide bisulphite as a feed additive for all animal species. Supplementary information. September 2012. VITAC EEIG Vitamins Authorisation Consortium.

3. Vitamin K_3 in the form of menadione sodium bisulphite and menadione nicotinamide bisulphite as a feed additive for all animal species. Supplementary information. January 2013. VITAC EEIG Vitamins Authorisation Consortium.

4. Vitamin K_3 in the form of menadione sodium bisulphite and menadione nicotinamide bisulphite as a feed additive for all animal species. Supplementary information. June 2013. VITAC EEIG Vitamins Authorisation Consortium.

5. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for vitamin K_3 (menadione).

6. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Vitamin K3 (Menadione)[38]

In the current application authorisation is sought for *Vitamin K_3* (*Menadione*) under the category/functional group 3(a) 'nutritional additives'/'vitamins, pro-vitamins and chemically well defined substances having similar effect' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use the active substances, *Menadione Sodium Bisulphite (MSB)* and *Menadione Nicotinamide Bisulphite (MNB)* for all animal species and categories, with a minimum content of 50 % and 44 % of *Menadione*, respectively. The *feed additive* is intended to be incorporated in *feedingstuffs* through *premixtures* or directly in *water* (only for *MSB*). However, the Applicant did not specify any maximum or minimum concentration of *MSB* and *MNB* in *feedingstuffs* or *water*.

For the determination of *Vitamin K_3* (*Menadione*) and its commercial derivatives *Menadione Sodium Bisulphite (MSB)* and *Menadione Nicotinamide Bisulphite (MNB)* in the *feed additive* the Applicant proposed a Method from the Association of German Agricultural Analytical Research Institutes (VDLUFA), validated for *premixtures* and *feedingstuffs*. The Applicant applied this method to the *feed additive* and reported a relative standard deviation of *repeatability* (RSD_r) of 1.7 % for *MSB* and *MNB* containing 50 % and 44 % of *Menadione*, respectively. Moreover, a *recovery* rate (R_{Rec}) ranging from 101 % to 104 % was calculated by EURL, based on the experimental data provided by Applicant.

Based on the performance characteristic presented, the EURL recommends for official control the validated method from VDLUFA (Bd.III 13.7.1) to determine *Vitamin K_3* in the *feed additive*.

For the determination of *Vitamin K_3* in *premixtures* and *feedingstuffs* the Applicant proposed the above mentioned VDLUFA method - validated for *premixtures* and *feedingstuffs*, for which an RSD_r ranging from 10 % to 20 % is reported.

An alternative ring-trial validated method for *premixtures* and *feedingstuffs* was published in the Italian Official Journal, based on Normal Phase High-Performance Liquid Chromatography (NP-HPLC) coupled to Ultraviolet (UV) detector, using a wavelength of 251 nm. The following performance characteristics were reported for *premixtures*, complementary *feedingstuffs* and complete *feedingstuffs* samples with a *Vitamin K_3* (*Menadione*) content ranging from 5.57 to 543 mg/kg:

- RSD_r ranging from of 2.9 to 6.8 % ;
- a relative standard deviation of *reproducibility* (RSD_R) ranging from 7.0 to 11.7%;
- R_{Rec} ranging from 89.2 to 111.4 %, and
- a limit of detection (LOD) and quantification (LOQ) of 1.2 and 3.8 mg/kg, respectively.

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated method, published in the Italian Official Journal, based on Normal Phase High-Performance Liquid Chromatography (NP-HPLC) coupled to UV detector to determine *Vitamin K_3* in *premixtures* and *feedingstuffs*, within the concentration range covered.

For the determination of the *Menadione Sodium Bisulphite (MSB)* in water, the Applicant applied the VDLUFA method. The following performances characteristics were reported for a concentration of 0.5 g *Menadione* /L:

[38] The full report is available on the EURL website. http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0099.pdf

- RSD_r ranging from 1.8 to 4.4 %, and

- R_{Rec} ranging from 97.9 to 99.2 %.

Based on the performance characteristics presented, the EURL recommends for official control the validated method from VDLUFA (Bd.III 13.7.1) to determine *Vitamin K_3* in *water*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.