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Safety and efficacy of an additive consisting of synthetic vitamin K_1 (phytomenadione) for horses (JARAZ Enterprises GmbH & Co. KG)

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Abstract

Following a request from the 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 a feed additive consisting of vitamin K_1 (phytomenadione) produced by chemical synthesis when used as a nutritional additive in complementary feed of horses at a maximum supplemental level of 14 mg vitamin K_1/horse per day. The use of vitamin K1 is safe when used as a feed additive for horses under the proposed conditions of use. The use of vitamin K1 in nutrition of horses under the proposed conditions of use is considered safe for the consumer and the environment. No exposure of users by inhalation is expected. The Panel cannot conclude on the potential of the additive to be a skin and eye irritant. Vitamin K1 is considered a moderate dermal sensitiser. Vitamin K1 is an effective source of vitamin K in horse nutrition. The Panel recommends that the specifications of the additive refer to the substance-related impurities listed in the most updated monograph of the European Pharmacopoeia.

Keywords: Vitamin K, phylloquinone, phytomenadione, horse, nutritional additive, feed

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] 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.

The European Commission received a request from JARAZ Enterprises GmbH & Co. KG.[2] for authorisation of the product vitamin K_1 (phytomenadione), when used as a feed additive for horses (category: nutritional additives; functional group: vitamins, pro-vitamins and chemically well-defined substances having a similar effect).

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). The particulars and documents in support of the application were considered valid by EFSA as of 20 March 2020.

According to Article 8 of Regulation (EC) No 1831/2003, 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. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product vitamin K_1 (phytomenadione) produced by chemical synthesis, when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

Vitamin K_1 produced by chemical synthesis is the subject of the assessment. Vitamin K_1 is currently not authorised as a feed additive in the EU market. Vitamin K (phytomenadione and/or metaquinone) is authorised in food (Regulation (EC) No 1925/2006[3] and related Directive 2002/46/EC[4], Regulation (EC) 609/2013[5]), in cosmetics (Commission Regulation (EU) No 344/2013)[6] and as veterinary drug (Commission Regulation (EU) 37/2010).

In 2014 the EFSA FEEDAP Panel adopted an opinion on the safety and efficacy of vitamin K3 (sodium bisulfite and nicotinamide bisulfite forms) and it is authorised as a feed additive in the EU. Dietary reference values for vitamin K (phytomenadione and menaquinones) in food were assessed by the EFSA Panel on dietetic products, nutrition and allergies (EFSA NDA Panel, 2017). Health claims related to vitamin K (phytomenadione and menaquinone) and maintenance of bone, blood coagulation and function of the heart and blood vessels had been assessed by the EFSA NDA Panel (2009). The Scientific Committee for Veterinary Medicinal Products expressed an opinion on phytomenadione (vitamin K_1) and menadione (vitamin K3) (EMEA, 1998). The scientific committee of food issued an opinion on the tolerable upper intake level of vitamin K (SCF, 2003). The Scientific Committee of Consumer Safety (SCCS, 2010) issued an opinion on the allergenic potential of vitamin K_1 when used as cosmetic. The Panel on Nutrition, dietetic products, Novel Food and Allergy of the Norwegian Scientific Committee For Food And Environment assessed the dietary intake and maximum limits for vitamin K in food supplements (VKM, 2018).

The European Pharmacopoeia has a monograph (01/2020:3011) dedicated to phytomenadione, racemic (European Pharmacopoeia, 2020).

[1] 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.
[2] JARAZ Enterprises GmbH & Co KG, Halligenstrasse 5, 49661 Cloppenburg, Germany.
[3] Commission 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. 30/12/2006. OJ L404, pp. 26–38.
[4] 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 183/51, 12.07.2002, pp. 51–57.
[5] Regulation (EC) 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181/35, 29.6.2013, pp. 35–56.
[6] Commission Regulation (EU) No 344/2013 of 4 April 2013 amending Annexes II, III, V and VI to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products. OJ L 114, 25.4.2013, p. 1–59.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[7] in support of the authorisation request for the use of vitamin K_1 (phytomenadione) as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the vitamin K1 (phytomenadione) in animal feed. The Executive Summary of the EURL report can be found in Annex A.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of vitamin K_1 (phytomenadione) is in line with the principles laid down in Regulation (EC) No 429/2008[9] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

Vitamin K_1 (also known as phylloquinone or phytomenadione) under assessment is produced by chemical synthesis and it is intended for use in feed for horses as a nutritional additive, functional group of vitamins, pro-vitamins and chemically well-defined substances having a similar effect.

Vitamin K describes a group of lipophilic vitamins that exist naturally in two forms: vitamin K_1 (found in green plants) and vitamin K_2 (a group of compounds with side chains of variable length, named as menaquinones [MK-*n*], 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 poly-isoprenoid side chain.

Vitamin K_3 (or menadione) is a synthetic form of vitamin K without a side chain as well as the vitamin K_4 (menadiol). 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 bone health (Gla-proteins) (EFSA NDA Panel, 2017).

3.1. Characterisation

3.1.1. Manufacturing process

Vitamin K_1 is produced by chemical synthesis [redacted]

[redacted]

[7] FEED dossier reference: FAD-2020-0006.
[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2020-0006_vitk1.pdf
[9] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

3.1.2. Characterisation of the active substance

Vitamin K_1 (International Union of Pure and Applied Chemistry [IUPAC] name 2-methyl-3-[(*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione; and Chemical Abstracts Service [CAS] number 84-80-0) has two geometrical isomers (*cis–trans* or *Z–E* isomers) and two asymmetric carbon atoms (C7 and C11) are each generating two enantiomers (R or S). Thus, there are eight diastereoisomers (four in the *trans*- and four in the *cis*-configuration). Synthetic vitamin K_1 is a mixture of *Z-E* isomers. It has a molecular weight of 450.68 Da. The empirical formula is $C_{31}H_{46}O_2$. It is a clear, yellow to amber viscous odourless liquid. Its density is 984 kg/m^3 and it is not miscible in water.[10] The structural formula (racemic) is indicated below (Figure 1).

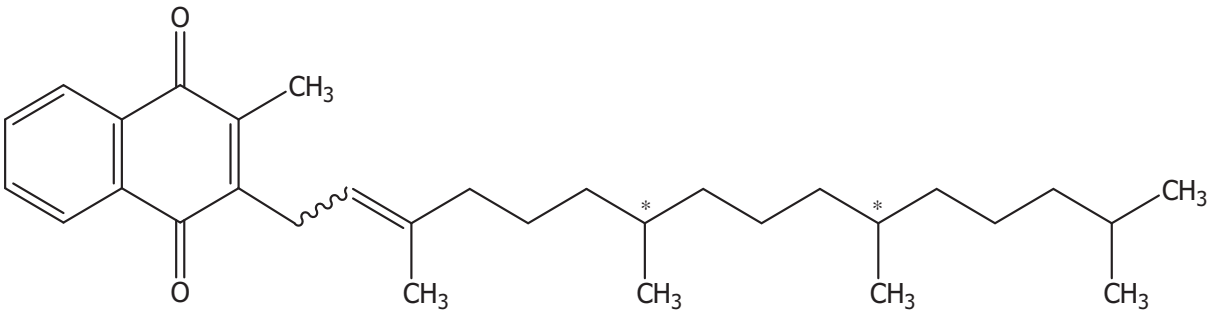

Figure 1: Structural formula of vitamin K_1 (phytomenadione). The asymmetric carbon atoms C7 and C11 are indicated with an asterisk

The European Pharmacopoeia monograph 01/2020:3011 (European Pharmacopoeia, 10th Edition, 2020) defines vitamin K_1 (racemic) as a mixture of *trans*-(*E*) and *cis*-(*Z*) phytomenadione. *E*-phytomenadione isomers should represent ≥ 85% and the sum of *E*- and *Z*-phytomenadione isomers should represent ≥ 97.0%.

Vitamin K_1 produced by chemical synthesis is specified by the applicant to contain ≥ 75% *E*-phytomenadione and ≤ 4% *E*-epoxyphytomenadione. The sum of *E*-phytomenadione, *E*-epoxyphytomenadione and *Z*-phytomenadione should represent ≥ 97%. Analysis of five batches showed that *E*-phytomenadione was on average 83.3% (range 81.8–85.6%), *Z*-phytomenadione was on average 16.5% (range 14.4–18.1%), and the sum of *E*-, *Z*-phytomenadione was on average 99.8% (range 99.2–100%).[11] *E*-Epoxyphytomenadione was 0.6% in all three batches analysed.[12]

The applicant proposed the following specifications for potential-related impurities, based on the European Pharmacopoeia monograph 01/2014:1036 (European Pharmacopoeia, 2016) and on the VICH guidelines:[13]

- 2-methylnaphthalene-1,4-dione (menadione/vitamin K_3) with a maximum limit established at 0.2%;
- (*E*)-1a-methyl-7a-[(3,7,11,15-tetramethylhexadec-2-en-1-yl]-1a,7a-dihydronaphtho[2,3-b] oxirene-2,7-dione, consisting of the *E*-epoxyphytomenadione isomers with a maximum limit of 4%;
- other impurities ('total impurities' in relation to the peaks of the three main constituents) with a maximum of 1.2%;
- the residual solvents methanol (maximum 0.3%), diethyl ether (maximum 0.5%) and ethanol (maximum 0.5%);
- Inorganic impurities that may result from the use of catalysts and other chemicals during the manufacturing are determined as sulfated ash (maximum 0.1% according to the European Pharmacopoeia).

[10] Technical dossier/Section III/Annex III 54.
[11] Technical dossier/Supplementary information September 2020/Annex 1. Analytical method VKFL AOAC 2017.
[12] Technical dossier/Section II/Annex II.1. Liquid chromatography according to the European Pharmacopoeia 2.2.29.
[13] In the current version of the European pharmacopoeia (V10.0), *E*-epoxyphytomenadione isomers have a maximum limit of 0.2%, and the maximum limit for 'unspecified impurities' is 0.15% for each impurity. In the European Pharmacopoeia V8.0, *E*-epoxyphytomenadione isomers were not considered as impurity.

Analysis of three batches (five batches for ether and absolute alcohol) showed compliance with the limits for related impurities listed in the European Pharmacopoeia monograph 01/2014:1036 (Table 1).[14]

Table 1: Specifications (European Pharmacopoeia monograph 01/20141036) and analysed concentrations of impurities in the additive under assessment

Item	Specification (%)	Range of analysed concentrations in the additive (%)
Eluting before *E*-epoxyphytomenadione	Simple impurity ≤ 0.15	0.02–0.03
	Total impurities ≤ 0.2	0.02–0.08
Eluting between *Z*-phytomenadione and *E*-phytomenadione	Single impurity ≤ 0.4	0.2 in all cases
	Total impurities ≤ 0.5	0.25–0.34
Eluting after *E*-phytomenadione	Single impurity ≤ 0.25	0.13 in all cases
	Total impurities ≤ 0.5	0.2–0.3
Total (other impurities)	≤ 1.2	0.5–0.7
Sulfated ash	≤ 0.1	0.02 in all cases
Methanol*	≤ 0.3	< 0.036 in all cases
Ether*	≤ 0.5	0.002–0.02
Absolute alcohol*	≤ 0.5	0.02–0.08

*: In house method; the symbol '<' corresponds to the limit of detection.

The FEEDAP Panel notes that specifications for impurities do not fully comply with the most updated monograph of the European Pharmacopoeia for phytomenadione (01/2020:30111). The analytical data provided comply with these criteria except for the concentration of *E*-epoxyphytomenadione, which is 0.6% in three batches instead of ≤ 0.2%.

The applicant also provided data on the levels of heavy metals (cadmium, lead and mercury) and arsenic in the product, analysed in three batches, which were in all cases below the limit of detection (LOD, < 0.1 mg/kg additive).[15]

The viscosity of vitamin K_1 was measured in three batches and ranged from 402 to 408 mPa s.[16]

3.1.3. Characterisation of a preparation of the additive

The active substance is marketed diluted and standardised in the form of a solid powder preparation formulated with carriers (e.g. maltodextrin, sugars), emulsifier(s) and antioxidant(s) (made up of sucrose, maize starch, polyoxyl 35 Castor oil and β-carotene) at a concentration of 42 g vitamin K_1/kg.[17] The certificate of analysis of the preparation states that dioxin, dioxin-like compounds and polychlorinated biphenyls (PCBs) meet the requirements as per Council Directive 2006/13/EC on undesirable substances.[18]

The dusting potential (Stauber–Heubach method) was measured in three batches of the vitamin K_1 preparation mentioned above. In all three cases no dust was formed (attributed to the hygroscopic characteristics of the preparation).[19]

Since the additive itself is a viscous liquid, no further tests for dusting potential are necessary.

3.1.4. Stability and homogeneity

The shelf life of the additive (two batches) was tested 7 and 21 months after manufacturing (one batch for each storage period). Samples were stored at –4°C in the original packaging (tight containers

[14] Technical dossier/Section II/Annex II.1 and supplementary information September 2020/Annex II.
[15] Technical dossier/Supplementary information September 2020/Annex II and 2020-09-11 Sin letter May 11-final reply/Reply to question 3.
[16] Technical dossier/Supplementary information September 2020/Annex II and 2020-09-11 Sin letter May 11-final reply/Reply to question 6.
[17] Technical dossier/Section II/Annex II.3.
[18] Commission Directive 2006/13/EC of 3 February 2006 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs. OJ L 32, 4.2.2006, p. 44–53.
[19] Technical dossier/Supplementary information September 2020/Annex III.

keeping the additive dry and protected from sunlight). No losses were observed at 7 months and losses after 21 months were < 1%.[20]

The shelf life of the additive in a preparation (composition not provided) containing 42,000 mg vitamin K_1/kg was measured in retention samples (three batches) tested at 8, 20 or 31 months after manufacturing (one batch for each storage period). Samples were stored at 15–25°C in containers as described above. No losses were observed in any of the samples at the end of the respective storage period.[21]

The stability of the additive (three batches) in a complementary feed for horses ([redacted]) was studied when supplemented at 140 mg vitamin K_1/kg and stored at 25°C in sealed white high-density polyethylene containers for 3 months. [redacted] At the end of storage period, no losses were observed (except one batch showing a loss of 0.6%).[22]

The capacity of the additive to distribute homogeneously in one of the complementary feeds described above (one batch tested) was studied by analysing 10 subsamples randomly selected. The coefficient of variation was 1%.[23]

3.1.5. Conditions of use

Vitamin K_1 may be placed on the market in form of a preparation. The applicant proposes its use for all horses via a complementary feed (moisture 12%) up to a maximum content of 140 mg vitamin K_1/kg. The complementary feed should provide 7–14 mg vitamin K_1/horse per day, independently of the background concentration of vitamin K_1 in the diet.

3.2. Safety

Vitamin K serves as co-factor for the vitamin K dependent carboxylase, which catalyses the post-translational synthesis of γ-carboxyglutamic acid (Gla) from glutamic acid residues contained in precursor proteins. The resulting vitamin K dependent proteins also referred to as Gla-proteins, are involved in blood clotting, bone metabolism and vascular health. Vitamin K has also been suggested to play a role in brain sphingolipid metabolism (NRC, 2007). Proteins resulting from the action of vitamin D, e.g. the calcitonin, require for full efficacy the carboxylation mediated by vitamin K.

3.2.1. Absorption, distribution, metabolism and excretion

Ingested vitamin K_1 is absorbed by an energy-dependent process from the proximal portion of the small intestine (Hollander, 1973).

By measuring plasma levels, Terachi et al. (2011) could demonstrate substantial absorption of synthetic vitamin K_1 in horses when given orally via feed (26 mg/kg feed).

Absorbed intestinal phytomenadione is transported by the lymphatic pathway in mammals. In humans, the intestinal absorption of vitamin K_1 may reach 80% (Shearer et al., 1974). Although vitamin K_1 is rapidly concentrated in liver, it has a relatively short biological half-life (< 1 day in rat) (NRC, 1987). No significant accumulation occurs in tissues being the liver and spleen the organs showing higher concentrations. The major route of excretion of intravenously administered radioactive vitamin K_1 appears to be faecal (NRC, 1987). When administered to rats, 60% phytomenadione is excreted in faeces and 10% in urine within 24 h (Griminger and Donis, 1960). A fraction of vitamin K is re-excreted into the intestine via bile.

3.2.2. Safety for horses

The applicant provided a tolerance study in horses and a literature review to support the safety of the additive for the target animals.[24] However, the tolerance study could not be considered due to serious limitations in the design (the animals were not randomly assigned into the groups resulting in different initial body weight and different blood parameters of the horses between the groups at the start of the study). Similarly, the extensive literature search could not be considered because of several limitations in the conduct and/or reporting (exclusion criteria not described, only 50 out of 160 hits of

[20] Technical dossier/Section II/Annex II.16.
[21] Technical dossier/Section II/Annex II.17.
[22] Technical dossier/Supplementary information September 2020/Annex IV.
[23] Technical dossier/Supplementary information September 2020/Annex VI.
[24] Technical dossier/Section III/Annex III.36.

a database were considered, 'targeted literature search' not properly documented, most of the studies were not related to horses),[25] and because the references selected by the applicant to support the safety of the additive for horses were studies not designed to address the evaluation of the safety of vitamin K_1. The argument of the applicant that horses eating green leaves of forage plants are usually exposed to concentrations of vitamin K_1 substantially higher than the recommended maximum use level proposed could not be considered appropriate because of uncertainties related to the bioavailability.

The product under assessment is highly purified. The analysis of five batches showed for the sum of *E*-phytomenadione and *Z*-phytomenadione – a mean value of 99.8% (99.2–100%).[26] The inclusion rate for vitamin K usually does not exceed 10 mg/kg complete feed. The inclusion rate of 140 mg vitamin K_1/kg complementary feed proposed by the applicant, refers to a maximum daily provision of 100 g complementary feed for a 500 kg horse, corresponding to a daily dose of 14 mg per horse or 1.4 mg/kg complete feed.[27]

Excess intake of phytomenadione appears to be essentially innocuous. Also, menaquinones and menadione have probably low oral toxicity. The NRC (1987) proposed that oral toxic levels of vitamin K are at least 1,000 times the dietary requirement.

— Considering all of the above, the FEEDAP Panel concludes that the use of vitamin K_1 under the proposed conditions of use is safe for horses. Setting a maximum content in feed is therefore not necessary.

3.2.3. Safety for the consumer

According to EMEA (1998), the possibility of residues in meat and milk is considered of no concern because natural and synthetic forms of vitamin K are rapidly metabolised and largely excreted as glucuronide or sulfate conjugated. For phytomenadione, no maximum residue limit (MRL) is required (Commission regulation (EU) No 37/2010).[28]

Tolerable upper intake level

There are no requirements of vitamin K established for humans. The EFSA NDA Panel (2017) could not derive a dietary reference value and set adequate intake guidelines based on representative dietary intake data from healthy individuals, which for vitamin K_1 in adult individuals was of 1 μg/kg body weight. A tolerable upper intake level (UL) is not established.

Consumer exposure

The UK expert group on vitamins and minerals (EVM, 2003) concluded that adverse effects were unlikely with a daily supplement of 1 mg vitamin K_1/day (equivalent to 14 μg/kg bw in a 70 kg adult). Formula-fed infants (0–6 months of age) have dietary intakes of vitamin K_1 larger than human-milk-fed infants (about 50 μg/day vs about 0.7 μg/day, FAO, 2011).

As indicated in the section on absorption, distribution, metabolism and excretion (ADME), vitamin K_1 is largely metabolised and rapidly excreted and no residues in meat or milk are expected (EMEA, 1998). No data were available on levels of vitamin K_1 in horse meat. Horse liver was reported to contain 3.3 mg/kg of vitamin K_1 (Duello and Matschiner, 1970). In absence of residue data in horses, some assumptions can be made based on the ADME of the substance and available literature on occurrence data.

Several tissues (e.g. pancreas, testis, vessels wall) can convert vitamin K_1 into menaquinone 4 (MK-4) (Thijssen et al., 1996; Ronden et al., 1998; Okano et al., 2008) which can reach relatively high concentrations in animal products. No substantial differences were seen between game (hare, deer), free-range animals and those from factory farms in terms of MK-4 concentration in meat (Schurgens and Vermeer, 2000). In horses, contrarily to other mammals and birds, vitamin K_1 conversion to menaquinone 4 is probably low, because supplementation of diets for 7 days with pure vitamin K_1

[25] Technical dossier/Section III/Annex III.9.
[26] Technical dossier/Supplementary information_September_2020/Annex_I_Isomer.
[27] When considering total exposure of the horse, it should be taken into account that the complete feed will routinely be supplemented with the already authorised vitamin k_3, so that vitamin K_1 and vitamin K_3 would be administered together. Considering the very low toxicity of orally administered vitamin K, this would not be of any concern for target animal safety.
[28] Commission Regulation (EU) No 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. 1–72.

leads to a quadratic increase (2- to 2.3-fold) of its plasmatic concentration (within 8 h post administration) and did not result in a significant increase of plasmatic MK-4 (Terachi et al. (2011).

It is not expected that the use of vitamin K_1 in horse nutrition at the proposed conditions of use will result in an increase in the consumer exposure to vitamin K_1.

Conclusions on safety for the consumer

The use of vitamin K_1 in nutrition of horses under the proposed conditions of use is considered safe for the consumer.

3.2.4. Safety for user

No studies performed with the additive under assessment were provided to support the safety for the user.

The additive is a viscous liquid, not forming dust and as such users will not be exposed by inhalation. The additive will be placed in the market in the form of a solid preparation which shows no dusting potential and therefore no inhalation exposure is expected.

The SCCS considered that vitamin K_1 is not safe when used in cosmetic products in a concentration up to 1% because case reports show that vitamin K_1 is a contact allergen in man. Data from the SCCS (2010) indicate that vitamin K_1 can be categorised as a dermal sensitiser.[29]

The FEEDAP Panel recognises that once authorised, multiple formulations of the additive can be placed in the market, and consequently, not all preparations can be directly tested for user safety. No information was provided on the irritation/sensitisation potential of any preparation. In absence of these data, the FEEDAP cannot conclude on the potential of the preparations to be toxic by inhalation or on their potential as skin/eye irritant.

Conclusions on safety for the user

No exposure by inhalation is expected. The FEEDAP Panel cannot conclude on the potential of the additive to be a skin and eye irritant. Vitamin K_1 is considered a dermal sensitiser.

3.2.5. Safety for the environment

Vitamin K_1 occurs widely in nature and it is synthesised by cyanobacteria, algae and higher plants (photosynthetic organisms able to perform oxygenic photosynthesis) (Nowicka and Kruk, 2010). Vitamin K_1 present in plants and in feed materials is excreted mainly as menaquinones in faeces and as menadione in small amounts in urine. Considering the huge amount of phytomenadione in nature, the use of vitamin K_1 in horses under the proposed conditions of use is not expected to substantially increase the concentration of vitamin K metabolites in the environment.

3.3. Efficacy

Dietary vitamin K requirements have not been determined for the horse (NRC, 1989). Phytomenadione content of forages along with menaquinones synthesised by intestinal bacteria, presumably meet requirements in all but the most unusual circumstances. Vitamin K deficiency in horses due to inadequate vitamin K consumption has not been identified by the NRC (2007). Vitamin K antagonists such as dicoumarol (e.g. from mouldy sweet clover hay) and warfarin can impair vitamin K metabolism.

Synthetic vitamin K_1 was shown to be absorbed in the intestine of horses (Terachi et al., 2011). Consequently, vitamin K_1 is considered as an efficacious source of vitamin K for the horse, when added to feed.

3.4. 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[30] and Good Manufacturing Practice.

[29] Technical dossier/Section III/Annex III.43.

[30] 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.

4. Conclusions

The use of vitamin K_1 is safe when used as a feed additive for horses under the proposed conditions of use.

The use of vitamin K_1 in nutrition of horses under the proposed conditions of use is considered safe for the consumer and the environment.

No exposure of users by inhalation is expected. The Panel cannot conclude on the potential of the additive to be a skin and eye irritant. Vitamin K_1 is considered a moderate dermal sensitiser.

Vitamin K_1 is an effective source of vitamin K in horse nutrition.

5. Recommendation

The Panel recommends that the specifications of the additive refer to the substance-related impurities listed in the most updated monograph of the European Pharmacopoeia.

6. Documentation as provided to EFSA/Chronology

Date	Event
07/02/2020	Dossier received by EFSA. Vitamin K_1 (phytomenadione) for horses. Submitted by JARAZ Enterprises GmbH & Co. KG.
06/05/2019	Reception mandate from the European Commission
20/03/2020	Application validated by EFSA – Start of the scientific assessment
11/05/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Methods of analysis, characterization of the additive, conditions of use, safety for horses.*
22/06/2020	Comments received from Member States
27/11/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
07/01/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
17/03/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
ANS	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
BW	body weight
CAS	Chemical Abstracts Service
CV	coefficient of variation
DM	dry matter
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FAO	Food Agricultural Organization
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
SCAN	Scientific Committee on Animal Nutrition
SCF	Scientific Committee on Food
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for vitamin K_1 (phytomenadione)

In the current application an authorisation is sought under Article 4 for vitamin K_1 (phytomenadione) as feed additive under the category/functional group 3(a) "nutritional additive"/ "vitamins, provitamins and chemically well-defined substances having a similar effect" according to the classification system of Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the feed additive for horses.

The product presented by the Applicant contains as active substance a mixture of *trans*-phytomenadione (not less than 75%, w/w), *cis*-phytomenadione (~ 20%, w/w) and *trans*-epoxy-phytomenadione isomers (not more than 4%, w/w). The sum of the three components is not less than 97% (w/w). The product is placed on the market as preparation with a typical content of 42 g vitamin K_1/kg of the preparation. It is intended to be used in complementary feed with a minimum content of 140 mg vitamin K_1/kg feed.

For the determination of vitamin K_1 (*trans*- and *cis*-isomers of phytomenadione, and *trans*-epoxy-phytomenadione) in the feed additive the Applicant proposed the method presented within the phytomenadione monograph of the European Pharmacopoeia, where quantification is based on the generic high performance liquid chromatography (HPLC) method as described in the test for related substances. The EURL recommends for official control the European Pharmacopoeia method described in the phytomenadione monograph to determine vitamin K_1 (*trans*- and *cis*-isomers of phytomenadione, and *trans*-epoxy-phytomenadione) in the feed additive.

For the determination of vitamin K_1 in the additive preparation and in complementary feed the Applicant presented a procedure based on the ring-trial validated CEN method (EN 14148) intended for foodstuffs. The analytical method is based on HPLC coupled with fluorescence detection (FLD). The Applicant's method is a slightly modified EN 14148 method in order to reach the same concentration range as specified in the CEN method and is routinely used by the Applicant.

In the frame of the ring-trial validation studies supporting the CEN method the following performance characteristics were reported for the quantification of vitamin K_1 in milk and infant formula samples with a content ranging from 0.05 to 1.2 mg/kg: a relative standard deviation for repeatability (RSDr) from 2.6 to 9.0% and a relative standard deviation for reproducibility (RSDR) from 4.3 to 10.9%.

Based on the findings above, the EURL recommends for official control the Applicant's method based on the HPLC-FLD ring-trial validated method EN 14148 to determine vitamin K_1 in the additive preparation and in complementary feed.

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, as last amended by Regulation (EU) 2015/1761), is not considered necessary.