European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of vitamin D_3 (cholecalciferol) as a feed additive for chickens for fattening, turkeys, other poultry, pigs, piglets (suckling), calves for rearing, calves for fattening, bovines, ovines, equines, fish and other animal species or categories, based on a dossier submitted by DSM[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The principal physiological role of vitamin D in all vertebrates is in calcium and phosphorus homeostasis. The classic clinical deficiency syndrome is rickets. The FEEDAP Panel notes that for turkeys for fattening, equines, bovines, ovines and pigs the maximum content for vitamin D_3 in feed does not provide any margin of safety, and that, except for pigs, the maximum content is above the upper safe level, according to National Research Council data when animals were fed a supplemented diet for more than 60 days. No safety concern was identified for the use of vitamin D_3 in chickens for fattening and fish. Not withstanding the long history of supplementing compound feed with vitamin D and the absence of publicly reported intolerances, the FEEDAP Panel is not in a position to draw final conclusions on the safety of vitamin D and considers the current maximum contents as temporarily acceptable. Any additional administration of vitamin D_3 via water for drinking represents a safety concern. Current nutritional surveys in 14 European countries showed that vitamin D intake is sufficiently below the upper safe limit. The FEEDAP Panel assumes that foodstuffs of animal origin were produced following current production practices, including vitamin D_3 supplementation of feed and concludes that the use of vitamin D in animal nutrition at the currently authorised maximum dietary content has not and will not cause the tolerable upper intake level to be exceeded. Vitamin D_3 is not an irritant to skin and eyes and is not a skin sensitiser. Inhaled vitamin D_3 is highly toxic; exposure to dust is harmful to persons handling the additive. No risk to the environment resulting from the use of vitamin D_3 in animal nutrition is expected. The vitamin D_3 under application is regarded as an effective dietary source of the vitamin in animal nutrition.

[1] On request from European Commission, Question No EFSA-Q-2011-00950, adopted on 13 November 2012.

[2] Panel members: Gabriele Aquilina, Alex Bach, Vasileios Bampidis, Maria De Lourdes Bastos, Gerhard Flachowsky, Josep Gasa-Gasó, Mikolaj Gralak, Christer Hogstrand, Lubomir Leng, Secundino López-Puente, Giovanna Martelli, Baltasar Mayo, Derek Renshaw, Guido Rychen, Maria Saarela, Kristen Sejrsen, Patrick Van Beelen, Robert John Wallace and Johannes Westendorf. One member of the Panel did not participate in the discussion on the subject referred to above because of potential conflicts of interest identified in accordance with the EFSA policy on declarations of interests. Correspondence: FEEDAP@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Fat-soluble Vitamins, including Georges Bories, Jürgen Gropp, Alberto Mantovani and the late Reinhard Kroker, for the preparatory work on this scientific opinion.

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KEY WORDS

Nutritional additive, vitamins and provitamins, vitamin D_3, cholecalciferol, safety.

SUMMARY

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 vitamin D_3 (cholecalciferol) as an additive to feed and water for drinking for chickens for fattening, turkeys, other poultry, pigs, piglets (suckling), calves for rearing, calves for fattening, bovines, ovines, equines, fish and other animal species and categories.

The principal physiological role of vitamin D in all vertebrates including humans is in calcium and phosphorus homeostasis. The classic clinical deficiency syndrome is rickets.

The FEEDAP Panel notes that for turkeys for fattening, equines, bovines, ovines and pigs the maximum authorised (and proposed) feed content for vitamin D_3 does not provide any margin of safety, and that, except for pigs, the current maximum authorised contents in feed for different species and categories are above the upper safe level, according to the National Research Council (NRC) data when animals were fed a supplemented diet for more than 60 days. On the other hand, no safety concern is identified for the use of vitamin D_3 in chickens for fattening and fish. Notwithstanding the long history of supplementing compound feed with vitamin D up to the maximum content and the absence of publicly reported intolerances, the FEEDAP Panel is not in a position to draw final conclusions on the safety of vitamin D in animal nutrition based on the NRC data collection, which has not been revised for 25 years.

The FEEDAP Panel considers the current maximum authorised contents to be temporarily acceptable for the target animals. In the view of the FEEDAP Panel, a complete review of the more recent literature is necessary to maintain or to revise the current maximum contents. Any administration of vitamin D_3 via water for drinking could exceed the safe amount of vitamin D_3 and therefore represents a safety concern.

Current nutritional surveys in 14 European countries have shown that vitamin D intake by consumers is sufficiently below the UL. The FEEDAP Panel assumes that foodstuffs of animal origin monitored in these studies were produced following current production practices, including vitamin D_3 supplementation of feed. It is concluded that the use of vitamin D in animal nutrition at the currently authorised maximum dietary content has not and will not cause the UL to be exceeded.

Vitamin D_3 is not an irritant to skin and eyes and not a skin sensitiser. For some formulations of vitamin D_3 there is a potential for workers to be exposed to high levels of vitamin D_3 by inhalation. Inhaled vitamin D_3 is highly toxic; exposure to dust is harmful to persons handling the additive.

Vitamin D is widely distributed in plants and animals, as a result of endogenous synthesis. It is susceptible to oxidation by light and air. No risk to the environment resulting from the use of vitamin D_3 in animal nutrition is expected.

The vitamin D_3 under application is regarded as an effective dietary source of the vitamin in animal nutrition.

The FEEDAP Panel made recommendations concerning (i) the specification of the product under application, (ii) labelling of the additive and (iii) the route of incorporation in complete and complementary feedingstuffs.

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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 time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company DSM nutritional Products Ltd[5] for (i) authorisation of a new use (i.e., use in water for drinking) and (ii) re-evaluation of vitamin D_3(cholecalciferol), when used as a feed additive for chickens for fattening, turkeys, other poultry, pigs, piglets (suckling), calves for rearing, calves for fattening, bovines, ovines, equines, fish and other animal species or categories (category: nutritional additive; functional group: vitamins, provitamins and chemically well-defined substances having similar effect) under the conditions mentioned in Table 1.

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 30 September 2011.

Vitamin D in the form of vitamin D_3 has been authorised without time limit under Council Directive 70/524/EEC[7] for its use in pigs, piglets, bovines, ovines, calves, equines, chickens for fattening, turkeys, other poultry, fish, other species or categories of animals as a nutritional additive.

The Scientific Committee on Food expressed an opinion on the tolerable upper intake level of vitamin D (EC, 2002). The Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) reviewed the UL of Vitamin D (EFSA, 2012a). The Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of Hy·D (calcifediol) based on 25-hydroxycholecalciferol/25-hydroxy-pre-cholecalciferol (EFSA, 2005) and another opinion on the safety and efficacy of 25-hydroxycholecalciferol as feed additive for poultry and pigs (EFSA, 2009a). The Panel on Food Additives and Nutrient Sources added to Food (ANS) issued an opinion on the inability to assess the safety of Vitamin D-enriched yeast added for nutritional purposes as a source of Vitamin D in food supplements and the bioavailability of Biotin from this source, based on the supporting dossier (EFSA, 2009b). The NDA Panel issued several opinions on the scientific substantiation of a health claim related to Vitamin D pursuant to Article 13(1) and Article 14 of Regulation (EC) No 1924/2006 (EFSA, 2008a,b; 2009c,d,e; 2010a,b and 2011a,b).

[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] DSM Nutritional Products Ltd, Switzerland, represented in the European Union by DSM Nutritional Products SP. z.o.o., Tarczynska 113, 96.320 Mszczonow, Poland.

[6] EFSA Dossier reference: FAD-2010-0146.

[7] 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.

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 safety for the target animals, consumer, user and the environment and the efficacy of vitamin D_3, when used under the conditions described in Table 1.

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

Additive	Vitamin D_3 - Cholecalciferol			
Registration number/EC No/No	E 671			
Category(ies)of additive	Nutritional additives			
Functional group(s) of additive	Vitamins, provitamins and chemically well defined substances having a similar effect			
Description				
Composition, description	Chemical formula	Purity criteria	Method of analysis	
Cholecalciferol + precholecalciferol	$C_{27}H_{44}O$	67.2 %	HPLC	
Trade name				
Name of the holder of authorisation				
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		mg/kg of complete feedingstuffs		
chickens for fattening			0.125	
turkeys			0.125	
other poultry			0.075	
pigs			0.050	
piglets (suckling)			0.250	
calves for rearing			0.250	
calves for fattening			0.250	
bovines			0.100	
ovines			0.100	
equines			0.100	
fish			0.075	
other animal species or categories			0.050	

Other provisions and additional requirements for the labelling			
Specific conditions or restrictions for use	For all animal species or categories; the simultaneous use of vitamin D_2 is prohibited. For use in feed and in water for drinking. For chickens for fattening and turkeys, the maximum content of the combination of vitamin D_3 - cholecalciferol with 25-hydroxycholecalciferol per kg of complete feedingstuff shall not exceed 0.125 mg. For other poultry, the maximum content of the combination of vitamin D_3-cholecalciferol with 25-hydroxcholecalciferol per kg of complete feedingstuff shall not exceed 0.080 mg. For pigs, the maximum content of the combination of vitamin D_3-cholecalciferol with 25-hydroxycholecalciferol per kg of complete feedingstuff shall not exceed 0.050 mg. For piglets (suckling), calves for rearing, calves for fattening the maximum concentration refers to milk replacer only.		
Specific conditions or restrictions for handling	For all animal species or categories, vitamin D_3 shall be used in the form of a preparation. The preparation of vitamin D_3 shall be incorporated in compound feedingstuffs via the use of a premixture.		
Post-market monitoring	-		
Specific conditions for use in complementary feedingstuffs	In complementary feed for all animal species or categories, the concentration of vitamin D_3-cholecalciferol per kg of complementary feed shall not exceed 5 mg.		
Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues

ASSESSMENT

This opinion is based in part on data provided by a single company involved in the production/distribution of vitamin D_3 (cholecalciferol). It should be recognised that these data cover a fraction of the existing additives containing vitamin D_3. The composition of additives is not the subject of the application. The FEEDAP Panel has sought to use the data provided, together with data from other sources, to deliver an opinion.

1. Introduction

The two major natural sources of vitamin D are cholecalciferol (vitamin D_3, which occurs in animals) and ergocalciferol (vitamin D_2, which occurs predominantly in plants). Vitamin D_3 is metabolised to the active steroid hormone 1,25-dihydroxyvitamin D_3 (25(OH)D_3) by successive hydroxylation in the liver and kidney. Vitamin D_2 is metabolised to 1,25-dihydroxyvitamin D_2 by the same enzyme systems. Vitamin D_3 is also produced by endogenous synthesis in mammalian species and birds but not in fish. 7-Dehydrocholesterol in the skin is converted by exposure to ultraviolet light and subsequently by a temperature-regulated reaction into vitamin D_3. Vitamin D_2 is formed by photochemical reaction from ergosterol in plants, fungi and lower life forms. Moreover, a few plants (e.g. Solanaceae, *Trisetum flavescens*) are known to produce vitamin D_3-related metabolites. Both forms of the vitamin can also be produced by chemical synthesis.

Cholecalciferol undergoes several hydroxylation steps in the organism, mainly in the liver and the kidneys, by which the hormonally active metabolites are formed. The principal physiological role of vitamin D in all vertebrates including humans is in calcium and phosphorus homeostasis. Vitamin D is a key regulator of transcellular calcium uptake. Furthermore, vitamin D modulates the expression of Na^+-dependent inorganic phosphate (P_i) transporters (i.e., in the intestine and the kidneys). Bone represents the largest store of calcium phosphate, and vitamin D directly affects osteoblast activity and osteoclast formation. The vitamin D-regulated metabolic pathways also play important roles in other biological processes not related to calcium and phosphorus homeostasis (i.e., in muscle function, autoimmune diseases and cardiovascular physiology). Vitamin D has been shown to affect cell proliferation and differentiation.

Vitamin D_3 deficiencies of are unlikely to occur in animals under present feeding practices. In young, growing animals vitamin D deficiency results in rickets, the classic vitamin D deficiency syndrome. A low serum level of 25(OH)D_3 represents an early biomarker of an inadequate vitamin D_3 supply.

Both vitamins, D_2 and D_3 are approximately equally effective in mammalian species, whereas in poultry D_2 is about 25 times less effective than D_3. One milligram of vitamin D_3 is equal to 40 000 international units (IU).

Vitamin D in the form of vitamin D_3 (E 671) 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 without a time limit in application of Article 9t (b) of Council Directive 70/524/EEC[8] concerning additives in feedingstuffs (2004/C 50/01) for its use in all animal species as a nutritional additive. A maximum limit of 2 000 IU/kg complete feed has been established for pigs for fattening, 4 000 IU/kg for bovines, ovines and equines, 5 000 IU/kg for chickens and turkeys for fattening, 3 000 IU/kg for other poultry and fish and 2 000 IU/kg for other animal species and categories. For piglets and calves, a maximum of 10 000 IU/kg milk replacer is set. The simultaneous use of vitamin D_2 and vitamin D_3 is prohibited.

The applicant asks for the re-evaluation of the use of vitamin D_3 (cholecalciferol) as a feed additive for chickens for fattening, turkeys, other poultry including laying hens, pigs, piglets (suckling), calves for

[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.

rearing, calves for fattening, bovines, ovines, equines, fish and other animal species or categories and for a new use of vitamin D_3 in water for drinking.

Vitamin D in the form of cholecalciferol and ergocalciferol is authorised for use in food (Regulation (EC) No 1925/2006,[9] amended by Regulation (EC) No 1170/2009)[10] and in food supplements (Directive 2002/46/EC, Annex II),[11] for addition for specific nutritional purposes in foods for particular nutritional uses (Regulation (EC) No 953/2009),[12] to processed cereal-based foods for infants and young children (Directive 2006/125/EC, Annex IV)[13] and to infant formulae and follow-on formulae when reconstituted as instructed by the manufacturer (Directive 2006/141/EC, Annex III).[14] Vitamin D is also listed as a pharmacologically active substance in veterinary medicinal products (Commission Regulation (EU) No 37/2010)[15] and is not subject to maximum residue levels when used in medicinal products for food-producing animals.

Cholecalciferol is described in the European Pharmacopeia (PhEur), Monograph (MG) 0072. Cholecalciferol concentrated, oily form, powder form and water-dispersible form are described in MGs 0575, MG 0574 and MG 0598, respectively.

2. Characterisation

Vitamin D_3 is synthesised by a photochemical electrocyclic ring-opening reaction of 7-dehydrocholesterol to form pre-vitamin D_3, which through a thermochemical [1,7] sigmatropic rearrangement reaction is converted to vitamin D_3. At the same time distillation of the remaining solvent takes place. This technical vitamin D_3 (crude vitamin D_3) is not isolated (vitamin D_3 + pre-vitamin D_3 at least 67.2 %) but is immediately diluted in plant oil (such as maize, peanut or soybean oil) to a standardised concentration of about 10 000 000 IU/g of vitamin D_3 (23.75–26.25 % vitamin D_3 + pre-vitamin D_3). The oily solution is used as such and for further formulation of powder forms.

2.1. Characterisation of the active substance

Vitamin D_3 (IUPAC name: (3β,5Z,7E)-9,10-secocholesta-5,7,10(19)-trien-3-ol; synonyms: 9,10-secocholesta-5,7,10(19)-trien-3-ol, cholecalciferol, calciol) has the Chemical Abstracts Service (CAS) number 67-97-0 and the European Inventory of Existing Chemical Substances (EINECS) number 200-673-2. The structural formula of cholecalciferol is shown in Figure 1.

[9] 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.
[10] 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.
[11] 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.
[12] Commission Regulation (EC) No 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.
[13] 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.
[14] 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.
[15] 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.

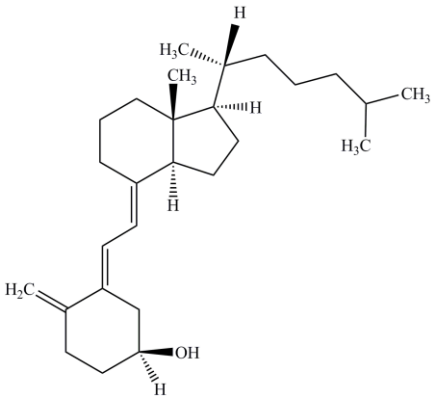

Figure 1: Structural formula of cholecalciferol

The molecular formula of cholecalciferol is $C_{27}H_{44}O$ and its molecular weight is 384.64. Vitamin D_3 is insoluble in water (< 1 mg/L at 20 °C) but is soluble in ethanol, acetone, ether and vegetable oils.

Cholecalciferol is described in the European Pharmacopoeia (PhEur 7.0, 0072) with a purity of 97.0–102.0 %. Related impurities, i.e., (*5E,7E*)-9,10-secocholesta-5,7-10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3, impurity A), cholesta-5-7-dien-3β-ol (7,8-dehydrocholesterol, provitamin D_3, impurity B), 9β,10α-ergosta-5,7-dien-3β-ol (lumisterol$_3$, impurity C), (*6E*)-9,10-secocholesta 5(10),6,8(14)-trien-3β-ol (isotachysterol$_3$, impurity D) and (*6E*)-9,10-secocholesta-5(10),6,8,-trien-3β-ol (tachysterol$_3$, impurity E), are described and limited by PhEur (total related impurities < 1 %, impurity A < 0.1 %).

Analytical data on the crude vitamin D_3 were provided for five batches. The fraction of vitamin D_3 and related compounds consisted of 75.1 ± 0.7 % vitamin D_3, 10.4 ± 0.4 % pre-vitamin D_3, 3.3 ± 0.5 % pro-vitamin D_3, 6.3 ± 0.4 % tachysterol, 1.0 % lumisterol, < 0.2 % for both trans-vitamin D_3 and isotachysterol and 3.4 ± 0.3 % other impurities. Solvents (methanol) measured by loss on drying were shown to reach a maximum of 5.7 %.[16]

The crude vitamin D_3 as described above does not comply with the PhEur. Deviation from the PhEur specifications is essentially a result of the presence of considerable amounts of precursors in the biosynthetic pathway leading to cholecalciferol (pro- and pre-vitamin D_3), both of which exert vitamin D activity in the body, and to a lesser extent to the higher quantity and different proportions of accompanying sterols (higher content of tachysterol, a metabolite in the biosynthetic pathway of vitamin D_3). Consequently, the FEEDAP Panel does not see any safety concern arising from the derived difference in composition between the product under application and another complying with PhEur.

2.2. Characterisation of the additive (formulated products)

The active substance vitamin D_3 is marketed as diluted and standardised preparations of liquid (oil based) or solid forms.

A liquid preparation (the crude vitamin D_3 diluted in plant oil) with a standard concentration of 10 000 000 IU vitamin D_3/g is used for the production of solid feed additives (the applicant has reported that it is also sold to clients as a chemical). This form of vitamin D_3 is prepared directly after the synthesis of the vitamin, by dilution with an edible oil carrier. The additive contains 23.75–26.25 % of vitamin D_3 + pre-vitamin D_3, a maximum of 10.6 % of related substances and a maximum of 76.25 % of carrier. Analysis of five production batches resulted in an average of 21.7 ± 0.2 % vitamin D_3 and 3.0 ± 0.1 % pre-vitamin D_3.[17] Three batches of the additive were analysed for heavy

[16] Technical dossier/Supplementary Information June 2012.
[17] Technical dossier/Section II/Annex 2.39 and Supplementary Information June 2012.

metals and arsenic and organic solvents. Arsenic was below 2 mg/kg, lead < 10 mg/kg, mercury < 0.1 mg/kg and cadmium < 1 mg/kg. Methanol, determined as loss on drying, is specified to be below 2 %. Analytical values were in the range of 0.6–0.9 %.[18] These values are above the VICH (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products) threshold of 0.3 % (EMA, 2010).

A solid preparation containing 500 000 IU vitamin D_3/g (12.5 mg/g) is produced by spray drying of the oily formulation with carriers (maltodextrin, plant protein, starch). Antioxidants are added to the oily formulation before spray drying resulting in 12.5 mg (ascorbic acid, alpha-tocopherol, ethoxyquin) per gram of solid product. The emulsion is spray dried in the presence of an anticaking agent (5 mg/g, colloidal silica). Analysis of five production batches showed an average of 13.0 ± 0.2 mg vitamin D_3 + pre-vitamin D_3/g. Methanol content was below 0.1 %.[19] Particle size examination (three batches) showed that the median particle diameter was between 50 and 150 µm. The proportion of particles with diameters of less than 10, 50 and 100 µm was 4 %, 22 %, and 47 % (v/v), respectively. Dusting potential, measured by the Stauber–Heubach method, was in the range of 6.2–8.0 g/m^3.[20]

Another spray-dried formulation contains 200 000 IU vitamin D_3/g (5 mg/g) and 1 000 000 IU vitamin A/g (357 mg retinyl acetate). Fructose and maltodextrin are used as carriers, and about 100 mg antioxidants (tocopherol, ethoxyquin, ascorbic acid) per gram and anticaking agents (colloidal silica, calcium silicate) are added. Analysis of five batches showed an average of 5.4 ± 0.1 mg vitamin D_3 + pre-vitamin D_3/g and 353 ± 2 mg retinyl acetate/g.[21] Methanol content was below 0.04 %.[16] No particles below 100 µm were found in five batches. The dusting potential was not determined.[22]

2.3. Stability and homogeneity

2.3.1. Shelf-life of the additives

The standardised liquid preparation (three batches, stored in sealed aluminium bags in the absence of light) was shown to have a shelf-life of nine and six months when kept at 25 °C and 40 °C, respectively.[23]

The shelf-life of the solid additive (500 000 IU vitamin D_3/g) was demonstrated to be 36 months at 5 °C, 15 °C and room temperature (one batch each) and 12 months at 15 °C for another two batches when stored in sealed aluminium bags in the absence of light. Storage at 35 °C resulted in losses of about 6 % after 12 and 24 months and of 15 % after 36 months.[24]

Shelf-life of another solid additive (200 000 IU vitamin D_3/g) was shown to be 15 months in three batches when kept at 15 °C or 25 °C in sealed aluminium bags in the absence of light.[25]

2.3.2. Stability of the additive when added to premixtures and compound feed

Three batches of the additive containing 500 000 IU vitamin D_3/g were incorporated in a premixture containing trace elements and choline chloride to provide 100 000 IU vitamin D_3/kg. The premixtures were kept in plastic bags at 25 °C up to six months. A reduction in the initial content was seen after three and six months of 10 % and 14 %, respectively.[26]

Three batches of the additive containing 500 000 IU vitamin D_3/g were incorporated in a piglet mash feed (to provide 9 000 IU vitamin D_3/kg). After three months' storage at 25 °C, between 70 % and

[18] Technical dossier/Section II/Annex 2.51.
[19] Technical dossier/Section II/Annex 2.40.
[20] Technical dossier/Section II/Annex 2.10.
[21] Technical dossier/Section II/Annex 2.41.
[22] Technical dossier/Section II/Annex 2.11.
[23] Technical dossier/Section II/Annexes 2.53 and 2.54.
[24] Technical dossier/Section II/Annexes 2.19 and 2.20.
[25] Technical dossier/Section II/Annex 2.21.
[26] Technical dossier/Section II/Annex 2.44.

80 % of the initial content was recovered in the mash feed. The same mash feed was pelleted and the pelleted feed stored under the same conditions. Pelleting did not affect stability, and losses during storage of pelleted feed were in the same range as for the mash feed.[27]

2.3.3. Homogeneity in feed

The capacity of the additive to homogeneously distribute in complete feedingstuffs was experimentally demonstrated for three batches on the basis of six samples per pelleted feed. The coefficient of variation (CV) for the three samples varies between 2.6 % and 4.0 %.

2.3.4. Stability and homogeneity in water for drinking

Stability of the additive (two batches) in water was examined after stepwise dilution of a solid additive (500 000 IU vitamin D_3/g) to a final concentration of 9 000 IU/L at 25 °C. No relevant loss of vitamin D_3 in water for drinking was observed for 48 hours. The homogeneous distribution of the active substance in water for drinking is supported by a CV of 3.5 % for two batches with six determinations each.[28]

2.4. Physicochemical incompatibilities in feed

Besides reduced stability in premixtures in the presence of choline chloride and trace minerals, no other physicochemical incompatibilities have been reported between vitamin D_3 and feed materials, carriers, other approved additives or medicinal products when the additive was added to premixtures and feed. No such incompatibilities are expected.

2.5. Conditions of use

Vitamin D_3 is intended for use in chickens for fattening, turkeys, other poultry, pigs, piglets (suckling), calves for rearing, calves for fattening, bovines, ovines, equines, fish and other animal species or categories. The applicant proposes to maintain the maximum contents already established by the 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)[29] for the different animal species and categories: 0.500 mg/kg (corresponding to 2 000 IU/kg) complete feed for pigs for fattening, 0.100 mg/kg (4 000 IU/kg) for bovines, ovines and equines, 0.125 mg/kg (5 000 IU/kg) for chickens and turkeys for fattening, 0.075 mg/kg (3 000 IU/kg) for other poultry and fish, 0.050 mg/kg (2 000 IU/kg) for other animal species and categories. The simultaneous use of vitamin D_2 and vitamin D_3 is prohibited. The applicant recommends incorporation of the additive through a premixture.

The applicant also proposed to label the vitamin D_3 supplementation of feedingstuffs in mg.

The applicant derived the maximum vitamin D_3 concentration for use in water for drinking from the maximum feed concentration and the amount of the daily ration, also taking into account the ratios of feed to water intake. The following maximum contents (mg/L) are proposed by the applicant: 0.017 for pigs, 0.083 for sucking piglets; 0.03 for other poultry including laying hens, 0.05 for chickens for fattening and turkeys and 0.025 for other animal species and categories. The recommendations for cattle (1 mg/L), dairy cows (2 mg/L), ovines (0.15 mg/L) and equines (0.8 mg/L) are calculated assuming that one litre of water for drinking provides the total daily supply.

2.6. 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 D_3 in animal feed. The executive summary of the EURL report can be found in Appendix A.

[27] Technical dossier/Section II/Annex 2.38.
[28] Technical dossier/Section II/Annex 2.22.
[29] OJ C 50, 25.2.2004, p. 1.

3. Safety

3.1. Safety for the target species

Requirements for vitamin D (NRC, see McDowell, 2000) are in the range of 200–1 200 IU/kg feed for poultry, 150–220 IU/kg for pigs, 250–2 400 IU/kg for fish, 275–1 400 IU/kg for ruminants, 300–600 IU/kg for horses, 500 IU/kg for cats and 22 IU/kg body weight for dogs.

Vitamin supplementation of commercial compound feed is mostly oriented towards recommended allowances in the range of 1 000–2 000 IU/kg for pigs, 1 500–5 000 IU/kg for poultry, 1 500–2 000 IU/kg for fish and 800–1 200 IU/kg feed for pets (AWT, 2002). A survey on vitamin supplementation of commercial feeds for pigs and poultry in Europe (Belgium, Denmark, Germany, Italy, Netherlands, Portugal, Spain and United Kingdom) identified a range of 115–20 000 IU vitamin D_3/kg as the level of commercial use (Gropp, 1994). A range for piglets and pigs of 1 000–2 000 IU/kg feed was reported by Whittemore et al. (2002).

Tolerance to high dietary levels of vitamin D_2 and D_3 is generally lower when administered over a long period of time, and vitamin D_3 is more toxic than vitamin D_2. However, most animal species appear to tolerate about 10 times their dietary requirements for a period of more than 60 days; up to 100 times the requirements may be tolerated for short periods of less than 60 days (NRC, 1987), as shown in Table 2.

Table 2 also shows the comparison between the estimated safe upper dietary levels of vitamin D_3 and the currently maximum authorised content for vitamin D_3 for the different animal species and categories.

Table 2: Estimation of safe upper dietary levels of vitamin D_3 (IU/kg feed) for animals according to NRC (1987) and comparison with the current maximum authorised content for different animal species

	Dietary requirements (NRC, 1987)	**Safe upper dietary level (NRC 1987)**		**Maximum authorised content[a]**
		Exposure time		
Species		**< 60 days**	**>60 days**	
Birds				
Chicken	200	40 000	2 800	5 000
Japanese quail	1 200	120 000	4 700	3 000
Turkey	900	90 000	3 500	5 000
Fish				
Catfish	1 000		20 000	3 000
Rainbow trout	1 800	–	1 000 000	3 000
Cow	300	25 000	2 200	4 000
Horse	400		2 200	4 000
Sheep	275	25 000	2 200	4 000
Pigs	220	33 000	2 200	2 000

(a) 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).[29]

3.1.1. Conclusions on target animal safety

The FEEDAP Panel notes that for turkeys for fattening, equines, bovines, ovines and pigs the current maximum authorised (and proposed) feed contents for vitamin D_3 do not provide any margin of safety, and that, except for pigs, the maximum contents are above the respective upper safe levels, according to NRC data, when fed a supplemented diet for more than 60 days. On the other hand, no safety concern was identified for the use of vitamin D_3 in chickens for fattening and fish.

Not withstanding the long history of supplementing compound feed with vitamin D up to the maximum content and the absence of publicly reported intolerances, the FEEDAP Panel is not in a position to draw final conclusions on the safety of vitamin D in animal nutrition, based on a data collection that has not been revised for 25 years. The FEEDAP Panel considers, therefore, the current maximum contents to be temporarily acceptable for the target animals. In the view of the FEEDAP Panel, a complete revision of the more recent literature is necessary to maintain or to revise the current maximum contents.

Any administration of vitamin D_3 via water for drinking could exceed the safe amounts of vitamin D_3 and therefore represents a safety concern.

3.2. Safety for the consumer

3.2.1. Metabolic and residue studies

Vitamin D_3 is a fat-soluble vitamin. Its metabolites have hormonal activity in the organism, which is less than that of $1,25(OH)_2D_3$. Consequently, the uptake and biological activity is subject to homeostatic control. Once absorbed from the lumen of the small intestine vitamin D_3 is transported to the liver where the hydroxylation to 25-hydroxyvitamin D_3 ($25(OH)D_3$) takes place. The plasma $25(OH)D_3$ reflects the long-term dietary supply of vitamin D_3 and/or endogenous formation from its precursors. In the kidneys $25(OH)D_3$ is further hydroxylated to the most active metabolite 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$) or to 24,25-dihydroxy-D_3, which is essentially inactive. Further hydroxylation take place in the liver and generates trihydroxy metabolites.

Based on a range of measurements, vitamin D_3 levels in tissues and products of animal origin have been reported under various dietary vitamin D concentrations to be 30–43 µg/kg in egg yolk (corresponding to about 8.1–11.6 µg/kg egg) (Mattila et al., 1999a,b), 7.47 ± 1.39 µg/kg in pig fat, 2.99 ± 0.88 µg/kg in pig skin, 2.67 ± 0.45 µg/kg in pig liver, 1.11 ± 0.31 µg/kg in pig meat (Jakobsen et al., 2007) and 1.3–2.1 µg/kg in milk (Norman, 2000).

Considerably higher levels are reported for fish flesh: 72–153 µg/kg for trout, 225–319 µg/kg for herring, 59–247 µg/kg for perch, 26–68 µg/kg for vendace (Mattila et al., 1999a) and 40–160 µg/kg for salmon.[30]

3.2.2. Assessment of consumer safety

The Panel on Dietetic Products, Nutrition and Allergies (NDA) recently revised the tolerable upper intake levels (ULs) of vitamin D for all relevant population groups based on a no observed adverse effect level (NOAEL) of 250 µg/day for hypercalcaemia in men (EFSA, 2012a). The UL for adults, including pregnant and lactating women, and adolescents was set at 100 µg/day. For children aged 1–10 years, a UL of 50–µg/day was proposed and for infants 25 µg/day.

According to the NDA opinion (EFSA, 2012a), data on vitamin D intakes from surveys in 14 European countries indicate that intakes in consumers of large amounts are below the revised ULs for vitamin D for all population subgroups (i.e., about 25 % of the UL for adults, 75 % for infants, 30 % for children and 8 % for adolescents).

Vitamin D_3 is present only in food of animal origin. Vitamin D_3 intake from food of animal origin was estimated by applying the model described for potential consumer exposure in food based on default values for chronic consumption (EFSA, 2012b). These values are derived from the EFSA

[30] http://www.nifes.no/index.php?page_id=168

Comprehensive European Food Consumption Database and represent the high-intake (95th percentile) of consumers only for each food item.[31]

Following the EFSA guidance for establishing the safety of additives for the consumer (EFSA, 2012b), the highest intake of vitamin D_3 by adults would result from the consumption of fish (salmon) and milk amounting to 23 % of the UL. All other food groups (liver, fat, meat and eggs) would each contribute less than 1 % of the UL for the 95th percentile of European consumers. For toddlers aged 1–3 years, the consumption of milk and fish would lead to 27 % of the UL for children (1–10 years) set by the NDA Panel. The details can be found in Appendix B. This estimate is in agreement with the overall estimate of the EFSA NDA Panel, based on surveys showing that adults consume about 25 % of the UL of vitamin D.

3.2.3. Conclusions on consumer safety

Since the NDA Panel's review is based on surveys of foodstuffs produced following current production practices, there is reason to assume that they contain vitamin D from supplemented feed and/or body synthesis. As there has not been a change in the supplementation of feed in recent decades, it is concluded that the continuous use of vitamin D in animal nutrition does not mean that the UL set by the EFSA NDA Panel will be exceeded.

3.3. Safety for the user

3.3.1. Effects on eyes and skin

A primary skin irritation test was performed using the standardised oily preparation in three New Zealand White rabbits. A single application of the compound was applied to intact and abraded skin according to Organization for Economic Cooperation and Development (OECD) Guideline 404. Treatment caused some reddening and oedema but the response was insufficient to trigger requirement of test material to be labelled as skin irritant.[32]

An acute eye irritation study using the standardised oily preparation in three New Zealand White rabbits was performed in compliance with OECD Guideline 405. Conjunctival redness with no alterations to the cornea was observed only at one hour post administration. Symptoms were not observed after 24 hours. Thus, the test substance was not regarded as an eye irritant.[33]

A Maurer optimisation test was performed to determine the sensitising potential using the standardised oily preparation in groups of 10 Himalayan White Spotted female guinea pigs. Challenge concentrations were not irritating in the negative control animals. The positive control group responded as expected. The results showed that the test material had no sensitising potential.[34]

3.3.2. Effects on the respiratory system

A solid preparation including 500 000 IU vitamin D_3/g (12.5 mg/g) showed a moderately high dusting potential. About 4 % of particles by volume were of respirable size (< 10 µm).

An exposure assessment for persons handling the additive in a premixtures factory following the principle laid down in the EFSA guidance for user safety (EFSA, 2012c) was performed. The details can be found in Appendix C. The calculation identified that a potential amount of 5.6 mg vitamin D_3/worker could be inhaled during an eight-hour working day. How much vitamin D_3 would really reach the alveolar surface could not be calculated owing to the absence of data on the particle size distribution of the dust.

[31] European Food Safety Authority; Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097 [34 pp.]. doi: 10.2903/j.efsa.2011.2097. Available online: www.efsa.europa.eu/efsajournal.htm

[32] Technical dossier/Section III/Annex 3.17.

[33] Technical dossier/Section III/Annex 3.16.

[34] Technical dossier/Section III/Annex 3.18.

A good laboratory practice-compliant acute inhalation toxicity study was performed in Wistar rats.[35] Groups of five males and five females were exposed for four hours to a dust of vitamin D_3 (98.6 % pure, mixed with an anti-caking agent, 1 % amorphous silica) at nominal air concentrations of 0 (amorphous silica), 1 150, 1 700, 2 200 and 4 600 mg/m^3. The concentrations of vitamin D_3 by analysis were much lower than the intended values, but there was inconsistency within the report in the values. The most commonly reported values were 0 (control), 134, 155, 203 and 377 mg/m^3. The dusts generated all had a mass median aerodynamic diameter of approximately 0.9 µm. Exposure was followed by a recovery period of 35 days, at the end of which survivors were killed and necropsied. The lungs and trachea were weighed and the lungs were examined microscopically. Mortality was zero in controls, but high in all treated groups, with a dose–response relationship in females (but not in males). An LC_{50} of between 130 and 380 mg/m^3 (extremely toxic) was estimated. No clinical signs were observed during treatment, and respiratory frequencies were unaffected. Mean body weight of the treated groups was lower than that of the controls, with no clear dose relationship, for three weeks following treatment. Increased lung weights were found in females at all dose levels but not in males. Post-mortem investigation showed changes in both sexes at all dose levels: pale kidneys with a rough surface, white areas on the myocardium and stomach and red spots on the lungs. Histology showed calcification of the alveolar walls of the lungs of some animals of both sexes at all doses tested. Foci of lymphocytic infiltration were found in the lungs of animals from all groups including controls and were not considered to be treatment related. Organs other than the lungs were not examined for histopathology. The high mortality at low levels of inhalation exposure to vitamin D_3 indicates that exposure to dust can be very hazardous. The level of exposure at which there were no adverse effects was not identified.

3.3.3. Conclusions on user safety

Vitamin D_3 is not an irritant to skin and eyes and not a skin sensitiser. For some formulations of vitamin D_3 there is a potential for workers to be exposed to high levels of vitamin D by inhalation. Inhaled vitamin D_3 is highly toxic; exposure to dust is harmful.

3.4. Safety for the environment

Both vitamin D forms, ergocalciferol and cholecalciferol, are widely distributed in plants and animals, respectively, as products of endogenous synthesis. Both forms are susceptible to oxidation by light and air. The use of vitamin D_3 in animal nutrition is not expected to substantially increase the concentration in the environment. Therefore, a risk to the environment arising from the use of cholecalciferol in animal nutrition is not expected.

4. Efficacy

According to Regulation (EC) No 429/2008, efficacy studies are not required for vitamins, pro-vitamins and chemically defined substances having similar effects that are already authorised as feed additives under Directive 70/524/EEC.

Owing to the long history of use and its established nutritional role in domestic animals, cholecalciferol is regarded as an effective source of vitamin D.

There is an interaction among vitamins D, A and E, i.e., at marginal dietary levels of vitamin D_3 (500 IU/kg) high or excessive concentrations of vitamin A and/or E may reduce the biological activity of vitamin D, thus potentially leading to reduced bone ash and increased risk for rickets (Aburto and Britton 1998a,b; Aburto et al., 1998).

Vitamin D has been used in animal nutrition globally for decades. Data on requirements, allowances and recommendations for feed supplementation are easily accessible through the standard literature for animal nutrition experts.

[35] Technical dossier/Supplementary information June 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[36] and good manufacturing practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The FEEDAP Panel notes that for turkeys for fattening, equines, bovines, ovines and pigs the current maximum authorised contents of vitamin D_3 in feeds do not provide any margin of safety, and that, except for pigs, the maximum contents are above the upper safe levels for the different species and categories, according to NRC data, when fed a supplemented diet for more than 60 days. On the other hand, no safety concern is identified for the use of vitamin D_3 in chickens for fattening and fish. Notwithstanding the long history of supplementing compound feed with vitamin D up to the maximum content and the absence of publicly reported intolerances, the FEEDAP Panel is not in a position to draw final conclusions on the safety of vitamin D in animal nutrition based on the NRC data collection, which has not been revised for 25 years.

The FEEDAP Panel considers the current maximum authorised contents to be temporarily acceptable for the target animals. Any administration of vitamin D_3 via water for drinking could exceed the safe amounts of vitamin D and therefore represents a safety concern.

Current nutritional surveys in 14 European countries showed that vitamin D intake by consumers is sufficiently below the UL. The FEEDAP Panel assumes that foodstuffs of animal origin monitored in these studies were produced following current production practices, including vitamin D_3 supplementation of feed. It is concluded that the use of vitamin D in animal nutrition at the currently authorised maximum dietary content has not and will not cause the UL to be exceeded.

Vitamin D_3 is not an irritant to skin and eyes and is not a skin sensitiser. For some formulations of vitamin D_3 there is a potential for workers to be exposed to high levels of vitamin D_3 by inhalation. Inhaled vitamin D_3 is highly toxic; exposure to dust is harmful to persons handling the additive.

Vitamin D is widely distributed in plants and animals as a result of endogenous synthesis. It is susceptible to oxidation by light and air. The use of vitamin D_3 in animal nutrition is not expected to substantially increase the concentration in the environment. Therefore, no risk to the environment resulting from the use of cholecalciferol in animal nutrition is expected.

The vitamin D_3 under application is regarded as an effective dietary source of the vitamin in animal nutrition.

RECOMMENDATIONS

As vitamin D_3 (cholecalciferol) is highly susceptible to oxidation, it is marketed for animal nutrition in stabilised forms. Any entry of vitamin D_3 (cholecalciferol) should appear as "Vitamin D_3, stabilised".

The FEEDAP Panel proposes the following specifications for the vitamin D_3 under application: purity minimum 75 % vitamin D_3, minimum 10 % pre-vitamin D_3, maximum 7 % tachysterol.

The FEEDAP Panel supports the applicant's proposal to label the vitamin D_3 supplementation of feedingstuffs in mg, in order to harmonise labelling of all authorised vitamin D active substances (cholecalciferol and 25-hydroxycholecalciferol).

The additive shall be incorporated into compound feedingstuffs via premixtures.

[36] OJ L 35, 8.2.2005, p. 1.

GENERAL REMARKS

The FEEDAP Panel notes that: (i) in most cases (turkeys for fattening, equines, bovines, ovines and pigs) the maximum authorised contents for vitamin D_3 do not provide any margin of safety; and (ii) except for pigs the maximum contents are above the respective upper safe levels, according to the NRC data, when fed a supplemented diet for more than 60 days. The FEEDAP Panel is not in a position to draw final conclusions on the safety of vitamin D in animal nutrition based on the NRC data collection, which has not been revised for 25 years. In the view of the FEEDAP Panel, a complete review of the more recent literature is necessary to maintain or revise the current maximum contents.

DOCUMENTATION PROVIDED TO EFSA

1. Vitamin D_3 in the form of cholecalciferol as a feed additive for chickens for fattening, turkeys, other poultry, pigs, piglets (suckling), calves for rearing, calves for fattening, bovines, ovines, equines, fish and other animal species or categories. November 2010. Submitted by DSM Nutritional Products Ltd.

2. Vitamin D_3 in the form of cholecalciferol as a feed additive for chickens for fattening, turkeys, other poultry, pigs, piglets (suckling), calves for rearing, calves for fattening, bovines, ovines, equines, fish and other animal species or categories. Supplementary information. June 2012. Submitted by DSM Nutritional Products Ltd.

3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for cholecalciferol.

4. Comments from Member States received through the ScienceNet..

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APPENDICES

APPENDIX A

Executive summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for vitamin D_3[37]

In the current grouped application, authorisation is sought under Article 4(1)[38,3] and 10(2)[1,2,3] for *vitamin D_3—cholecalciferol*, under the category/functional group 3(a) 'nutritional additives'/'vitamins, pro-vitamins and chemically well defined substances having similar effect', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *vitamin D_3* for all animal species and categories, as requested in FAD-2010-0165.

Vitamin D_3 is produced by chemical synthesis with a minimal purity of 65 % and is placed on the market as oily or powder preparations. *Vitamin D_3* is intended to be incorporated in *feedingstuffs* through *premixtures* or directly in *water* at levels ranging from 0.05 to 0.250 mg/kg (or 2–10 kIU/g), thus complying with Directive 70/524/EEC.

All Applicants (FAD-2010-0146, -0156, -0165) submitted a set of European Pharmacopoeia methods for the determination of *vitamin D_3* in the *feed additives* (EurPh 6.0 01/2008:0072, 0575, 0574, 0598), where *identification* is based on thin-layer chromatography and ultraviolet and visible absorption spectrophotometry; while *quantification* is based on liquid chromatography coupled to spectrophotometry at 254 nm. Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia methods based on liquid chromatography coupled to spectrophotometry for the determination of *vitamin D_3* in the feed additive.

For the determination of *vitamin D3* in premixtures and feedingstuffs, Applicant (FAD-2010-0165) submitted the ring-trial validated method by the Association of German Agricultural Analytical Research Institutes (VDLUFA), based on high-performance liquid chromatography coupled to an ultraviolet detector (HPLC-UV). The following performance characteristics were reported by VDLUFA, for *Vitamin D_3* concentrations ranging from 0.06 to 410 mg/kg (or from 2.23 to 16 400 kIU/kg):

- a relative standard deviation for *repeatability* (RSD_r) ranging from 2.2 to 8.2%;
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 5.8 to 14%; and
- a limit of quantification (LOQ) of 0.025 mg/kg *feedingstuffs* (or 1000 IU/kg).

Applicant (FAD-2010-0146) suggested for the determination of *vitamin D_3* in *feedingstuffs* and *water* to apply the CEN ring-trial validated method intended for foodstuffs (EN 12821), claiming that this method tested for low-fat milk samples should apply to a simpler water matrix without any major interfering substances. The CEN method was ring trial validated using a variety of food commodities (i.e. low-lactose ultra heat-treated milk, liquid infant formulas and margarine) and two certified reference materials (CRM 122 and CRM 421) to cover a *vitamin D_3* concentration range from 4 to 140 μg/kg (or 0.16 to 5.6 kIU/kg). The following performance characteristics were reported (when excluding cooking oil and fish oil data):

- RSD_r ranging from 2.4 to 6.7 %;
- RSD_R ranging from 5.5 to 12 %; and
- a limit of quantification of 0.025 mg/kg (or 1000 IU/kg).

[37] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-Vit-D3-Group.pdf

[38] FAD-2010-0146; [2] FAD-2010-0156; [3] FAD-2010-0165

Based on the experimental evidence and performance characteristics presented, the EURL recommends for official control the ring-trial validated VDLUFA method (VDLUFA 1997, Methodenbuch, Method 13.8.1) for the determination of *vitamin D3* in *premixtures* and *feedingstuffs*; together with the ring-trial validated EN 12821 method based on reverse-phase high-performance liquid chromatography coupled with ultraviolet detection (RP-HPLC-UV) for the determination of *vitamin D_3* in *feedingstuffs* and *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.

APPENDIX B

Exposure of consumers to vitamin D_3 resulting from the consumption of food of animal origin

The calculation of the exposure of consumers to vitamin D_3 resulting from the consumption of food of animal origin is based on:

1. The concentrations in relevant tissues/products were considered as described in Section 3.2.1: pig meat (1.11 ± 0.31 µg/kg), pig liver (2.67 ± 0.45 µg/kg) and pig fat (7.47 ± 1.39 µg/kg) according to Jakobsen et al., 2007; fish flesh (40–160 µg/kg),[39] eggs (30–43 µg/kg in egg yolk, corresponding to about 8.1–11.6 µg/kg egg) according to Mattila et al., 1999a,b; and milk (1.3–2.1 µg/kg, according to Norman, 2000).

2. For calculating the intakes by relevant tissues/products, the concentrations were taken as the arithmetic mean ± 2 standard deviations or the highest single value in the case of fewer than six animals.

3. For risk assessment, the intake of toddlers and adults should be calculated for all food items listed in the table—the sum of the two highest values is then taken as total intake.

4. Default values for daily food consumption are derived from the EFSA Comprehensive European Food Consumption Database and represent the high-intake (95th percentile) consumers only for relevant food items listed in the table for chronic intake by toddlers and adults.[40]

Table 1: Consumer daily exposure to vitamin D_3 resulting from the consumption of food of animal origin

	Concentration in food (µg/kg)	Toddlers[(a)]		Adults[(b)]	
		Chronic intake[(c)] (g)	Exposure (µg)	Chronic intake[(c)] (g)	Exposure (µg)
Meat[(d)]	1.72	90	0.15	290	0.50
Liver	3.57	–	–	60	0.21
Fat	10.25	-	–	30	0.31
Milk[(e)]	2.1	1050	**2.21**	1500	**3.15**
Eggs	11.6	35	0.41	70	0.81
Fish (salmon)	160	65	**10.40**	125	**20.0**

(a) Toddlers: 1–3 years of age, 12 kg body weight.
(b) Adults: 18–65 years of age, 60 kg body weight.
(c) Chronic intake is the 95th percentile of the distribution of average individual consumption levels (over the survey period) for consumers only from all available EU national surveys.
(d) Meat including processed meat products.
(e) Milk including dairy products.

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APPENDIX C

POTENTIAL EXPOSURE OF USERS HANDLING VITAMIN D_3

There are different operations in a premixture factory during which the worker could be exposed to dust:

- taking the additive from its bag for weighing in the dispensary
- emptying bags of previously weighed material in the hopper or mixers
- packing the final premixture.

Default values/positions:

- a factory with a large throughput can prepare 40 premixture batches per day (eight hours per shift)
- the maximum time for weighing/emptying is 20 seconds
- total breathed air per worker of 10 m^3 per eight hours = 1.25 m^3/hour
- the percentage of premixtures that contain the additive: 100 %
- dusting potential measured: worst case 8.01 g/m^3
- concentration of the active substance in dust: 1.27 %.

ESTIMATE OF RISK MITIGATION

- Estimated reduction (%) in exposure due to the use of personal protection equipment (coverall, goggles, gloves and masks of the type P2 or P3).

CALCULATION OF EXPOSURE BY INHALATION DURING A WORKING DAY

Batches with potential exposure	40 (batches) × 1 (fraction of batches containing additive) = 40 (batches)
Time of exposure	40 × 20 seconds = 800 seconds An uncertainty factor of 2 should be introduced
Inhaled air during exposure (Ia), m^3	1.25 m^3 per hour × 2 × 800/60/60 in hours = 0.55
Active substance in air (Asa), g/m^3	8.0 (dust in g/m^3) × 0.0127 (1.27 % active substance in dust) = 0.102
Active substance inhaled (Asi), mg/day	0.102 (Asa) × 0.55 (Ia) × 1 000 = 55.9
Reduced by filter mask (Asir), mg/day	55.9 (Asi) × 0.1 (by mask type P2) = 5.6 mg (21 200 IU)

The aerosol fraction relevant for occupational health, related to the whole transported aerosol, cannot be further refined as data on the particle fractions in the dust are not available.