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Safety and efficacy of a feed additive consisting of vitamin B12 (cyanocobalamin) produced by fermentation with *Ensifer adhaerens* CGMCC 19596 for all animal species (Hebei Huarong Pharmaceutical Co. ltd)

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Abstract

The vitamin B12 (in the form of cyanocobalamin) under assessment is produced by fermentation with *Ensifer adhaerens* CGMCC 19596 and it is intended to be used as a nutritional additive for all animal species. Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of cyanocobalamin, produced by fermentation with *E. adhaerens* CGMCC 19596. Cyanocobalamin produced by other strains of *E. adhaerens* is already authorised for use in animal species. [redacted] However no viable cells nor DNA of the production strain were detected in the additive. Therefore, cyanocobalamin produced by *E. adhaerens* CGMCC 19596 does not raise safety concerns as regards to the production strain. The FEEDAP Panel concluded that cyanocobalamin produced by fermentation with *E. adhaerens* CGMCC 19596 is considered safe for all animal species. The use of cyanocobalamin in animal nutrition is of no concern for consumer safety. Cyanocobalamin is non-irritant to skin and non-irritant to eyes. No conclusions could be drawn on the potential of the additive to be a skin sensitiser. The potential endotoxin activity of the additive is unlikely to represent a hazard for users. The use of the additive under assessment in animal nutrition is considered safe for the environment. Cyanocobalamin produced by *E. adhaerens* CGMCC 19596 is effective in meeting animal's nutritional requirements when administered via feed.

Keywords: nutritional additive, vitamins and provitamins, vitamin B12, cyanocobalamin, *Ensifer adhaerens* CGMCC 19596, safety

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from CSPC Dermay Europe GmbH on behalf of Hebei Huarong Pharmaceutical Co., Ltd.[2] for the authorisation of the additive consisting of Vitamin B12 (cyanocobalamin) produced by fermentation with *Ensifer adhaerens* CGMCC 19596, when used as a feed additive for all animal species (category: nutritional additive; functional group: vitamins, pro-vitamins and chemically well-defined substances having similar effect).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 8 December 2021.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of vitamin B12 (cyanocobalamin), when used under the proposed conditions of use (see **Section 3.1.7**).

1.2. Additional information

The additive is vitamin B12, in the form of cyanocobalamin, produced by fermentation with *Ensifer adhaerens* CGMCC 19596, declared to be non-genetically modified. Vitamin B12 (cyanocobalamin) produced by a different strain of *E. adhaerens* is currently authorised as a nutritional additive for use in all animal species without maximum content.[3] Cyanocobalamin produced by fermentation is described in the European Pharmacopoeia in Monograph (MG) 0547 (PhEur, 11th Ed).

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) has previously issued opinions on the safety and efficacy of vitamin B12 (in the form of cyanocobalamin) produced by *E. adhaerens* (EFSA FEEDAP Panel, 2015, 2018a, 2020).

2. Data and Methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of vitamin B12, in the form of cyanocobalamin, as a feed additive. The dossier was received on 26 July 2021 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00571.

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

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the vitamin B12 in animal feed.[5]

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Hebei Huarong Pharmaceutical Co. Ltd. represented in EU by CSPC Dermay Europe GmbH, Gurlittstraße 24, 20099 Hamburg, Germany.
[3] Commission Implementing Regulation (EU) 2022/1249 of 19 July 2022 concerning the authorisation of vitamin B12 in the form of cyanocobalamin produced by *Ensifer adhaerens* CNCM I-5541 as a feed additive for all animal species. OJ L 191/10, 20.7.2022, p. 3
[4] FEED dossier reference: FAD-2021-0041.
[5] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2021-0041_en

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of vitamin B12 is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019) and EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2021).

3. Assessment

The current opinion deals with the assessment of vitamin B12 in the form of cyanocobalamin, produced by fermentation with *E. adhaerens* (CGMCC 19596). It is intended to be used as a nutritional additive (functional group: vitamins, pro-vitamins and chemically well-defined substances having a similar effect) in feed for all animal species. The additive corresponds to the active substance.

3.1. Characterisation

3.1.1. Characterisation of the production strain

Vitamin B12 in the form of cyanocobalamin is produced by fermentation with the strain of *E. adhaerens* CGMCC 19596, declared by the applicant to be non-genetically modified, which is deposited in the collection of the Chinese General Microbiological Collection Center (GCMCC), with the accession number CGMCC 19596.[7]

[redacted][8]

[redacted][9]

The production strain was tested for antibiotic susceptibility by broth microdilution. The battery of antibiotics used included those recommended by EFSA for Enterobacteriaceae (EFSA FEEDAP Panel, 2018c). [redacted][10]

The WGS data of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes, using the [redacted] and genes encoding for toxins and virulence factor[redacted] No hits of concern were identified. However, the Panel notes that the thresholds used in the searches were higher than those recommended by the EFSA Statement (EFSA, 2021). Therefore, the potential presence of genes encoding for AMR, toxins or virulence factors cannot be fully excluded.[11]

[6] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
[7] Technical dossier/Section II/Annex II_14.
[8] Technical dosser/EFSA SIN reply B12/Annex 3.
[9] Technical dossier/Section II/Annex II_12.
[10] Technical dossier/Section II/Annex II_16.
[11] Technical dossier/Annex II_12 and EFSA Sin_reply_B12; Annex 2.1a and Annex 2.1b and Annex 2.2.

The applicant also conducted a literature search[12] to identify any potential report on the toxicity of *E. adhaerens*. The following databases were searched: PubMed, Web of science and Scopus. All databases were searched by words in the title, the abstract, the keywords and the text. Search terms contained 'Ensifer adhaerens' and words like 'toxin*', 'safety', 'virulence factor*', 'pathogenicity', etc. Search period was reported to be from 2011 onwards. The search did not produce any relevant results.

3.1.2. Manufacturing process

[redacted][13]

3.1.3. Characterisation of the active substance

Cyanocobalamin (International Union of Pure and Applied Chemistry (IUPAC) name: cobalt(3+); [(2*R*,3*S*,4*R*,5*S*)-5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-(hydroxymethyl) oxolan-3-yl] [(2*R*)-1-[3-[(1*R*,2*R*,3*R*,5*Z*,7*S*,10*Z*,12*S*,13*S*,15*Z*,17*S*,18*S*,19*R*)-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-1*H*-corrin-24-id-3-yl]propanoylamino] propan-2-yl] phosphate; cyanide; Synonyms: vitamin B12, cobalamin, α-(5,6-dimethyl benzimidazole-1-yl)cobamidcyanide) is identified with the Chemical Abstracts Service (CAS) number 68-19-9 and the European Inventory of Existing Chemical Substances (EINECS) number 200-680-0. Cyanocobalamin has a molecular weight of 1355.37 g/mol, a molecular formula $C_{63}H_{88}CoN_{14}O_{14}P$ and its structural formula is shown in Figure 1.

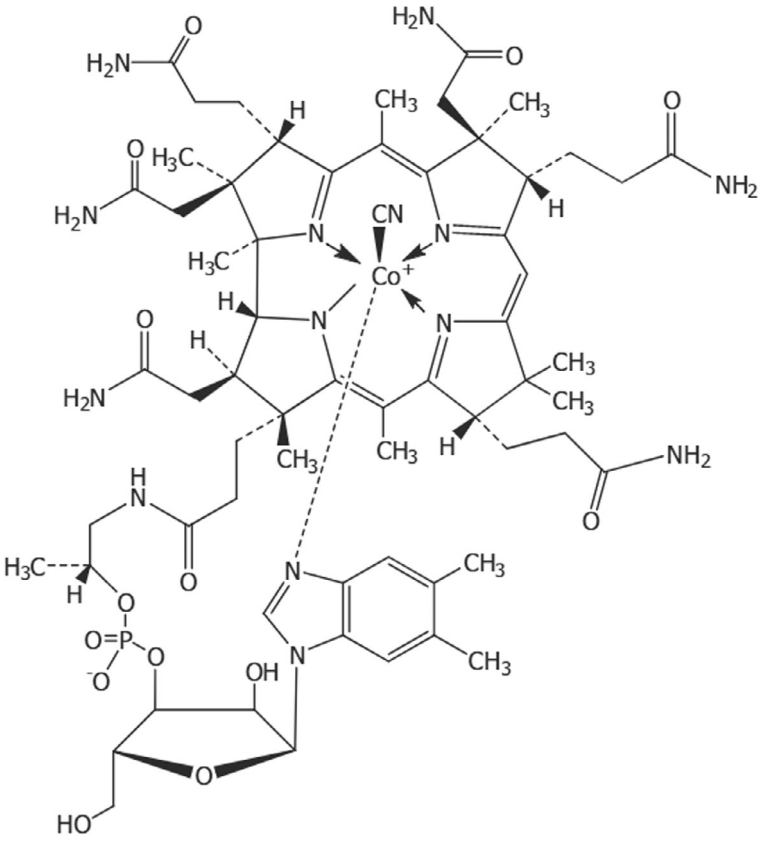

Figure 1: Structural formula of cyanocobalamin

[12] Technical dossier/Section III/Annex III_1.
[13] Technical dossier/Section II/Annex II.18.

3.1.4. Characterisation of the additive

The additive is specified to contain a minimum of 96.0% cyanocobalamin (on a dry basis) and loss on drying should be ≤ 12%.

Analytical data to confirm the specification were provided for five independent batches[14] of the additive, showing the average value of 99.2% (range 99.0–99.4%) for cyanocobalamin, on a dry basis.[15] Loss on drying was in average 6.3% (range 3.8–7.3%).

Content of cyanocobalamin in the additive and loss on drying were in line with requirements from the European Pharmacopoeia with regards to cyanocobalamin produced by fermentation.[16]

Per manufacturer's specification, maximum levels of organic impurities were ≤ 0.5% of 13-hydroxycyanocobalamin, ≤ 2% of 34-methylcyanocobalamin, ≤ 0.5% for 8-epi-cyanocobalamin, ≤ 0.5% of any other unidentified impurity (on a dry basis) and ≤ 0.5% of residual solvent (acetone). Five batches[17] of the additive were tested for these impurities and they all showed compliance with the specification. Three batches[18] of the additive were analysed for inorganic impurities: lead content was 0.15 mg/kg, 2.5 mg/kg and below the limit of quantification (LOQ); cadmium, mercury, fluorine and arsenic content was below the limit of detection (LOD).[19] Cyanide residues were tested in three batches and were all below LOD.[20]

Dioxins and the sum of dioxins plus dioxin like PCBs concentrations[21] were 0.0089–0.047 ng WHO-PCDD/F-TEQ/kg and 0.0089–0.047 ng WHO-PCDD/F-PCB-TEQ/kg; non dioxin-like PCBs were below LOQ of 0.5 μg/kg in three batches tested.

The analysis of mycotoxins fumonisins (B1 + B2), deoxynivalenol, T-2 toxin, ochratoxin A, zearalenone was carried out in three batches and showed values below the corresponding LOQ.[22] Aflatoxins (B1, G1, B2, G2) were tested in three different batches[23] and all resulted below LOQ.[24]

Microbiological contamination was tested in three batches[18] by determination of total plate count, yeasts and moulds and Enterobacteriaceae which were in all cases below the LOD (≤ 10 CFU/g), and in line with the specification. *Bacillus cereus* and *Salmonella* spp. were non detected in 10 g of sample.

The levels of endotoxins (lipopolysaccharides) were tested in 10 batches and results were all below the LOD.[25]

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

The potential presence of viable cells of the production strain was investigated in three batches of the additive (three replicates per batch). In each replicate, 3 g of vitamin B12 sample was diluted in 12 mL of sterile buffered peptone water, mixed, and 5 mL of this solution was plated on five different tryptic soy agar (TSA) plates (1 mL/plate). The plates were incubated at 30°C for 6 days. A proper positive control was included in the analysis. No cells of the production strain were detected in the analysed samples.[26]

The potential presence of DNA from the production strain was investigated in 1 g samples of three batches of the vitamin B12, in triplicate. The primers targeted a gene having 75% similarity to *aac(3) IIb* gene and the expected size of the amplicon was of 703 bp. The DNA extraction protocol included a lysis step. Positive and negative controls were included. The LOD in samples spiked with genomic DNA of the production strain was < 1 ng/g of product. No DNA was detected in any of the analysed samples.[27]

[14] Technical dossier/EFSA_Sin_Reply_B12_Q7.
[15] Technical dossier/Section II/Annex II_1 to Annex II_5 and EFSA_Sin_Reply_B12.
[16] Vitamin B12 produced by fermentation is described in the European Pharmacopoeia (PhEur 11th Ed, Monograph 0547) with a purity of 96.0–102.0% in the dried substance, less than 3% total related impurities, loss on drying< 12%.
[17] Technical dossier/Section II/Annex II_1, Annex II_2, Annex II_3, Annex II_4 and Annex II_5.
[18] Technical dossier/Section II/Annex II_3, Annex II_4 and Annex II_5.
[19] Limit of quantification: LOQ(Pb) = 0.1 mg/kg; Limit of detection: LOD(Cd) = 0.01 mg/kg; LOD(Hg) = 0.01 mg/kg; LOD (As) = 0.04 mg/kg; LOD(F) = 3 mg/kg.
[20] Technical dossier/Section II/Annex II_6; Annex II_30; LOD(CN^-) = 1.6 mg/kg.
[21] Technical dossier/Section II/ Annex_II_3, Annex_II_4, and Annex_II_5.
[22] LOQ(fumonisin B1 + B2) = 0.05 mg/kg; LOQ(deoxynivalenol) = 0.1 mg/kg, LOQ(T-2 toxin) = 2 μg/kg; LOQ(ochratoxin A) = 5 μg/kg; LOQ(zearalenone) = 0.01 mg/kg; EFSA SIn_ADR Reply.
[23] Technical dossier/Section II/Annex II_7, Annex II_8 and Annex II_9.
[24] LOQ(aflatoxin B1) = 3 μg/kg, LOQ(aflatoxin B2) = 3 μg/kg, LOQ(aflatoxin G1) = 2 μg/kg and LOQ(aflatoxin G2) = 2 μg/kg.
[25] Technical dossier/Section II/Annex II 10; LOD = 3.2 endotoxin units (EU)/mg.
[26] Technical dossier/Annex II_17 and EFSA SIN reply B12/Annex 1.1,
[27] Technical dossier/ EFSA_Sin_Reply/Annex 1.2.

3.1.5. Physico-chemical properties of the additive

The additive is a dark red crystalline powder, without a specific odour. The additive is sparingly soluble in water (12.5 g/L)[28] and in ethanol, and practically insoluble in acetone, ether and chloroform. Cyanocobalamin has a melting point > 300°C. Its bulk density[29] is reported to be 400–800 kg/m^3. The anhydrous substance is very hygroscopic.

The dusting potential of three batches of the additive was determined using the Stauber–Heubach method and showed an average value of 276.7 mg/m^3 (range 200–325 mg/m^3).[30] Particle-size distribution of the dust of three batches of the additive from Stauber–Heubach test was carried out by laser-diffraction. The fractions of particles having a diameter < 100 μm, < 50 μm, < 10 μm and < 1 μm were on average 100%, 99.9%, 31.7% and 3.18%, respectively.

The particle size of the additive was analysed by the laser diffraction method; the fractions of particles having a diameter < 100 μm, < 50 μm, <10 μm and < 1 μm were on average 53.46%, 19.3%, 3.26% and 0.38%, respectively.

It is noted that the data available do not allow to exclude the presence of small/nano particles as foreseen in the Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA Scientific Committee, 2021). Therefore, the applicant was requested to provide information choosing any of the appraisal routes as indicated by the aforementioned guidance document.

The applicant provided a dissolution test at pH 7.0 (target test concentration 1 mg/mL).[31] The result of the dissolution test (98% in 10 min) met the criteria of Section 2.3.2 of the Guidance on Particle – TR (EFSA Scientific Committee, 2021). The Panel noted that an ultrafiltration step was not included and, therefore, the test was not fully compliant with the above-mentioned guidance. However, when considering the large difference between the water solubility (12.5 g/L) and the expected use levels of cyanocobalamin (up to 150 μg/kg complete feed, see Section 3.1.7), the Panel concluded that any potential cyanocobalamin nanoparticles present in the additive would be fully solubilised in the gastrointestinal tract and, therefore, a conventional risk assessment should be sufficient.

3.1.6. Stability and homogeneity

3.1.6.1. Shelf life

Three batches of the additive were stored for 60 months at 25°C and 60% relative humidity (RH).[32] Samples were stored in packaging that simulates the commercial packaging (double-layer polyethylene (PE) bag as primary packaging and aluminium bottle as outer packaging).[33] Loss at the end of the storage period was 0.2% (on dry basis).

Three other batches were stored for 6 months at 40°C/75% RH.[34] There was no loss reported at the end of the accelerated stability study period.

3.1.6.2. Stability

Stability of cyanocobalamin was tested in vitamin–mineral premix for 6 months and in pig feed (meal and pellets) for 3 months.[35]

Three different commercial vitamin–mineral premixture formulas (for pigs, ruminants and fish), were used to test the stability of the additive at inclusion rates of 1,250 mg/kg, 1,500 mg/kg and 30,000 mg/kg of cyanocobalamin, respectively when stored at room temperature and at 40°C. Losses after 6 months were on average 6.7% at room temperature. Loss of vitamin B12 after the storage period at 40°C was up to 22.8%.

Stability of cyanocobalamin was tested in pig feed (meal and pellets) at inclusion level of 55 μg/kg. Composite samples were taken both from the press meal as well as the pelleted feed in 3 replicate feed batches. The temperature of the meal after passing the conditioner was 71°C and of the pellets

[28] The value is below the threshold set in Section 2.3.1. of the Guidance on Particle – TR (EFSA Scientific Committee, 2021).
[29] Technical dossier/Section II/Section II-Identity.
[30] Technical dossier/Section II/Annex II_15.
[31] Technical dossier/EFSA SIn_ADR Reply/Annexes/Annex 8.
[32] Technical dossier/Section II/Annex II_20.
[33] Technical dossier/EFSA Sin_ADR Reply.
[34] Technical dossier/Section II/Annex II_21.
[35] Technical dossier/Section II/Annex II_22; SIn_ADR_Reply(1)/Annexes/Annex_5.

after pelletisation was 82°C. Loss of the active substance in both meal and pellets after 3 months was up to 4.8%.

3.1.6.3. Homogeneity

The capacity for homogeneous distribution of the additive was studied in one batch of vitamin–mineral premixture supplemented with cyanocobalamin at 9 mg/kg.[36] The coefficient of variation for cyanocobalamin content in 10 subsamples of the mineral premixture was 3.7%.

3.1.7. Conditions of use

Vitamin B12 (cyanocobalamin) is intended for use in feed for all animal species and categories without a maximum content and withdrawal period. The typical use levels have been reported to be in a range of 3–150 μg cyanocobalamin/kg complete feed, depending on the species (Gropp, 1994; Whittemore et al., 2002; Leeson and Summers, 2005; EFSA FEEDAP Panel, 2015). Cyanocobalamin should be incorporated into feed in the form of feed premixtures.

3.2. Safety

3.2.1. Safety of the production organism

The identity of the production strain as *E. adhaerens* was confirmed. The production strain *E. adhaerens* CGMCC 19596 was shown to be resistant to [redacted]. The interrogation of its WGS data did not conclusively exclude the presence of genes encoding for AMR, toxins or virulence factors. [redacted] However, since viable cells and DNA of the production strain were not detected in the additive, it can be concluded that vitamin B12 in the form of cyanocobalamin, produced by *E. adhaerens* CGMCC 19596 does not raise safety concerns as regards the production strain.

3.2.2. Safety for the target species

Vitamin B12 requirements for different animal species and safe supplementation levels were addressed in a previous opinion (EFSA FEEDAP Panel, 2015). Vitamin B12 itself is considered safe for the target species at current use levels. The endotoxin activity of the additive under evaluation was below the LOD of [redacted] IU/mg. These values are compared with ca. 1,000 IU/mg commonly found in feedingstuffs (Cort et al., 1990). Therefore, at the usual conditions of use of the additive in feed, the endotoxins potentially added by the additive would be insignificant compared with the background in feed. This specific product is extensively purified, ensuring that the active substance represents more than 96% (on a dry matter basis) and that the remainder is almost exclusively attributable to substance-related impurities (max 3%). Taking into account the extensive purification process and the absence of viable cells and DNA in the final product, the FEEDAP Panel considers that the use of the additive would not represent any safety concern for the target species. Moreover, the low inclusion level of vitamin B12 in animal feed provides further reassurance of the safety for target animals.

3.2.3. Safety for the consumer

Vitamin B12 has been considered of very low toxicity by several international bodies and it has a history of safe long-term use in humans (IOM, 1998; European Commission, 2000; EVM, 2003; EFSA NDA Panel, 2015). A previous EFSA opinion (EFSA FEEDAP Panel, 2015) addressed the absorption, distribution (including partitioning to eggs), metabolism and excretion of vitamin B12. It also tackled toxicology, occurrence of vitamin B12 in food and average vitamin B12 human intake in the EU. The use of vitamin B12 as a feed additive is not expected to modify substantially the content of vitamin B12 already present in food of animal origin. No concerns for the consumer are expected from the use of the cyanocobalamin in animal nutrition at current use levels.

[36] Technical dossier/Section II/Annex II_23.

3.2.4. Safety for the user

3.2.4.1. Effect on respiratory system

The dusting potential of the additive under assessment was up to 325 mg/m^3. Exposure of the users by inhalation is likely.

Users can suffer from occupational respiratory disease depending on the level of endotoxins in air and dust (Rylander et al., 1999; Thorn and Kerekes, 2001). The endotoxin content[37] in the additive is below [redacted] International Units (IU)/g (LOD). The exposure of persons handling the additive to endotoxins in the dust was calculated (Appendix A) based on the EFSA FEEDAP Guidance on user safety (EFSA FEEDAP Panel, 2012; Wallace et al., 2016) and taking the endotoxin LOD value as worst-case scenario. The health-based recommended threshold for the quantity of inhaled endotoxins per working day is 900 IU, derived from provisional occupational exposure limits given by the Dutch Expert Committee on Occupational Safety (DECOS) (Health Council of the Netherlands, 2010). Based upon the calculation of the potential endotoxin content in dust, the inhalation exposure is calculated as [redacted] endotoxin IU per working day, indicating no risk of exceeding the recommended limit of exposure by inhalation to endotoxins for persons handling the additive.

3.2.4.2. Effect on eyes and skin

The applicant conducted a literature search[38] to assess the potential of vitamin B12 (cyanocobalamin) to be irritant to skin and eyes or a skin sensitiser. The literature search was carried out using PubMed, Web of Science and Scopus. No relevant articles were identified.

The skin irritation potential of the additive was investigated in an *in vitro* skin irritation study[39] performed according to Organisation for Economic Co-operation and Development (OECD) technical guidance (TG) 439. The results of the study showed that the additive is classified as non-irritant to the skin (UN GHS 'No Category').

The eye irritation potential of the additive was investigated in an *in vitro* eye irritation study[40] performed according to OECD TG 492. Based on the results of the study, the additive is considered non-irritant to eyes (UN GHS 'No Category').

In the absence of data, the FEEDAP Panel cannot conclude on the skin sensitisation potential of the additive.

3.2.5. Conclusions on safety for the user

Cyanocobalamin is non-irritant to skin and eyes. Exposure of the users by inhalation is likely. No conclusions could be drawn on the potential of the additive to be a skin sensitiser. The potential endotoxin activity of the additive does not represent a hazard for users handling the additive when exposed by inhalation.

3.2.6. Safety for the environment

Vitamin B12 occurs in bacteria and animals. Neither viable cells nor DNA of the production strain were detected in the additive. The use of cyanocobalamin in animal nutrition is not expected to substantially increase its concentration in the environment.

3.3. Efficacy

Vitamin B12 has been globally used in animal nutrition for decades. Owing to the long history of use and its established nutritional role in domestic animals, cyanocobalamin is regarded as effective in covering the animal's requirement when administered via feed. Data on feed additive requirement, allowances and recommendations are easily accessible as standard literature for animal nutrition experts. The additive is considered to be an effective source of vitamin B12 in animal nutrition.

[37] Technical dossier/Section III/Annex III_4.
[38] Technical dossier/Section III/Annex III_5.
[39] Technical dossier/Supplementary information/Annexes/Annex 9.1.
[40] Technical dossier/Supplementary information/Annexes/Annex 9.2.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[41] and Good Manufacturing Practice.

4. Conclusions

[redacted] However, no viable cells nor DNA of the production strain were detected in the additive. Therefore, cyanocobalamin produced by *E. adhaerens* CGMCC 19596 does not raise safety concerns as regards to the production strain.

The use of the additive under assessment in animal nutrition at the recommended use levels is considered safe for the target species and for consumers.

Cyanocobalamin produced by *E. adhaerens* CGMCC 19596 is not irritant to skin and eyes. No conclusion on the potential of the additive to be a skin sensitiser could be drawn. The potential endotoxin activity of the additive does not represent a hazard for users handling the additive when exposed by inhalation.

The use of the additive under assessment in animal nutrition does not cause concern for the environment.

Cyanocobalamin produced by *E. adhaerens* CGMCC 19596 is efficacious in meeting animals' nutritional requirements when administered via feed.

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Abbreviations

ANI	average nucleotide identity
CAS	Chemical Abstracts Service
CD	Commission Decision
CFU	colony forming unit
CV	coefficient of variation
DECOS	Dutch Expert Committee on Occupational Safety
DM	dry matter
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
HSE	Health and Safety Executive
IU	International Units
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofurna
PE	polyethylene
RH	relative humidity
TSA	tryptic soy agar
TEQ	toxic equivalent
UN GHS	United Nations' Globally Harmonized System (of Classification and Labelling of Chemicals)
TG	technical guidance
WGS	whole genome sequence
WHO	World Health Organization

Appendix A – Safety for the user

Calculation of maximum acceptable levels of exposure to endotoxins from feed additives: the probable exposure time according to EFSA Guidance (EFSA FEEDAP Panel, 2012) for additives added in premixtures assumes a maximum of 40 periods of exposure per day, each comprising 20 s: 40 × 20 = 800 s/day. With an uncertainty factor of 2, maximum inhalation exposure would occur for 2 × 800 = 1,600 s = 0.444 h/day. Assuming a respiration volume of 1.25 m^3/h, the inhalation volume providing exposure to potentially endotoxin-containing dust would be 0.444 (h/day) × 1.25 (m^3/h) = 0.556 m^3 per day. This volume should contain no more than 900 IU endotoxin, so the dust formed from the product should contain no more than 900 IU/0.556 m^3 = 1,619 IU/m^3.

Calculation of endotoxin content of dust: Two key measurements are required to evaluate the potential respiratory hazard associated with the endotoxin content of the product (the dusting potential of the product, expressed in g/m^3, and the endotoxin activity of the dust, determined by the Limulus amoebocyte lysate assay (expressed in IU/g)). If data for the dust are not available, the content of endotoxins of the product can be taken instead. If the content of endotoxins of the relevant additive is: a IU/g and the dusting potential is b g/m^3, then the content of endotoxins of the dust, c IU/m^3, is obtained by simple multiplication a × b. This resulting value is further used for calculation of the potential inhalatory exposure of users to endotoxins from the additives under assessment (Table A.1) (EFSA FEEDAP Panel, 2012).

Table A.1: Estimation of user exposure to endotoxins from vitamin B12 produced by *Ensifer adhaerens* (CGMCC 19596)

Calculation	Identifier	Description	Value	Source
	a	Endotoxin content IU/g product	[redacted]	Technical dossier
	b	Dusting potential (g/m^3)	0.325	Technical dossier
a × b	c	Endotoxin content in the air (IU/m^3)	[redacted]	
	d	No of premixture batches made/working day	40	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
	e	Time of exposure (s) per production of one batch	20	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
d × e	f	Total duration of daily exposure/worker (s)	800	
	g	Uncertainty factor	2	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
f × g	h	Refined total duration of daily exposure/worker (s)	1,600	
h/3,600	i	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m^3) per eight-hour working day	10	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
j/8 × i	k	Inhaled air during exposure (m^3)	0.56	
c × k	l	Endotoxin inhaled (IU) during exposure per eight-hour working day	[redacted]	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3) per eight-hour working day	90	Health Council of the Netherlands (2010)
m × j	n	Health-based recommended exposure limit of total endotoxin exposure (IU) per eight-hour working day	900	