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SCIENTIFIC OPINION

Scientific Opinion on safety and efficacy of vitamin B_{12} (cyanocobalamin) produced by *Ensifer adhaerens* when used as a feed additive for all animal species[1]

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

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 03 July 2018, replaces the earlier versions published on 22 May 2015 and on 09 March 2016[4]

ABSTRACT

The product under consideration is vitamin B_{12} (cyanocobalamin) produced by *Ensifer adhaerens*. There are uncertainties regarding the genetic basis of the antibiotic resistance found in the production strain and regarding the possible presence of its DNA in the product. However, the product under assessment is extensively purified ensuring that the active substance represents more than 99 % on a dry matter basis and that the remainder is almost exclusively attributable to substance-related impurities. Taking into account the extensive purification process, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considers it unlikely that any remnants of DNA from the production strain would remain in the final product to an extent that would give rise to any safety concerns. The use of vitamin B_{12} produced by *E. adhaerens* as a nutritional additive is safe for the target animals. Its use in animal nutrition does not give rise to safety concerns for consumers. In the absence of data, the FEEDAP Panel considers it prudent to assume that vitamin B_{12} is an irritant to skin and eyes, is a skin sensitiser and is hazardous by inhalation. The use of vitamin B_{12} produced by *E. adhaerens* in animal nutrition does not pose a risk to the environment. Vitamin B_{12} produced by *E. adhaerens* is regarded as effective in meeting animals' requirements when administered orally.

KEY WORDS

nutritional additive, vitamins and pro-vitamins, vitamin B_{12}, cyanocobalamin, *Ensifer adhaerens*, safety

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[1] On request from the European Commission, Question No EFSA-Q-2012-00455, adopted on 30 April 2015.

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

[4] Due to technical issues on 9 March 2016, the published summary of the opinion was not replaced by the full opinion amended following the adoption of the decision of the Commission on confidentiality claims submitted by the applicant, in accordance with Article 18(6) of Regulation (EC) No 1831/2003. The output published on 03 July 2018 corresponds exactly to what was aimed to be published on 09 March 2016.

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SUMMARY[5]

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 B_{12} in the form of cyanocobalamin produced by *Ensifer adhaerens* as a feed additive for all animal species. Cyanocobalamin is intended for addition to feed and for use in water for drinking.

The product under consideration is vitamin B_{12} (cyanocobalamin), used as a nutritional feed additive for all animal species and categories, produced by *Ensifer adhaerens*. There are uncertainties regarding the genetic basis of the antibiotic resistance found in the production strain and regarding the possible presence of its DNA in the product. However, the FEEDAP Panel notes that, at the end of the fermentation process, living cells are absent, cobalamin is isolated from the fermentation medium, it is adsorbed to a resin, chemically converted to cyanocobalamin, eluted with acetone and crystallised, and that the product (dry matter) consists of 99 % cyanocobalamin and 1 % cobalamin-related substances. Taking into account the above, the FEEDAP Panel considers it unlikely that any remnants of DNA from the production strain would remain in the final product.

Requirements and practical supplementation levels are established for domestic animals; however, owing to the microbial synthesis of cyanocobalamin in the rumen, no dietary requirements are established for ruminants. Vitamin B_{12} produced by *E. adhaerens* is safe for the target animals provided that the current use levels for vitamin B_{12} are not exceeded. Setting a maximum content for vitamin B_{12} in complete feed is not considered necessary.

The limited toxicological studies with vitamin B_{12} show that it has a low toxicity and do not indicate a genotoxic potential. The use of the additive in animal nutrition will not significantly alter the vitamin B_{12} content of food of animal origin. The FEEDAP Panel considers that the use of vitamin B_{12} produced by *E. adhaerens* in animal nutrition is not of any safety concern for consumers.

In the absence of data, the FEEDAP Panel considers it prudent to assume that cyanocobalamin is an irritant to skin and eyes, is a skin sensitiser and is hazardous by inhalation.

The use of vitamin B_{12} produced by *E. adhaerens* in animal nutrition does not pose a risk to the environment.

Vitamin B_{12} produced by *E. adhaerens* is regarded as effective in meeting animal's requirements when administered orally.

The FEEDAP Panel made recommendations on the specifications of the product and on the restricted distribution of the pure substance on the market.

[5] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 1831/2003[6] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7; in addition, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from Lohmann Animal Health GmbH & Co KG[7] for authorisation of a new use (i.e. use in water for drinking) and re-evaluation of authorisation of vitamin B_{12} in the form of cyanocobalamin, when used as a feed additive for target species (nutritional additive; functional group: vitamins, pro-vitamins and chemically well-defined substances having similar effect) under the conditions mentioned in 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.[8] 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 4 May 2012.[9]

Vitamin B_{12} in the form of cyanocobalamin has been authorised without time limit under Council Directive 70/524/EEC[10] for its use for all animal species as a nutritional additive.

The Scientific Committee on Food (SCF) issued an opinion on the tolerable upper intake level of vitamin B_{12} (EC, 2000). The Panel on Food additives and Nutrient Sources added to Food (ANS) published a scientific opinion on 5′-deoxyadenosylcobalamin and methylcobalamin as sources for vitamin B_{12} added as a nutritional substance in food supplements (EFSA, 2008) and a statement on the inability to assess the safety of vitamin B_{12}-enriched yeast added for nutritional purposes as a source of vitamin B_{12} in food supplements and the bioavailability of vitamin B_{12} from this source, based on the supporting dossier (EFSA, 2009). The Panel on Additive and Products or Substances used in Animal Feed (FEEDAP) published a scientific opinion on the use of cobalt compounds as additives in animal nutrition (EFSA FEEDAP Panel, 2009). The Panel on Dietetic Products, Nutrition and Allergies (NDA) published a scientific opinion on the substantiation of several health claims related to vitamin B_{12} pursuant to Article 13(1) of Regulation (EC) No 1924/2006 (EFSA NDA Panel, 2009, 2010).

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 B_{12}, when used under the conditions described in Table 1.

[6] 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.
[7] Lohmann Animal Health GmbH & Co KG, Heinz-Lohmann-Str 4, D-27472, Cuxhaven, Germany.
[8] EFSA Dossier reference: FAD-2010-0173.
[9] A new mandate was received in EFSA in April 2012.
[10] 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.

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

Additive	B_{12} in the form of Cyanocobalamin			
Registration number/EC No/No	EC-No.: 200–680–01			
Category(ies) of additive	Category 3: Nutritional Additives.			
Functional group(s) of additive	Functional group a: Vitamins, pro-vitamins and chemically well-defined substances having a similar effect.			
Description				
Composition, description	Chemical formula	Purity criteria	Method of analysis	
B_{12} in the form of Cyanocobalamin	$C_{63}H_{88}CoN_{14}O_{14}P$	minimum of 96 % Cyanocobalamin (in dry matter).	European Pharmacopoeia, 6th edition: monograph 0547	
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		
All animal species and categories	-	-	-	-
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use	-			
Specific conditions or restrictions for handling	-			
Post-market monitoring	-			
Specific conditions for use in complementary feedingstuffs	-			
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 B_{12} in the form of cyanocobalamin obtained by fermentation. It should be recognised that these data cover only a fraction of existing additives containing vitamin B_{12} in the form of cyanocobalamin. The application is for the active substance and the composition of the additive formulations is not the subject of the application. The Panel has sought to use the data provided, together with data from other sources, to deliver an opinion.

1. Introduction

Cobalamin belongs to the corrinoid structural group, which includes the vitamin active forms hydroxycobalamin, adenosylcobalamin and methylcobalamin, and several rather inactive analogues, such as cobamide and cobinamide.

While a number of vitamin B_{12}-dependent metabolic functions have been identified in microorganisms, only two vitamin B_{12}-dependent enzymes, methylmalonyl–coenzyme A (CoA) mutase and 5-methyltetrahydrofolatehomocysteine methyltransferase, have been described in vertebrates (Kennedy et al., 1991). Methylmalonyl–CoA mutase is involved in the conversion of methylmalonyl-CoA into succinyl-CoA, finally releasing energy from proteins and fatty acids. 5-Methyltetrahydrofolatehomocysteine methyltransferase, also known as methionine synthase, catalyses the conversion of homocysteine to methionine. A third coenzyme function of vitamin B_{12} (for leucine mutase) has also been proposed (McDowell, 2000; Suttle, 2010).

Cyanocobalamin does not occur in nature, but is used in many pharmaceuticals and supplements, and as a food and feed additive, because of its better stability and lower cost. In the body, the pro-vitamin is converted to the physiological forms methylcobalamin and adenosylcobalamin.

Vitamin B_{12} in the form of cyanocobalamin is included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 and is foreseen for re-evaluation.

The applicant asks for the re-evaluation of the use of vitamin B_{12} (nutritional additive, functional group vitamins, pro-vitamins and chemically well-defined substances having similar effects) in the form of cyanocobalamin for use in feed for all animal species and categories and for a new use in water for drinking.

Cyanocobalamin is described in the European Pharmacopoeia (PhEur) in Monograph (MG) 0547 (European Pharmacopoeia, 2010).

Vitamin B_{12} is also listed as a pharmacologically active substance in veterinary medicinal products and is not subject to maximum residue levels when used in food-producing animals.[11] Cyanocobalamin is also listed as an ingredient in cosmetic products as a skin-conditioning agent.[12]

Vitamin B_{12} in the form of cyanocobalamin is authorised for addition to food[13] and food supplements,[14] and for the addition for specific nutritional purposes to foods for particular nutritional

[11] 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.
[12] Commission Decision 2006/257/EC of 9 February 2009 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97 5.04.2006, p. 1.
[13] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404 30.12.2006, p. 26, last amended by Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. OJ L 314 1.12.2009, p. 36.
[14] 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.

uses,[15] to processed cereal-based foods and baby foods for infants and young children,[16] and to infant formulae and follow-on formulae when reconstituted as instructed by the manufacturer.[17]

2. Characterisation

2.1. Manufacturing process

2.1.1. Characterisation of the production organisms

The vitamin B_{12} under assessment is produced by fermentation by a strain of *Ensifer adhaerens*, which is deposited in the China Center of Industrial Culture Collection, with the deposition number CICC 11008s.[18] The strain was identified as *E. adhaerens* by its 16S ribosomal RNA (rRNA) gene sequence.[19] *E. adhaerens* belongs to the class Alphaproteobacteria and the family and is a motile, Gram-negative, non-spore-forming, aerobic microorganism. The cells are usually rod-shaped. Strains of most species within the genus *Ensifer* identified so far are characteristically able to invade temperate-zone and tropical-zone leguminous plants and to incite the production of root nodules.

According to the applicant, *E. adhaerens* CICC 11008s is not genetically modified. No other information is provided on its origin, toxicity or pathogenicity. The strain seems to be resistant to at least carbenicillin, kanamycin and streptomycin, neomycin, and nalidixic acid.[20] However, none of the analyses performed to confirm this was carried out in accordance with the guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012). Furthermore, the applicant did not provide information about the strain's sensitivity to the other antibiotics (ampicillin, vancomycin, clindamycin, tetracycline or chloramphenicol) mentioned in this guidance. Uncertainty remains regarding the genetic basis of the antibiotic resistance observed in *E. adhaerens* CICC 11008s, as well as potential resistance to other antibiotics.

2.1.2. Active substance[21]

Cobalamin is produced by aerobic fermentation. At the end of the fermentation process, the production strain is inactivated. After cell separation and extraction, cobalamin is adsorbed to a resin and cyanidised with sodium cyanide to give cyanocobalamin. Vitamin B_{12} is obtained after elution of the resin with acetone.

The production strain could not be detected in a test volume of 0.1–0.2 mL of a 10 % (w/v) dilution of a non-identified sample of the final product, or in a test volume of 0.1–0.2 mL of the fermentation broth after filtration. Tests were done by incubation on non-selective solid medium at 30 °C for seven days.[22] The final product was found not to exert antimicrobial activity.[23]

The applicant examined whether the genes from the production strain conferring kanamycin and streptomycin resistance were absent in three batches of the active substance. Polymerase chain reaction (PCR) analyses could not detect these genes in cyanocobalamin.[24] The applicant also provided data from PCR analyses in which no amplicon corresponding to the 16S rRNA gene was observed in the active substance.[25] However, the quality of the relevant pictures was very poor, the

[15] Commission Regulation (EC) 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269, 14.10.2009, p. 9.
[16] 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.
[17] 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.
[18] Technical dossier/Supplementary information March 2013/Annex 2.18-III.
[19] Technical dossier/Supplementary information May 2013/ Annex 2.18.
[20] Technical dossier/Supplementary information May 2013/ Annex 2.18.
[21] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[22] Technical dossier/Supplementary information September 2013/Annex 2.21.
[23] Technical dossier/Supplementary information September 2013/Annex 2.22.
[24] Technical dossier/Supplementary information January 2015/Annex 2.26.
[25] Technical dossier/Supplementary information January 2015/Annex 2.26 to 2.28.

bands corresponding to the molecular marker and most of the positive controls were very weak, which casts doubts on the sensitivity of the analysis, and the study report was incomplete. These data alone are not sufficient to exclude the presence of minor amounts of DNA fragments in the active substance. However, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) notes that, at the end of the fermentation process, living cells are absent, cobalamin is isolated from the fermentation medium, it is adsorbed to a resin, chemically converted to cyanocobalamin, eluted with acetone and crystallised, and the product (dry matter) consists of 99 % cyanocobalamin and 1 % cobalamin-related substances (see section 2.2).

Considering the above, the FEEDAP Panel considers it unlikely that any remnants of DNA from the production strain would remain in the final product.

2.1.3. Additives

The active substance cyanocobalamin is marketed in different formulations, that is as the pure substance and as blended additives containing 1.0 % or 0.1 % cyanocobalamin.

2.2. Characterisation of the active substance

Cyanocobalamin (International Union of Pure and Applied Chemistry (IUPAC) name: cobalt(3+);[(2R,3S,4R,5S)-5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-(hydroxymethyl)oxolan-3-yl][(2R)-1-[3-[(1R,2R,3R,5Z,7S,10Z,12S,13S,15Z,17S,18S,19R)–2,13,18-tris(2-amino-2-oxoethyl)-7,12,17-tris(3-amino-3-oxopropyl)–3,5,8,8,13,15,18,19-octamethyl-2,7,12,17-tetrahydro-1H-corrin-24-id-3-yl]propanoylamino]propan-2-yl]phosphate; cyanide; synonyms: α-(5,6-dimethyl benzimidazole-1-yl)cobamid cyanide)) is identified with Chemical Abstracts Service (CAS) number 68-19-9 and European Inventory of Existing Chemical Substances (EINECS) number 200-680-0. The structural formula of cyanocobalamin is shown in Figure 1.

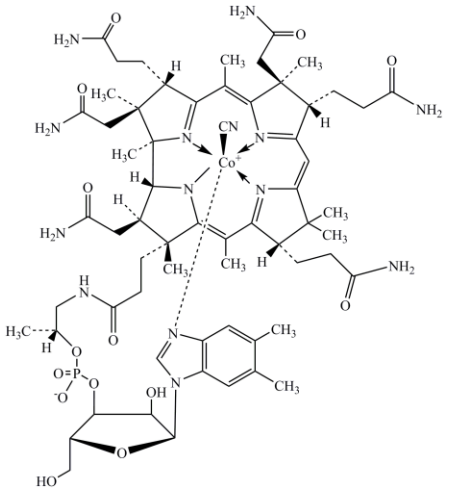

Figure 1: Structural formula of cyanocobalamin

The molecular formula of cyanocobalamin is $C_{63}H_{88}CoN_{14}O_{14}P$ and its molecular weight is 1 355.38. It has a melting point of 300 °C (with decomposition), a bulk density of 0.6 g/cm^3 and is sparingly soluble in water (1.25 g dissolves in about 100 mL of water) and 95 % ethanol. The anhydrous substance is very hygroscopic.

Cyanocobalamin is a dark-red crystalline powder and is practically odourless and tasteless. Cyanocobalamin is described in the PhEur (PhEur 7.0, MG 0547) as having a purity of 96–102 % with < 3 % total related substances in the dried substance, and a loss on drying of < 12 % (European Pharmacopoeia, 2010).

The product, cyanocobalamin, is specified by the applicant as having a minimum purity of 96 %. Analysis of five production batches showed an average of 99.0 % ± 0.1 % cyanocobalamin and 0.8–1.2 % cobalamin-related impurities (mostly corrinoids) in the dried product, and the loss on drying amounted to 2.1–4.9 %.[26] All the analytical values complied with the thresholds of PhEur.

The concentrations of heavy metals (cadmium, mercury and lead) and arsenic were determined in three additional batches of the active substance: cadmium was < 0.01 mg/kg, mercury was < 0.05 mg/kg, lead was in the range of 0.06–0.09 mg/kg and arsenic was < 2 mg/kg.[27] Data for residual solvents (acetone) were below the thresholds proposed by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (EMA, 2010).[28] Control measures are in place.

One batch of the active substance was analysed for particle size distributions by laser diffraction. The percentages of particles with diameters of < 10 and < 50 µm were 7.4 and 74 %, respectively.[29] Another batch showed a dusting potential of 0.46 g/m^3.[30]

One batch of the blended additive containing 1.0 % cyanocobalamin showed a dusting potential of 7 g/m^3.[31]

2.3. Stability and homogeneity

2.3.1. Shelf life

The applicant provided information on the recovery of cyanocobalamin 96 % when the product (three batches) was kept in polyethylene bags at 25 °C for up to 48 months and at 40 °C for up to six months, respectively. No losses were found.[32]

2.3.2. Stability of cyanocobalamin in premixtures, feed and water for drinking

A vitamin–mineral premixture (without choline chloride) containing an intended concentration of 20 mg cyanocobalamin/kg from one batch of the product was stored at 20 °C in polyethylene bags for six months. No reduction of the content of cyanocobalamin was observed.[33]

According to data published by Whitehead (2002), the cyanocobalamin recovery in premixtures containing choline chloride and trace elements was 98 % after one month of storage and 89 % after six months (average monthly loss of 2 %). In premixtures without choline, monthly losses of vitamin B_{12} were 0.04 % (Coelho, 1996).

Cyanocobalamin (one batch) was incorporated into a pig feed at a nominal concentration of 60 µg/kg via a premixture (5 mg/kg); the feed was then pelleted at 80 °C. Pelleted feed was stored at 20 °C in polyethylene bags for three months. The results, despite their variability (from 94 to 122 %), did not indicate a loss of vitamin B_{12} during feed processing or storage.

According to Whitehead (2002), the recovery of cyanocobalamin in feedingstuffs is inversely related to the temperature of expanding, ranging from 99 % at 93 °C for 30 seconds to 90 % at 149 °C for 30 seconds.

[26] Technical dossier /Section II.
[27] Technical dossier/Supplementary information March 2013/Annex 2.18-IV.
[28] Technical dossier/Section II.
[29] Technical dossier/Section II/Annex 2.16.
[30] Technical dossier/Supplementary information May 2013/Annex 2.20.
[31] Technical dossier/Supplementary information September 2013 /Annex 2.23.
[32] Technical dossier/Section II.
[33] Technical dossier/Section II.

Marchetti et al. (1999) reported some stability data for crystalline cyanocobalamin added to commercial fish feed. Pelleting at 68 °C reduced the initial concentration of 0.26 mg/kg by 12 % and by 15 % after extrusion at 96 °C. Upon storage in paper bags at room temperature, the concentration of cyanocobalamin in pelleted fish feed remained stable for 90 days and the concentration in extruded feed showed an additional loss of about 23 % after 90 days.

The stability of cyanocobalamin (one batch) in water for drinking was demonstrated when added at 10 mg/L and stored for 24 hours at 15 °C.[34] For another batch, the stability was demonstrated after storage for 48 hours at 20 °C.[35]

2.3.3. Homogeneity

Based on a statistical method (Jansen, 1992), the coefficient of variation for homogeneity of cyanocobalamin in poultry feed was calculated to be around 0.03 %. However, this method was developed to test the working accuracy of mixing equipment and is not accepted by the FEEDAP Panel as a valid method for assessing the homogeneity of distribution of additives in feeds.

The FEEDAP Panel expresses its reservation concerning the incorporation of cyanocobalamin additives directly into feed. The small quantities of cyanocobalamin could only be homogeneously distributed in feed if added via premixtures.

The solubility of cyanocobalamin (12.5 g/L) exceeded the concentration at which cyanocobalamin is expected to be added to water for drinking by at least two orders of magnitude. Therefore, homogeneity in water for drinking does not need to be demonstrated.

2.4. Physico-chemical incompatibilities in feed

No physico-chemical incompatibilities or interactions have been reported between cyanocobalamin and feed materials, carriers, other approved additives (except choline chloride) or medicinal products when the additive is added to premixtures and feed. No such incompatibilities or interactions are expected.

2.5. Conditions of use

Cyanocobalamin is intended for use in all animal species and categories without a maximum content and withdrawal period. According to the applicant, the active substance is proposed to be administered via feed (premixtures, complete or complementary feed) or water for drinking.

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 B_{12} (cyanocobalamin) in animal feed. The Executive Summary of the EURL report can be found in Appendix A.

3. Safety

3.1. Safety for the target species

According to the National Research Council (NRC), requirements for vitamin B_{12} are in the range of 3–10 µg/kg poultry feed (NRC, 1994), 5–20 µg/kg feed for pigs (NRC, 2012), 15–53 µg/kg feed for fish (NRC, 2011) and 20 and 26 µg/kg feed for cats and dogs, respectively (NRC, 2006, 2007a). Similar ranges for vitamin B_{12} requirements have been proposed by the German Society of Nutrition Physiology (Gesellschaft für Ernährungsphysiologie, GfE): 10 µg/kg diet (dry matter, DM) for poultry (GfE, 1999, 2004) and 10–40 µg/kg diet (DM) for pigs (GfE, 2008). Owing to the microbial synthesis

[34] Technical dossier A/Section II/Annex 2.14.
[35] Technical dossier/Supplementary information March 2013/Annex 2.19.

of vitamin B_{12} in the rumen and in the caecum, which requires cobalt (EFSA FEEDAP Panel, 2009; GfE, 2003), no dietary requirements have been established for ruminants (NRC, 1996, 2001, 2007b; GfE, 1995, 2001, 2003) or horses (GfE, 2014). For non-ruminating calves, the requirement for vitamin B_{12} in milk replacers is 70 µg/kg DM (NRC, 2001). Requirements for laboratory animals are 50 µg/kg diet for rats and 10 µg/kg diet for mice (NRC, 1995).

Vitamin supplementation of commercial compound feed is mostly oriented towards recommendations which are in the range of 15–60 µg/kg for pigs, 10–40 µg/kg for poultry, 20–40 µg/kg for fish and 30–40 µg/kg feed for pets (AWT, 2002). A survey on the vitamin supplementation of commercial feeds for pigs and poultry in Europe (Belgium, Denmark, Germany, Italy, Netherlands, Portugal, Spain and the United Kingdom) identified a range of 3–150 µg cyanocobalamin/kg as the commercial use level (Gropp, 1994; Whittemore et al., 2002).

In 1987, the NRC concluded that "insufficient data are available to support estimates of the maximum dietary tolerable levels of vitamin B_{12}. Data from a single chick study suggest that 3 times the vitamin B_{12} requirement of that species can be included safely in the diet. Mouse data suggest that dietary levels of at least several hundred times the requirements are safe" (NRC, 1987).

For nutritional additives produced by fermentation, the risks associated with the residues of the fermentation process in the final product need to be assessed. The production strain *E. adhaerens* has not been properly characterised in terms of virulence and antibiotic resistance, and uncertainties remain regarding the genetic basis of the antibiotic resistance. However, this specific product is extensively purified, ensuring that the active substance represents more than 99 % on a dry matter basis and that the remainder is almost exclusively attributable to substance-related impurities (see section 2.2). Taking into account the extensive purification process, the FEEDAP Panel considers it unlikely that any remnants of DNA from the production strain would remain in the final product to an extent that would give rise to any safety concerns. Moreover, the low inclusion level of vitamin B_{12} in animal feed provides further reassurance of the safety for target animals.

3.1.1. Conclusions on the safety of vitamin B_{12} for target species

The FEEDAP Panel concludes that the use of vitamin B_{12} produced by *E. adhaerens* CICC 11008s as a nutritional additive is safe for the target animals. The introduction of a maximum content for vitamin B_{12} does not appear necessary.

3.2. Safety for the consumer

3.2.1. Absorption, distribution, metabolism and excretion

Vitamin B_{12} is an essential micronutrient for monogastric animals. In contrast, ruminants, horses and rabbits do not depend on an oral vitamin B_{12} supply; gastrointestinal microbiota produce cobalamin provided that there is a sufficient intake of cobalt (EFSA FEEDAP Panel, 2009).

The intestinal absorption of vitamin B_{12} requires binding to protein factors (haptocorrin and intrinsic factor (IF)). The cobalamin–IF complex is absorbed by a receptor-mediated process in the ileum. The capacity of this active transport mechanism limits the absorption rate of cyanocobalamin. In addition, about 1 % of the dose is absorbed by passive diffusion. Cobalamin is converted to the active forms methyl- or adenosylcobalamin in enterocytes and, after release, is transported by specific binding proteins in the blood. Vitamin B_{12} is mainly stored in the liver. The main excretion route is via bile; urinary excretion is minimal. More details are given in Appendix B. The available data on monogastric animals indicate that vitamin B_{12} metabolism in pigs is similar to that in humans. Henderickx et al. (1964) reported a total retention of 20–23 % of dietary vitamin B_{12} in pigs; about 65 % of the absorbed vitamin was stored in the liver.

3.2.2. Deposition in eggs

Data describing deposition in edible tissues/products are available only for eggs. The relationship between dietary vitamin B_{12} and vitamin B_{12} deposition in eggs has been studied in laying hens. Leeson and Caston (2003) found a vitamin content of 0.87 and 3.35 µg/60 g egg after a three-month feeding period with a diet supplemented with 10 and 100 µg vitamin B_{12}/kg feed, respectively. Johnson and Korver (2008) found a vitamin B_{12} concentration of 0.72 and 0.92 µg/60 g egg after six weeks of supplementation with 16 and 116 µg vitamin B_{12}/kg, respectively. In another study (two factorial design, 60 weeks' duration), vitamin B_{12} levels were 14.23 and 22.33 µg/kg per whole egg (corresponding to 0.85 and 1.34 µg/60 g egg) in groups fed diets supplemented with 12.3 and 25 µg/kg, respectively (Pérez-Vendrel et al., 2004). The vitamin B_{12} levels in eggs increased from 41 µg/kg to 221 µg/kg when dietary vitamin B_{12} levels were increased from 30 to 250 µg/kg (Denton et al., (1954), reviewed by Naber, 1993), and levels in egg yolk increased from 13 µg/kg to 48 µg/kg when dietary vitamin B_{12} levels were increased from 4 to 16 µg/kg (Squires and Naber (1992), reviewed by Naber, 1993). Although an increase in vitamin B_{12} in eggs, related to the level of vitamin B_{12} supplementation, may occur, the results of the available studies are highly variable and no data were provided on the background content of vitamin B_{12}. Overall, the data suggest a considerable influence of the vitamin B_{12} level in feed for laying hens on the vitamin B_{12} content of eggs.

3.2.3. Toxicology

Vitamin B_{12} has been considered of very low toxicity by several international bodies. According to the Scientific Committee on Food (SCF; EC, 2000), "no adverse effects have been associated with excess vitamin B_{12} intake from food or supplements in healthy individuals. Vitamin B_{12} has a history of safe long-term use as a therapeutic agent given in high dosages per os, or via intramuscular injections, for treatment of disorders associated with impaired vitamin B_{12} absorption, such as in gastrectomy and malabsorption. In vitamin B_{12} replacement therapy oral or intramuscular dosages between 1 000–5 000 µg vitamin B_{12} are used, with no supportive evidence of adverse effects up to at least 5 years. The usual treatment in PA (pernicious anaemia) patients is 1 000 µg administered intramuscularly once every 1 to 3 months, but oral dosages of 300–1000 µg daily could also provide adequate treatment (Berlin et al., 1968; Hathcock and Troendle, 1991). At these dosage rates the cobalt and cyanide contributions are toxicologically insignificant (see Hathcock and Troendle, 1991). It should be noted, however, that these studies were not designed to find adverse effects". The SCF notes that "…there are no clearly defined adverse effects produced by vitamin B_{12} that can be used to define a LOAEL or NOAEL, which can be used as a basis for deriving an UL".

Recently, the US Food and Drug Administration (FDA, 2014) came to similar conclusions as the SCF: "Vitamin B_{12} has shown no toxicity to animals at several thousand times their nutritional requirements. In man, pernicious anaemia patients have received daily doses for years 10 to 20 times that of the highest estimate of average daily consumption. The only reaction to vitamin B_{12} so far demonstrated in man is the development of sensitivity that can become manifest as allergy or anaphylaxis after parenteral administration of relatively high doses".

In humans, the acute toxicity seems very low (EVM, 2003). High oral doses of cyanocobalamin given to healthy females of child-bearing age or to patients with seasonal affective disorder did not affect plasma homocysteine levels at doses of up to 4.5 mg cyanocobalamin/day for 14 days, 2.0 mg cyanocobalamin/day for up to one year or 1.0 mg cyanocobalamin/day for several years. Less information is available on toxicity following the oral administration of the hydroxocobalamin form of vitamin B_{12}. However, no adverse effects were reported in individuals administered 0.3 mg hydroxocobalamin/day for up to 12 months. No adverse effects were reported in a controlled study in which 125 individuals received 6.0 mg/day methylcobalamin for up to 12 weeks (EVM, 2003). This limited evidence suggests that adverse effects are unlikely to result from long-term treatment with 1 mg methylcobalamin/day (EVM, 2003).

Long-term studies in animals to evaluate carcinogenic potential have not been done. Two previous studies in rats (quoted by the SCF (EC, 2000)) suggest that, under certain experimental conditions,

vitamin B_{12} might promote tumour growth. However, there is no evidence from the long-term use of cyanocobalamin in patients with pernicious anaemia that this compound is carcinogenic (FDA, 2014). The FEEDAP Panel is aware that a meta-analysis demonstrated a modest but statistically significant association between high plasma concentrations of cobalamin and prostate cancer risk (Collin et al., 2010); however, this finding was not supported by a large and more recent study carried out in Norway (de Vogel et al., 2013).

No studies have investigated the reproductive or developmental toxicity of vitamin B_{12}. However, the long-term use of cyanocobalamin in patients with pernicious anaemia provided no indication that such effects might occur (FDA, 2014).

The FEEDAP Panel is in agreement with the assessments described above, that is that vitamin B_{12} has a very low toxicity.

3.2.4. Assessment of consumer safety

Vitamin B_{12} is found in animal products only. The concentrations found in foods of animal origin are summarised below; they are derived from Indyk et al. (2002), Ortigues-Marty et al. (2005), Souci et al. (2008), CIV-INRA (2010), Szterk et al. (2012) and Gille and Schmid, (2015). The food richest in vitamin B_{12} is liver: concentrations are reported for beef calves (380–820 µg/kg), beef cattle (499–782 µg/kg), pigs (on average 390 µg/kg), lamb (on average 350 µg/kg) and poultry (on average 260 µg/kg). The levels of cyanocobalamin in other edible tissues and products of animal origin are 210 µg/kg in beef kidney, 190 µg/kg in fish flesh (mackerel), 115 µg/kg in crab meat, 5–80 µg/kg in beef, lamb and veal meat, 20 µg/kg in pork meat, 2–6 µg/kg in poultry meat, 8–31 µg/kg in whole egg[36] (8–33 µg/kg egg yolk), and 0.1–7 µg/kg in cow's milk (levels in cheese are 5- to 10-fold higher).

Because vitamin B_{12} is not present in plants, breakfast cereals are frequently fortified with vitamin B_{12} (200 µg/kg) and can serve as a vitamin B_{12} source for vegans/vegetarians.

Based on data from 13 dietary surveys in nine European Union countries, average cobalamin intakes range from 2.2 to 4.0 µg/day in toddlers aged one to three years, and from 4.2 to 8.6 µg/day in adults (EFSA NDA Panel, 2015).

The provision of vitamin B_{12} to food-producing animals has not changed for several decades (the supplementation of vitamin B_{12} to non-ruminants and cobalt to ruminants is well established). Therefore, the FEEDAP Panel considers that the available human intake data already include the use of vitamin B_{12} in animal nutrition.

For nutritional additives produced by fermentation, the risks associated with the residues of the fermentation process in the final product need to be assessed. The production strain *E. adhaerens* has not been properly characterised in terms of virulence and antibiotic resistance, and uncertainties remain regarding the genetic basis of the antibiotic resistance. However, this specific product is extensively purified, ensuring that the active substance represents more than 99 % on a dry matter basis and that the remainder is almost exclusively attributable to substance-related impurities (see section 2.2). Taking into account the extensive purification process, the FEEDAP Panel considers it unlikely that any remnants of DNA from the production strain would remain in the final product to an extent that would give rise to any safety concerns for consumers.

3.2.5. Conclusions on consumer safety

The toxicity of vitamin B_{12} is low. Humans have been administered cyanoconalamin, hydroxycobalamin and methylcobalamin at milligram levels for many years without adverse effects. Current levels of intake (up to 9 µg/day) of vitamin B_{12} arising from consumption of animal tissues

[36] Naber (1993) reported up to 221 µg/kg whole egg; however, these values were obtained with dietary vitamin B_{12} concentrations (250 µg/kg) considerably exceeding practical use levels.

and products, which already include current supplementation practice of feed with vitamin B_{12}, do not pose a health concern.

The FEEDAP Panel considers that the use of highly purified vitamin B_{12} produced by *E. adhaerens* CICC 11008s in animal nutrition at the current use levels is safe for consumers.

3.3. Safety for the user

3.3.1. Effects on the respiratory system

The dusting potential of cyanocobalamin was low (< 0.5 g/m^3). The proportion of particles with diameters of < 10 and < 50 µm was 7 and 74 %, respectively. A formulated additive containing 1.0 % cyanocobalamin showed a dusting potential of 7 g/m^3. Inhalation toxicity studies were not available and nor were endotoxin activity data. Consequently, the FEEDAP Panel concludes that there is a potential for inhalation exposure of workers handling the additive and an associated health risk.

3.3.2. Effects on the eyes and skin

No studies on the skin/eye irritation potential or on the skin sensitisation potential of cyanocobalmin were provided.

3.3.3. Conclusions on user safety

In the absence of data, the FEEDAP Panel considers it prudent to assume that cyanocobalamin is an irritant to skin and eyes, is a skin sensitiser and is hazardous by inhalation.

3.4. Safety for the environment

Cobalamin occurs in nature in bacteria and in the animal kingdom. Its use in animal nutrition is not expected to substantially increase its concentration in the environment.

However, there are uncertainties regarding the genetic basis of the antibiotic resistance found in the production strain *E. adhaerens* CICC 11008s, and regarding the possible presence of its DNA in the product. However, taking into account the extensive purification process, the FEEDAP Panel considers it unlikely that any remnants of DNA from the production strain would remain in the final product to an extent that would give rise to a safety concern for the environment.

Therefore, a risk for the environment resulting from the use of vitamin B_{12} produced by *E. adhaerens* CICC 11008s in animal nutrition is not foreseen.

4. Efficacy

Vitamin B_{12} has been used globally in animal nutrition for decades. Because of its long history of use and its established nutritional role in domestic animals, cyanocobalamin is regarded as effective in meeting animal requirements when administered orally. Data on requirements, allowances and recommendations for feed supplementation are easily accessible as standard literature for animal nutritionists.

5. Post-market monitoring

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

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

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS[38]

The product under consideration is vitamin B_{12} (cyanocobalamin) produced by *E. adhaerens* strain CICC 11008s. There are uncertainties regarding the genetic basis of the antibiotic resistance found in the production strain and the possible presence of its DNA in the product. However, the FEEDAP Panel notes that, at the end of the fermentation process, living cells are absent, cobalamin is isolated from the fermentation medium, it is adsorbed to a resin, chemically converted to cyanocobalamin, eluted with acetone and crystallised, and that the product (dry matter) consists of 99 % cyanocobalamin and 1 % cobalamin-related substances. Taking into account the above, the FEEDAP Panel considers it unlikely that any remnants of DNA from the production strain would remain in the final product.

The use of vitamin B_{12} produced by *E. adhaerens* CICC 11008s as a nutritional additive is safe for the target animals.

The use of vitamin B_{12} in animal nutrition does not give rise to safety concerns for consumers.

In the absence of data, the FEEDAP Panel considers it prudent to assume that cyanocobalamin is an irritant to skin and eyes, is a skin sensitiser and is hazardous by inhalation.

The use of vitamin B_{12} produced by *E. adhaerens* CICC 11008s in animal nutrition does not pose a risk to the environment.

Vitamin B_{12} produced by *E. adhaerens* CICC 11008s is regarded as effective in meeting animals' requirements.

RECOMMENDATIONS

The specifications of the product should conform to the values considered as key elements in the safety assessment. It is recommended that the purity of the dry matter be set to ≥ 99.0 % cyanocobalamin and ≤ 1.0 % substance-related impurities. The absence of DNA from the production strain should be ensured.

The active substance should be used only via premixtures to ensure homogeneous distribution in feed.

DOCUMENTATION PROVIDED TO EFSA

1. Vitamin B_{12}/Cyanocobalamin as a feed additive for all animal species. October 2010. Submitted by Lohmann Animal Health GmbH & Co KG.

2. Vitamin B_{12}/Cyanocobalamin as a feed additive for all animal species. Supplementary information. March 2013. Submitted by Lohmann Animal Health GmbH & Co KG.

3. Vitamin B_{12}/Cyanocobalamin as a feed additive for all animal species. Supplementary information. May 2013. Submitted by Lohmann Animal Health GmbH & Co KG.

4. Vitamin B_{12}/Cyanocobalamin as a feed additive for all animal species. Supplementary information. September 2013. Submitted by Lohmann Animal Health GmbH & Co KG.

5. Vitamin B_{12}/Cyanocobalamin as a feed additive for all animal species. Supplementary information. February 2014. Submitted by Lohmann Animal Health GmbH & Co KG.

[38] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

6. Vitamin B_{12}/Cyanocobalamin as a feed additive for all animal species. Supplementary information. July 2014. Submitted by Lohmann Animal Health GmbH & Co KG.

7. Vitamin B_{12}/Cyanocobalamin as a feed additive for all animal species. Supplementary information. January 2015. Submitted by Lohmann Animal Health GmbH & Co KG.

8. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for vitamin B_{12} (cyanocobalamin).

9. Comments from Member States received through the ScienceNet.

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Appendix - Absorption, distribution, metabolism and excretion of vitamin B_{12}

A detailed description of the general features of absorption and metabolism of vitamin B_{12} can be found in the assessment of the Scientific Committee on Food (SCF) (EC, 2000), the Expert Group on Vitamins and Minerals (EVM, 2003) and in the draft EFSA scientific opinion on dietary reference values for cobalamin (vitamin B_{12}) (EFSA NDA Panel, 2015, currently undergoing public consultation; available online: http://www.efsa.europa.eu/en/consultations/call/150310a.pdf). The FEEDAP Panel review is mainly based on these references. Most (quantitative) data refer to human studies; details obtained from animal studies are scarce.

Ingested vitamin B_{12} is released from a feed matrix by the action of digestive enzymes and gastric acid, and becomes bound to haptocorrin—a binding protein (also known as R-protein) secreted by salivary glands and the gastric mucosa. As the pH rises further along the gut, and under the influence of pancreatic enzymes, vitamin B_{12} is released from haptocorrin and becomes complexed with intrinsic factor (IF)—a glycoprotein secreted by gastric parietal cells. The cobalamin–IF complex binds to a specific cell wall receptor of the ileal enterocytes and is internalised by endocytosis. Once inside the cell, the IF is degraded and the liberated vitamin is converted to the methyl or the 5′-deoxyadenosyl form. These forms of vitamin B_{12} are bound to transcobalamin (TC) II and then exported into the portal blood.

Although a remarkable variability has been observed, at intakes of around 1 µg the absorption is estimated to be about 40 % in healthy individuals (EFSA NDA Panel, 2015). Ileal receptors are saturated when the dose/meal contains from 1.5 to 2.5 µg of vitamin B_{12}. At daily intakes of around 1 µg, about 50 % is absorbed; at dosages of around 25 µg, only 5 % is absorbed. Some proteins in feeds may bind to cobalamin and decrease its absorption. Very small amounts of vitamin B_{12} (1–1.5 %) not bound to IF may be absorbed by passive diffusion; consequently, the relative importance of this process increases at high intake levels.

Vitamin B_{12} is an exceptional B-vitamin as it can be stored in significant amounts, especially in the liver (about 50 % of the body pool (about 2–3 mg) in humans) and, to a lesser extent, in the kidney. It does not accumulate in other tissues. The main route of excretion is through the bile, but there is a considerable reabsorption of these biliary cobalamin losses in the ileum (enterohepatic circulation). The transformation of stored cobalamin into an active form takes place mainly in liver and, to a lesser extent, in the kidney (McDowell, 2000).

Urinary excretion is minimal, and increases only if the plasma binding capacity is exceeded, e.g. following parenteral or intravenous administration. Average daily losses via faeces are estimated to be about 0.5 µg in humans. Total daily losses are estimated to be about 0.1 % of the total body pool (for reviews see Scott, 1997; Ellenbogen and Cooper, 1991).

In the general circulation, most cobalamin is bound to TC I and TC III (80 %). Twenty per cent of cobalamin is bound to TC II, and only this form is available for uptake into tissues other than the liver, e.g. bone marrow and brain. Uptake into cells occurs through receptor-mediated endocytosis involving specific TC II cell wall receptors. Once inside the tissues/cells, the complex is degraded by the lysosomes and the released cobalamin is metabolised. In the cytosol, methylcobalamin binds to methionine synthase; in the mitochondria, deoxyadenosylcobalamin binds to methylmalonyl–CoA mutase.

Annex – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Vitamin B_{12} (Cyanocobalamin)[1]

In the current three applications (FAD-2010-0173, 0199 and 0326), authorisation is sought under Articles 4(1) and 10(2) for *Vitamin B_{12}/cyanocobalamin* 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. Authorisation is sought for the use of the *feed additive* for all animal species and categories.

According to the Applicants, *Vitamin B_{12}/cyanocobalamin* is produced by fermentation, using different bacterial strains and later reaction with cyanide to form a dark red crystals or crystalline powder of *cyanocobalamin*, with a minimum purity of 96 %. The active substance (*Cyanocobalamin*) will be marketed in several preparations: - for Applicant FAD-2010-0173 the *feed additive* consists of the pure active substance containing a minimum of 96 % *cyanocobalamin*; - Applicant FAD-2010-0199 describes a "crude Vitamin B_{12}" preparation as organic or inorganic carriers including from 0.1 to 5 % *cyanocobalamin*, while –Applicant FAD-2010-0326 refers to a "feed grade Vitamin B_{12}" preparation containing from 30 to 40 % *cyanocobalamin*. *Vitamin B_{12}* is intended to be incorporated in *feedingstuffs* through *premixtures* or directly in *water*. No minimum or maximum concentrations in *feedingstuffs* or in *water* are specified, however the typical concentration ranges from 10 to 80 g/kg compound feed, depending on the target species.

For the characterisation of *cyanocobalamin per se*, Applicants FAD-2010-0173 submitted the European Pharmacopoeia method (Eur. Ph. 6.0, 01/2008:0547), where identification is based on spectrophotometry or thin-layer chromatography (TLC); quantification is based on spectrophotometry (UV/VIS), while purity is assessed by liquid chromatography followed by spectrophotometry (LC-UV/VIS). Even though no performance characteristics of the method are provided, the EURL recommends for official control the European Pharmacopoeia method for the characterisation of *cyanocobalamin per se*.

For the determination of *cyanocobalamin* in *water*, the Applicant (FAD-2010-0173) proposed the European Pharmacopoeia UV/VIS method mentioned above without providing any experimental data to support such a claim. Therefore, the EURL can neither evaluate nor recommend this method to determine *Vitamin B_{12}* in *water*.

For the determination of *cyanocobalamin* in *premixtures* and *feedingstuffs*, the Applicants submitted several microbiological essays, such as the AOAC and US Pharmacopoeia methods. Even though the validation and verification data provided by Applicants FAD-2010-0173 and FAD-2010-0199 seems to be acceptable some NRLs expressed their concern about the applicability of the proposed microbiological method for the quantification of *cyanocobalamin* in *premixtures* and *feedingstuffs.* Alternative HPLC methods have been published in the scientific literature, related to the determination of *Vitamin B_{12}* in food commodities. As they were not tested on feed samples, they are not recommended by the EURL for official control.

[1] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2010-017301990326?search&form-return