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SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of vitamin B_{12} (cyanocobalamin) produced by fermentation with *Ensifer adhaerens* CGMCC 21299 for all animal species (NHU Europe GmbH)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of vitamin B_{12} (cyanocobalamin) produced by fermentation with a non-genetically modified strain of *Ensifer adhaerens* (CGMCC 21299), when used as a nutritional additive for all animal species. No viable cells or DNA of the production strain were detected in the additive. Therefore, cyanocobalamin produced by fermentation with *E. adhaerens* CGMCC 21299 does not raise safety concerns as regards to the production strain. The Panel on Additives and Products or Substances used in Animal Feed concluded that cyanocobalamin produced by fermentation with *E. adhaerens* CGMCC 21299 is considered safe for all animal species, for the consumers and the environment. Due to the presence of nickel, the additive is considered a skin and respiratory sensitiser. Inhalation and dermal exposure are considered a risk. Due to the lack of data, the Panel could not conclude on the potential of the additive to be an eye irritant. Cyanocobalamin produced by fermentation with *E. adhaerens* CGMCC 21299 is effective in meeting animal's nutritional requirements when administered via feed.

KEYWORDS

cyanocobalamin, efficacy, *Ensifer adhaerens*, nutritional additives, safety, vitamin B12

CONTENTS

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 NHU Europe GmbH[2] for the authorisation of the additive consisting of cyanocobalamin (vitamin B_{12}) produced by *Ensifer adhaerens* CGMCC 21299, when used as a feed additive for all animal species (category: nutritional additives; 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). The dossier was received on 16 June 2023 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00409. The particulars and documents in support of the application were considered valid by EFSA as of 16 October 2023.

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 cyanocobalamin (vitamin B12) produced by fermentation with *Ensifer adhaerens* CGMCC 21299, when used under the proposed conditions of use (see **Section 3.1.7**).

1.2 | Additional information

The additive is a preparation containing cyanocobalamin (vitamin B_{12}), produced by fermentation with *Ensifer adhaerens* CGMCC 21299. It has not been previously authorised as a feed additive in the European Union.

Vitamin B12 (cyanocobalamin), produced with a different strain of *E. adhaerens*, is currently authorised as a nutritional additive for use in all animal species without maximum content.

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 B_{12} (in the form of cyanocobalamin) produced with other strains of *E. adhaerens* (EFSA FEEDAP Panel, 2015, 2018a, 2020, 2023a).

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[3] in support of the authorisation request for the use of cyanocobalamin (vitamin B_{12}) produced by fermentation with *Ensifer adhaerens* CGMCC 21299 as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 16 October 2023 to 16 January 2024; the comments received were considered for the assessment. In accordance with Article 38 of the Regulation (EC) No 178/2002[4] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[5] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 23 February to 15 March 2024. The comments received have been carefully evaluated and considered during the current assessment (Appendix B).

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

[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]NHU Europe GmbH, Daimlerstrasse 14–16 Bardowick – Germany.

[3]Dossier reference: FEED-2023-15991.

[4]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[5]Decision https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed.[6]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of cyanocobalamin (Vitamin B_{12}) produced by fermentation with *Ensifer adhaerens* CGMCC 21299 is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023b), 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 FEEEDAP 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, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), 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 B_{12} in the form of cyanocobalamin, produced by fermentation with *E. adhaerens* CGMCC 21299. 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.

3.1 | Characterisation

3.1.1 | Characterisation of the production organism

Vitamin B_{12} in the form of cyanocobalamin is produced by fermentation with a non-genetically modified strain of *E. adhaerens*, which was originally isolated from soil and is deposited in the China General Microbiological Culture Collection Center (GCMCC) with the accession number CGMCC 21299.[8]

[redacted]

The susceptibility of the production strain to the relevant antibiotics was tested by broth microdilution against the list of antimicrobials described for Enterobacteriaceae in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a, 2018b, 2018c).[10] The measured minimum inhibitory concentration values for kanamycin, streptomycin, colistin and fosfomycin were higher than the cut-off values specified in the guidance. Therefore, the strain is considered resistant to kanamycin, streptomycin, colistin and fosfomycin.

[redacted] No hits of concern above the thresholds recommended by EFSA (EFSA, 2021) were identified.

[redacted] No hits of concern above the thresholds recommended by EFSA (EFSA, 2021) were identified.

3.1.2 | Manufacturing process

[redacted]

[6] https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en

[7] 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.

[8] Annex_II_17.

[9] Annex_II_16a and Annex_II_16b.

[10] Annex II_20.

[11] Annex II_16a.

[12] Annex II_16a.

[redacted]

3.1.3 | Characterisation of the active substance

Cyanocobalamin (International Union of Pure and Applied Chemistry 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 3yl]propanoylamino] propan-2-yl] phosphate; cyanide; Synonyms: vitamin B_{12}, cobalamin, a-(5,6-dimethyl benzimidazole-1-yl)cobamidcyanide) is identified with the Chemical Abstracts Service number 68-19-9 and the European Inventory of Existing Chemical Substances 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.

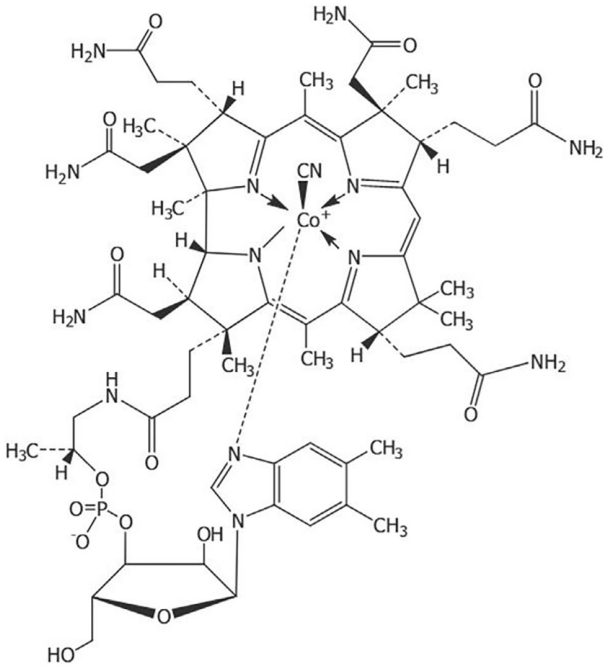

FIGURE 1 Structural formula of cyanocobalamin.

The active substance is specified to have a purity not less than 96%–100.5% and loss on drying < 12%.

Analytical data to confirm the specification were provided for eight batches of the active substance, showing the following average values: cyanocobalamin: 97.87% (range: 96.52%–98.8%), loss on drying: 3.23% (range: 2.1%–4.89%).[14]

Per manufacturer's specification, maximum levels of organic impurities were: total impurities ≤ 3%, Impurity A – 7β,8β-Lacto-cyanocobalamin ≤ 0.7%, Impurity B – 50-Carboxycyanocobalamin ≤ 0.5%, Impurity C – 34-Methylcyanocobalamin ≤ 1.5%, Impurity D – 32-Carboxycyanocobalamin ≤ 0.5%, Impurity E – 8-epi-Cyanocobalamin ≤ 0.5%, Impurity F – Unknown structure (cyanocobalamin isomer) ≤ 0.5%, any other unidentified impurities ≤ 0.2%, arsenic ≤ 10 mg/kg, lead ≤ 0.5 mg/kg, mercury ≤ 0.1 mg/kg, cadmium ≤ 0.2 mg/kg, total plate count ≤ 1000 colony forming unit (CFU)/g additive, mould and yeast ≤ 100 CFU/g additive, *E. coli* negative in 10 g, *Salmonella* negative in 25 g, *Staphylococcus aureus* negative in 25 g, residual solvent (acetone) ≤ 5000 mg/kg.

The purity of cyanocobalamin, the limits for impurities A, B, C, D, E, F, unspecified impurities and loss on drying were in line with requirements from the European Pharmacopoeia (version 11th) with regards to cyanocobalamin produced by fermentation.[15]

Five batches were tested for these impurities, and they all showed compliance with the specifications: total impurities 0.58% (range: 0.53%–0.72%), Impurity A 0.122% (range: 0.09%–0.17%), Impurity C 0.088% (range: 0.08%–0.09%), Impurity

[13]Annex_II_22a.

[14]Annex_II_02a.

[15]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%.

E 0.15% (range: 0.12%–0.18%), any other unidentified impurities 0.13% (range: 0.1%–0.16%). Impurity B, Impurity D and Impurity F were not detected in any of the batches tested.[16]

Three batches[17] were tested for possible presence of arsenic, lead, cadmium and mercury. All the results were below the respective limit of quantification (LOQ).[18] The same three batches[19] were further tested for possible presence of iron, copper, nickel, zinc, tin, aluminium, chromium. All the results were below the respective LOQ[20] with the exception of copper (< 0.5 mg/kg), zinc (< 0.5 mg/kg) and chromium (< 0.5 mg/kg).

Cobalt was also analysed and showed values ranging from 38,300 to 45,300 mg/kg (average: 41,566 mg/kg).

Microbiological contamination was tested in three batches and showed the following values, total plate count: ≤ 10 CFU/g additive, mould and yeast ≤ 100 CFU/g additive, *E. coli* absence in 10 g, *Salmonella* absence in 25 g, *Staphylococcus aureus* absence in 25 g, aerobic spore-formers < 10 CFU/g, anaerobic spore-formers < 10 CFU/g, mesophilic sulfite red. Clostridia < 10 CFU/g, coagulase positive staphylococci absence in 25 g, *Listeria monocytogenes* absence in 25 g.[21]

Possible presence of mycotoxins[22] was investigated in three batches and showed values below the respective LOQ.[23]

Polycyclic aromatic hydrocarbons (PAHs) and nitrosamines were also tested in three batches and were below the corresponding LOQs. In the same batches, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (PCBs) were below the corresponding LOQ.[24]

The calculated upper bound (UB) concentrations for the sum of dioxins was 0.056 ng WHO PCDD/F-toxic equivalent (TEQ) (World Health Organization PCDD) and PCDF-TEQ/kg, 0.059 ng WHO PCB-TEQ/kg for the sum of dioxin-like PCBs and was 0.116 ng WHO PCCD/F + PCB-TEQ/kg for the sum of dioxins and dioxin-like PCBs (all values are expressed based on 88% dry matter).[25]

Pesticides were not detected in a multiresidue analysis in one batch.

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

The capacity of the production strain to produce substances with antimicrobial activity was investigated in three batches of cyanocobalamin and one batch of the fermentation liquor from *E. adhaerens* CGMCC 21299.[26] A disk diffusion method and the following indicator strains were used: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis* ATCC 6633. No antimicrobial activity was detected.

The presence of viable cells[27] of the production strain was evaluated in three batches of cyanocobalamin, [redacted] No growth of the production strain was detected.

The presence of DNA[28] from the production strain was tested in three batches of cyanocobalamin, [redacted] No DNA of the production strain was detected.

The levels of endotoxins (lipopolysaccharides) were tested in six batches of the active substance and ranged between [redacted] International Units (IU)/g.[29]

Cyanocobalamin is a dark red crystalline powder or dark red crystals and is soluble in ethanol. The water solubility is 12.5 g/L.[30]

3.1.4 | Characterisation of the additive

The additive is composed by the active substance (cyanocobalamin) ≤ 1% mixed with a carrier (such as corn starch).

[18]LOQ: arsenic 0.1 mg/kg, lead 0.01 mg/kg, cadmium 0.01 mg/kg, mercury 0.01 mg/kg.

[16]Annex_II_02b.

[17]Annex_II_07.

[19]Annex_II_07.

[20]LOQ: iron 2 mg/kg, nickel, 2 mg/kg, tin 0.1 mg/kg, aluminium 0.5 mg/kg.

[21]Annex_II_05a and Annex_II_5b.

[22]Annex_II_10a.

[23]LOQ: Deoxynivalenol 2 µg/kg; HT2-Toxin 4 µg/kg; T2-Toxin 0.4 µg/k; Ochratoxin A, Fumonisin B1 1 µg/kg; Zearalenone 4 µg/kg; Fumonisin B2 10 µg/kg.

[24]LOQ: PAHs (16 congeners) < 0.2–< 0.5 µg/kg; nitrosamines (9 compounds): < 0.5 µg/kg. PCCF/PCDD: < 0.010–< 0.500 ng/kg; PCBs: < 0.5–< 20 ng/kg.

[25]Annex_II_07. Upper bound concentrations are calculated on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification. TEQ = toxic equivalency factors for PCDD/Fs and DL-PCBs established by WHO in 2005 (van den Berg et al., 2006).

[26]Annex II_21.

[27]Annex_II_24_Viable-cells-absence_confidential.pdf.

[28]Annex_II_23_DNA-presence-test_confidential.pdf.

[29]Annex_II_15.

[30]For solubility terms, see Table 2 of the Guidance on Particle-TR (EFSA Scientific Committee, 2021).

The applicant proposed the following specifications of the additive under assessment: cyanocobalamin ≤ 1%, lead ≤ 10 mg/kg, arsenic ≤ 2 mg/kg, cadmium ≤ 1 mg/kg, mercury ≤ 0.1 mg/kg and loss on drying ≤ 12%. Five batches of the additive were analysed, and the content of cyanocobalamin ranged from 1.00% to 1.03% (average: 1.01%). The loss on drying ranged from 6.84% to 7.74% (average: 7.15%).[31] Three batches of the additive[32] were tested for possible presence of arsenic, lead, cadmium and mercury. All the results were below the respective LOQ.[33]

The analyses demonstrated compliance of the additive with the proposed specifications.

Three batches[34] were further tested for iron 17.6 mg/kg (range: 10.9–30.2 mg/kg), copper 0.39 mg/kg (range: 0.35–0.42 mg/kg), nickel 0.38 mg/kg (range: 0.31–0.5 mg/kg), zinc 1.56 mg/kg (range: 1.45–1.75 mg/kg), tin 0.07 mg/kg (range 0.07–0.1 mg/kg), aluminium 5.37 mg/kg (range 4.85–6.35 mg/kg), chromium 0.22 mg/kg (two batches showed values below the LOQ of 0.2 mg/kg), cobalt 577 mg/kg (range: 564–588 mg/kg), vanadium 0.08 mg/kg (range: 0.07–0.09 mg/kg, one batch showed values below the LOQ of 0.05 mg/kg).

Possible presence of mycotoxins[35] was investigated in the same three batches of the additive and showed values below the respective LOQ.[36]

PAHs and nitrosamines were also tested[37] on three batches and all the results were below the corresponding LOQ with the exception of one batch where N – Nitrosodimethylamine was reported as 1.8 µg/kg. In the same batches, pesticides were not detected in a multiresidue analysis and dioxins and PCBs were below the corresponding LOQ.

The calculated UB concentrations for the sum of dioxins was 0.056 ng WHO PCDD/F-TEQ (World Health Organization PCDD) and PCDF TEQ/kg, 0.059 ng WHO PCB-TEQ/kg for the sum of dioxin-like PCBs and was 0.116 ng WHO PCCD/F + PCB-TEQ/kg for the sum of dioxins and dioxin-like PCBs (all values are expressed based on 88% dry matter).[38]

Three batches of the additive were tested for possible presence of microbiological impurities and showed the following values, total plate count: ≤ 10 CFU/g additive, mould and yeast: ≤ 100 CFU/g additive, aerobic spore-formers: < 10 CFU/g, anaerobic spore-formers: < 10 CFU/g, mesophilic sulfite red. Clostridia: < 10 CFU/g, Coagulase pos. *Staphylococci*: negative in 25 g, *E. coli*: negative in 10 g, *Salmonella*: negative in 25 g, *Staphylococcus aureus*: negative in 25 g, *Listeria monocytogenes*: negative in 25 g.[39]

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities, do not raise safety concerns, with the exception of nickel (see Section 3.2.3). The presence of viable cells and DNA was excluded in the cyanocobalamin used to formulate the additive.

The levels of endotoxins (lipopolysaccharides) were tested in six batches of the additive and results ranged between [redacted] IU/g.[40]

3.1.5 | Physical properties of the additive

The bulk density was determined in three batches of the additive and was on average 544 kg/m^3 (502–571 kg/m^3).[41]

The dusting potential of three batches[42] of the additive was determined using the Stauber–Heubach method and showed values on average of 2036 mg/m^3 (range 1490–2335 mg/m^3) (mg airborne dust per m^3 of air).

Particle size of the additive was measured by means of laser diffraction on the same three batches of the additive. On average, 0.25% of the particles were < 1 µm, 7.48% < 10 µm, 72.15% < 50 µm and 96.56% < 100 µm.[43]

The water solubility of cyanocobalamin (12.5 g/L) is below the threshold set by 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). However, when considering the vitamin B_{12} requirements for different animal species (EFSA FEEDAP Panel, 2015), it is expected that nanoparticles of cyanocobalamin if present in the additive would be fully solubilised in the gastrointestinal tract of the target species; therefore, no further characterisation of the fraction of small/nano particles is needed.

[31] Annex_II_02c.

[32] Annex_II_08.

[33] LOQ: arsenic 0.04 mg/kg, lead 0.015 mg/kg, cadmium 0.01 mg/kg, mercury 0.005 mg/kg.

[34] Annex_II_08.

[35] Annex_II_09.

[36] LOQ: Deoxynivalenol 2 µg/kg, HT2-Toxin 4 µg/kg, T2-Toxin 0.4 µg/kg, Ochratoxin A 1 µg/kg, Zearalenone 4 µg/kg, fumonisin B1 1 µg/kg and fumonisin B2 10 µg/kg.

[37] Annex_II_08; LOQ: PAHs (16 congeners) < 0.2–< 0.5 µg/kg; nitrosamines (9 compounds): < 0.5 µg/kg; pesticides: < 0.0030–< 0.050 mg/kg.

[38] Annex_II_07. Upper bound concentrations are calculated on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification. TEQ = toxic equivalency factors for PCDD/Fs and DL-PCBs established by WHO in 2005 (van den Berg et al., 2006).

[39] Annex_II_06.

[40] Annex_II_15.

[41] Annex_II_14.

[42] Annex_II_14.

[43] Annex_II_14.

3.1.6 | Stability and homogeneity

The shelf life of the active substance (three batches) was studied when stored at 25°C for (24 months). Losses at the end of the storage period were on average 0.3%.[44]

The shelf life of the additive (three batches) was studied when stored at 25°C for (24 months). No losses were recorded at the end of the storage period.[45]

The stability of the additive (three batches) in a vitamin–mineral premixture for pigs was studied when supplemented at 0.08% and stored at 25 and 40°C for (6 months). The recovery rate was 75.8% and 49.09%, respectively.

The stability of the active substance (one batch) in mash feed for pigs was studied when supplemented at 0.03% and stored at (25°C) for 3 months. No losses were recorded at the end of the storage period.[46]

The stability of the active substance (three batches) in pelleted pigs feed (pelleting temperature: 75–85°C) was studied when supplemented at 0.03% and stored at 25 and 40°C for 3 months. No losses were recorded at the end of the storage period.[47]

The capacity for homogeneous distribution of cyanocobalamin in premixtures was studied in 21 subsamples of 1 batch added to the vitamin–mineral premixture for pigs. The coefficient of variation was 0.04%.[48] No data were provided on the capacity of the additive to homogenously distribute in feed.

3.1.7 | Conditions of use

The additive is intended for use in feed for all animal species without a maximum or minimum level. The additive must be incorporated into feed through premixtures.

3.2 | Safety

Safety concerns from the additive may derive either from the active substance or from the residues of the fermentation process/production strain remaining in the final product. The product under assessment is highly purified (less than 1% unidentified material). The production strain *Ensifer adhaerens* CGMCC 21299 was not genetically modified and was shown not to carry genes of concern. In addition, its viable cells and DNA were not detected in the final product. It can be concluded that no safety concerns for target animals, consumers and the environment would arise from the use of *Ensifer adhaerens* CGMCC 21299 as a production strain.

To identify any relevant information available on the safety of vitamin B_{12} and the production organism, the applicant has conducted an extensive literature search.[49] A first search was conducted in 2021 using relevant agricultural, aquacultural, medical and veterinary databases. In 2023, an updated search was done using the LIVIVO database and an additional manual search was done in Google Scholar. Details on the keywords used and the inclusion and exclusion criteria were provided.

A total of 79 papers were retrieved: seven papers on the safety of *E. adhaerens* (not the strain under assessment), 14 for the safety of the target species, 46 for the consumers, 11 for the users and one for the environment. None of these papers were considered relevant for the assessment of vitamin B_{12} produced by fermentation with *E. adhaerens* CGMCC 21299.

3.2.1 | Safety for the target species

In the current application dossier, the applicant did not submit any tolerance studies conducted in the target species with the additive under assessment.

Vitamin B_{12} requirements for different animal species and safe supplementation levels were addressed in a previous opinion (EFSA FEEDAP Panel, 2015). Vitamin B_{12} itself is considered safe for the target species at current use levels. The highest endotoxin activity of the additive under evaluation measured was 48 IU/g. These values are compared with ca. 1,000,000 IU/g 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. The active substance is extensively purified, ensuring that cyanocobalamin represents more than 96% of the active substance (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 B_{12} in animal feed provides further reassurance of the safety for target animals.

[44]Annex_II_RFI-02_ShelfLife_VB12-pilot and Annex II RFI-04 ShelfLife VB12.
[45]Annex_II_RFI-01_ShelfLife_VB12-pilot and Annex II RFI-03 ShelfLife VB12.
[46]Annex_II_25_Homogeneity-and-stability_confidential.pdf.
[47]Annex_II_25_Homogeneity-and-stability_confidential.pdf.
[48]Annex_II_25_Homogeneity-and-stability_confidential.pdf.
[49]Annex_III_01_Literature_search_confidential.pdf.

3.2.2 | Safety for the consumer

Vitamin B_{12} has been considered of very low toxicity by several international bodies and it has a history of safe long-term use in humans (EFSA NDA Panel, 2015; European Commission, 2000; EVM, 2003; IOM, 1998). A previous EFSA opinion (EFSA FEEDAP Panel, 2015) addressed the absorption, distribution (including partitioning to eggs), metabolism and excretion of vitamin B_{12}. It also tackled toxicology, occurrence of vitamin B12 in food and average vitamin B_{12} human intake in the EU. The use of vitamin B_{12} as a feed additive is not expected to modify substantially the content of vitamin B_{12} 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.

3.2.3 | Safety for the user

3.2.3.1 | *Effect on respiratory system*

The highest dusting potential of the additive under assessment measured was 2335 mg/m^3, therefore 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 & Kerekes, 2001). The endotoxin content in the additive is up to ■ IU/g. The exposure of persons handling the additive to endotoxins in the dust was calculated (Appendix A) (Wallace et al., 2016) taking the highest endotoxin value measured 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 (HCN, 2010). Based upon the calculation of the potential endotoxin content in dust, the inhalation exposure is calculated as 62 endotoxin IU per working day, indicating no risk of exceeding the recommended limit of exposure by inhalation to endotoxins for persons handling the additive.

The nickel content in the additive was up to 0.5 mg/kg. Considering the dusting potential of 2335 mg/m^3 and assuming a similar proportion of nickel in the dust as in the additive, the nickel content in the dust would be up to 0.0012 mg Ni/m^3. This value would not exceed the transitional limit value of 0.1 mg Ni/m^3 for the inhalable fraction and 8 h time-weighted average (8 h TWA) exposure established in Directive (EU) 2022/431.[50] However, being nickel present in the additive, it should be considered a respiratory sensitiser.

3.2.3.2 | *Effect on eyes and skin*

No studies conducted with the additive under assessment were submitted by the applicant. The applicant identified 11 papers from the literature search conducted. None of these papers were considered relevant by the FEEDAP Panel as they did not provide information on the final formulation of the additive under assessment.

Due to the presence of nickel in the additive (up to 0.5 mg/kg from three batches), and given its well-known sensitisation potential, the additive is considered a skin sensitiser.

3.2.3.3 | *Conclusions on safety for the user*

Due to the presence of nickel the additive is considered a skin and respiratory sensitiser. Inhalation and dermal exposure are considered a risk. Due to the lack of data, the Panel could not conclude on the potential of the additive to be an eye irritant.

3.2.4 | Safety for the environment

Vitamin B_{12} 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. Therefore, the use of the additive is considered safe for the environment.

3.3 | Efficacy

Vitamin B_{12} 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

[50]Directive (EU) 2022/431 of the European Parliament and of the Council of 9 March 2022 amending Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work. 16.3.2022, OJ 88/1. According to Directive (EU) 2022/431, the limit value for the inhalable fraction of nickel until 18 January 2025 is 0.1 mg/m^3 (measured as nickel). After this date limit values of 0.05 mg/m^3 and 0.01 mg/m^3 (measured as nickel) shall apply for the respirable and the inhalable fractions, respectively.

administered via feed. Data on vitamin B_{12} 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 B_{12} in animal nutrition.

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[51] and Good Manufacturing Practice.

4 | CONCLUSIONS

No viable cells or DNA of the production strain were detected in the additive. Therefore, cyanocobalamin produced by fermentation with *E. adhaerens* CGMCC 21299 does not raise safety concerns as regards to the production strain.

The use of the additive under assessment in animal nutrition to cover the nutritional needs of the animals is considered safe for the target species, for consumers and the environment.

Due to the presence of nickel the additive is considered a skin and respiratory sensitiser. Inhalation and dermal exposure are considered a risk. Due to the lack of data, the Panel could not conclude on the potential of the additive to be an eye irritant.

Cyanocobalamin produced by fermentation with *E. adhaerens* CGMCC 21299 is efficacious in meeting animals' nutritional requirements when administered via feed.

ABBREVIATIONS

AMR	antimicrobial resistance
CFU	colony forming unit
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GCMCC	Chinese General Microbiological Collection Center
LOQ	limit of quantification
PAHs	polycyclic aromatic hydrocarbons
PCB(s)	polychlorinated biphenyls
PCDD(s)	polychlorinated dibenzo-*p*-dioxins
PCDF(s)	polychlorinated dibenzofurans
PhEur	European Pharmacopoeia
TEQ	toxic equivalent
UB	upper boundWGSwhole genome sequencing

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

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[51]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.

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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 = 1600 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 = 1619 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 amoebocytelysate 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 $a \times b$. 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 B_{12} produced by fermentation with *Ensifer adhaerens* (CGMCC 21299).

Calculation	Identifier	Description	Value	Source
	a	Endotoxin content IU/g product	■	Technical dossier
	b	Dusting potential (g/m^3)	2.3	Technical dossier
a*×*b	*c*	Endotoxin content in the air (IU/m^3)	112	
	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)	1600	
***h*/3600**	*i*	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m^3) per 8-h working day	10	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
j*/8×*i	*k*	Inhaled air during exposure (m^3)	0.556	
c*×*k	*l*	Endotoxin inhaled (IU) during exposure per 8-h working day	62	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3) per 8-h working day	90	Health Council of the Netherlands (2010)
m*×*j	*n*	Health-based recommended exposure limit of total endotoxin exposure (IU) per 8-h working day	900	

APPENDIX B

Outcomes of the public consultation on the non-confidential version of the technical dossier

Comment 1: Generally for a wild strain to become a industrial use one, it needs lots of strain screening and costs years of work under non-GMO technique. Since there is no information about when this strain is isolated, thus, about the strain history and history of modification. It is better to provide when this strain is isolated and its original production level for B12. By comparing this, the EFSA could determine whether there is any chance of GMO modification during screening. If it takes a short time from transforming a wild strain to an industrial level, it may raise the concern of using GMO technique.

Comment 2: Besides, by reviewing the non-GMO evidence provided, the GMO screen in the product is not considered as enough, since the applicant said there is no visible cell or DNA detected in the product. Further study shall needed.

EFSA evaluation: According to Commission Regulation (EC) No 429/2008 and the Guidance on the characterisation of microorganisms used as feed additives or as production organisms, information on the origin of the organism under assessment and its history of modifications should be provided. In the 'Detailed identification and characterisation of the additive(s)' the applicant submitted information on the history of *Ensifer adhaerens* CGMCC 21299, that is, the strain was not genetically modified and isolated from soil in China. The information is deemed sufficient to establish the history of the strain. Further studies/information, including the isolation date of the strain and comparison of vitamin B12 production levels, are not considered necessary or required by the above-mentioned Regulation/Guidance.

Comment 3: Following a mandate (M-2019-0199) from the European Commission, EFSA has published a Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles, including nanoparticles (EFSA SC Guidance on particle–TR). Despite the particle size analysis was previously provided by the applicant within the dossier, no scientific evidence that the additive meets the dissolution rate criteria indicated in the EFSA Guidance was provided and found.

EFSA evaluation: the water solubility of the additive is reported by the applicant in the dossier to be 12.5 g/L. The FEEDAP Panel is aware that this value is below the threshold set by 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). However, when considering the vitamin B_{12} requirements for different animal species (EFSA FEEDAP Panel, 2015), the FEEDAP Panel expects that nanoparticles of cyanocobalamin, if present in the additive, would be fully solubilised in the gastrointestinal tract of the target species; therefore, no further information on water solubility or on characterisation of the fraction of small/nanoparticles is needed.