SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of riboflavin-5’-phosphate ester monosodium salt (vitamin B₂) (from riboflavin 98%, produced by Bacillus subtilis KCCM 10445) for all animal species (Hubei Guangji Pharmaceutical Co. Ltd)

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of vitamin B₂ in the form of riboflavin-5’-phosphate ester monosodium salt. The additive is manufactured by chemical synthesis from riboflavin 98%, produced by fermentation with a genetically modified strain of Bacillus subtilis (KCCM 10445). Riboflavin 98% has been previously evaluated by the FEEDAP Panel for its use as feed additive for all animal species. Since the additive under assessment contains the phosphate ester monosodium salt of a riboflavin (98%) preparation already considered safe, the FEEDAP Panel concluded that the addition of the salt does not add any toxicological property to the final additive. Therefore, the additive is safe for the target species, the consumer and the environment. The additive is not a skin/eye irritant and it is not considered a respiratory sensitiser. Riboflavin is a known photosensitiser which may elicit skin and eye photoallergic reactions. The additive under assessment is effective in covering the animals’ requirements for vitamin B₂ when administered via feed and/or water for drinking.

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Keywords: vitamin B2/riboflavin, Bacillus subtilis, nutritional additive, vitamins, safety, efficacy

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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 Hubei Guangji Pharmaceutical Co. Ltd represented in the EU by Guang Ji Pharmaceutical Belgium SA NV\(^2\) for the authorisation of the additive consisting of riboflavin-5’-phosphate ester monosodium salt (vitamin B\(_2\)), when used as a feed additive for all animal species (category: nutritional additive; functional group: Vitamins, provitamins and chemically well-defined substances having a similar effect).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). 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 31 March 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 riboflavin-5’-phosphate ester monosodium salt (vitamin B\(_2\)), when used under the proposed conditions of use (see Section 3.1.6).

1.2. **Additional information**

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued two opinions on the safety and efficacy of vitamin B\(_2\) (80%) as riboflavin produced by *B. subtilis* KCCM 10445 for all animal species (EFSA FEEDAP Panel, 2014, 2018a). In its opinion of 2014 (EFSA FEEDAP Panel, 2014), the Panel concluded that neither the production strain nor its recombinant DNA were detected in the final product, and therefore, the additive did not give rise to any safety concern with regard to the genetic modification of the production strain. In the context of official controls, viable cells and recombinant DNA from the production strain were found in reference samples other than those used to produce the data included in the dossier submitted to EFSA. Based on the new data provided, the FEEDAP Panel issued a second opinion in 2018 (EFSA FEEDAP Panel, 2018a) and concluded that the product vitamin B\(_2\) (80%) as riboflavin posed a risk for the target species, consumers, users and the environment due to the presence of viable cells and DNA of a genetically modified strain-harbouring genes coding for resistance to antimicrobials of human and veterinary importance.

The FEEDAP Panel issued an opinion on the safety and efficacy of vitamin B\(_2\) as riboflavin and riboflavin-5’-phosphate ester monosodium salt, produced by either *B. subtilis* DSM 17339 or *B. subtilis* DSM 23984 (EFSA FEEDAP Panel, 2016). More recently, EFSA has issued another opinion on safety and efficacy of vitamin B\(_2\) (98%) and vitamin B\(_2\) (80%) as riboflavin produced by *B. subtilis* KCCM 10445 for all animal species (EFSA FEEDAP Panel, 2021).

Riboflavin produced by fermentation using different production strains is currently authorised for its use in all animal species as a nutritional additive.\(^3\) Following the EFSA opinion issued in 2018, the authorisation of vitamin B\(_2\) (80%) produced by *B. subtilis* KCCM 10445 has been denied by Commission Implementing Regulation (EU) 2018/1254\(^4\).

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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. Hubei Guangji Pharmaceutical Co. Ltd represented in the EU by GUANG JI PHARMACEUTICAL BELGIUM SA NV, Rue Laid Burniat 3, 1348, Louvain-la-Neuve, Belgium.
3. Commission Implementing Regulation (EU) 2019/901 of 29 May 2019 concerning the authorisation of riboflavin produced by *Ashbya gossypii* (DSM 23096), riboflavin produced by *Bacillus subtilis* (DSM 17339 and/or DSM 23984) and riboflavin 5’-phosphate sodium salt produced by *Bacillus subtilis* (DSM 17339 and/or DSM 23984) (sources of vitamin B2) as feed additives for all animal species. OJ L 144, 3.6.2019, p. 41.
4. Commission Implementing Regulation (EU) 2018/1254 of 19 September 2018 concerning the denial of authorisation of riboflavin (80%) produced by *Bacillus subtilis* KCCM-10445 as a feed additive belonging to the functional group of vitamins, pro-vitamins and chemically well-defined substances having similar effect. OJ L 37, 20.9.2018, p. 5-8.
Riboflavin-5’-phosphate ester monosodium salt for all animal species

Riboflavin sodium phosphate is included in the current edition of the European Pharmacopoeia (PhEur) monograph 10.0/0786 (PhEur, 2020).

Riboflavin is included in the current edition of the PhEur monograph 10.0/0292 (PhEur, 2021).

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\(^5\) in support of the authorisation request for the use of riboflavin-5’-phosphate ester monosodium salt (vitamin B\(_2\)) as a feed additive.

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. The Executive Summary of the EURL report can be found in Annex A.\(^6\)

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the additive is in line with the principles laid down in Regulation (EC) No 429/2008\(^7\) 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) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

This application deals with vitamin B\(_2\) in the form of riboflavin-5’-phosphate ester monosodium salt produced by chemical synthesis from riboflavin 98%. Riboflavin 98%, used as a substrate in the manufacturing process, is produced by fermentation with a genetically modified strain of \textit{B. subtilis} (KCCM 10445) (EFSA FEEDAP Panel, 2021, 2022). The product under assessment is intended to be used as a nutritional additive (functional group: vitamins, provitamins and chemically well-defined substances having a similar effect) in feed and water for drinking for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the riboflavin production organism

The riboflavin 98%, used as a reagent in the production process, is produced by fermentation with a genetically modified strain of \textit{B. subtilis} (KCCM 10445). The strain was characterised in full in the first assessment by the FEEDAP Panel (EFSA FEEDAP Panel, 2014). The identity of the production strain and the characterisation of its genetic modification were more recently evaluated by the FEEDAP Panel in 2021 (EFSA FEEDAP Panel, 2021).

Viable cells and recombinant DNA of this production strain were not detected in the riboflavin 98%.\(^9\)

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\(^5\) FEED dossier reference: FAD-2020-0094.

\(^6\) The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2020-0094_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.

\(^8\) Technical dossier/Section II/Annexes II_3-7-8
In its assessment of 2021, the FEEDAP Panel could not conclude on the correspondence of the strain deposited under accession number KCCM 10445 with the strain currently in use for production due to the differences compared to the genetic modification steps reported in the opinion of 2014.

In the current submission, the applicant conducted a bioinformatic analysis to compare the whole-genome sequencing (WGS) data, including the plasmid sequence, of the deposited strain and the one that is currently in use for the production of the additive under assessment. The applicant sequenced the WGS of the two strains, assembled and then compared them by alignment. The WGS alignment did not show significant deletions, insertions or inversions. Moreover, the differences found between the genome of the deposited strain and that used for production were inspected and the position of all the variants and indels, and their putative effect on the B. subtilis genes was investigated: and might be due to errors of the sequencing/assembly technology used.

The small number of nucleotide differences between the genome sequence of the strain deposited under accession number KCCM 10445 and the strain used for production of the additive under assessment, strongly support that they belong to different cultivars of the same strain. Therefore, the Panel concludes that the correspondence between the two strains is now confirmed.

### 3.1.2. Manufacturing process

Riboflavin 98% (produced by fermentation with a genetically modified strain of Bacillus subtilis KCCM 10445).

### 3.1.3. Characterisation of the additive

Riboflavin 5′-phosphate ester monosodium salt, produced by chemical synthesis, consists of an orange-yellow crystalline powder.

The International Union of Pure and Applied Chemistry (IUPAC) name is riboflavin-5′-phosphate sodium; monosodium (2R, 3R, 4S)-5-(3′)10′-dihydro-7,8-dimethyl-2,4-dioxo-10-(benzo[γ]pteridinyl)-2,3,4-trihydroxypentyl phosphate; monosodium salt of 5′-monophosphate ester of riboflavin2; riboflavin sodium salt; riboflavin sodium phosphate, the Chemical Abstracts service (CAS) number is 130-40-5, the European Inventory of Existing Chemical Substances (EINECS) number is 204-988-6, the molecular weight is 478.33 g/mol and the molecular formula is C17H20N4NaO9P.11

The structural formula of the active substance is presented in Figure 1.
Riboflavin sodium phosphate is described in the PhEur 10.0, 0786 with a purity of 73–79% in the dried substance, ≤6% (dried substances) for the sum of impurities A, B and C (riboflavin diphosphates, 3’,4’,5’- and 4’,5’-diphosphate, respectively), ≤6% impurity D (riboflavin), inorganic phosphate ≤1.5% and loss on drying ≤8.0%.

In line with the PhEur, the additive is specified to contain a minimum of 73% riboflavin on dry matter basis. Free riboflavin is ≤6.0%, riboflavin diphosphate is ≤6.0%, inorganic phosphate is ≤1.5% and loss on drying is ≤8.0%.

Analytical data to confirm the specifications were provided for six batches of the additive, analysed according to the methods described in the PhEur. Riboflavin from riboflavin 5-phosphate sodium in the dry product was on average 74.7% (74.4–75.6%) corresponding to 95% riboflavin 5-phosphate, loss on drying 4.35% (4.2–4.5%). Free riboflavin was 2.58% (2.5–2.8%). Based on the analytical results provided, the additive is produced in compliance with the proposed specifications and with the specifications of PhEur 10.0 (riboflavin from 73% to 79%).

The pH of a 1% solution is 5.0–6.5. The specific rotation is +38.0–43.0. Residual solvents are in compliance with PhEur 10.0 specifications.

Lumiflavin (7,8,10-trimethylbenzo[g]pteridine-2,4(3H,10H)-dione) is a toxic yellow photoderivative of riboflavin, produced by ultraviolet irradiation of riboflavin in alkaline solution. The lumiflavin content, measured on five batches of the additive, did not exceed 0.025%. This value is in compliance with the specific limit set in the PhEur.

Three batches of the additive were analysed for impurities. Cadmium, lead, mercury and arsenic levels were all below the respective limit of quantification (LOQ). The analysis of mycotoxins, including aflatoxins (B1, G1, B2, G2), ochratoxin A, fumonisins (B1 and B2), deoxynivalenol, HT-2-Toxin, T-2 Toxin and zearalenone showed values below the LOQ.

Microbiological quality was analysed by determination of Enterobacteriaceae (<10 colony forming units (CFU)/g), Escherichia coli (<10 CFU/g), Salmonella spp. (not detected in 25 g), Bacillus cereus (<10 CFU/g), yeasts (<10 CFU/g) and moulds (<10 CFU/g).

The detected amounts of the above-described substances and microorganisms do not raise safety concerns.

3.1.4. Physical properties of the additive

The additive appears as an orange-yellow crystalline powder with a density of 1,040 kg/m³, a bulk density of 0.85 g/ml, and the solubility of the additive (analysed in 3 batches) in water is 81.1 g/l.

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12 Technical dossier/Section II/Annex II_1.
13 Technical dossier/Section II/Annex II_5.
14 Arsenic: 0.1 mg/kg; cadmium: 0.01 mg/kg; lead: 0.1 mg/kg; mercury: 0.005 mg/kg.
15 Technical dossier/Section II/Annex II_4.
16 Aflatoxin: 0.1 µg/kg; fumonisin: 20 µg/kg; ochratoxin: 0.5 µg/kg; deoxynivalenol: 20 µg/kg; HT-2-Toxin, T-2 Toxin and zearalenone: 10 µg/kg.
17 Technical dossier/Section II/Annex II_6.
18 Technical dossier/Section II/Annex II_10-11.
The dusting potential of 3 batches of the additive was determined using the Stauber–Heubach method and showed values on average of 16,172 mg/m³ (range 15,714–16,537 mg/m³).\(^\text{19}\)

The particle size of the additive has been investigated on three batches.\(^\text{20}\) The majority of the particle measured between 0.3 and 300 µm. No particles were below 0.3 µm.

### 3.1.5. Stability and homogeneity

#### 3.1.5.1. Shelf life

Three batches of the additive were stored at 40°C in tight containers, protected from light, for 6 months. No loss of riboflavin at the end of the storage period was observed.

Three batches of the additive were stored at 25°C (relative humidity: 60°C) in polyethylene bags for 36 months. No loss of riboflavin at the end of the storage period was observed.\(^\text{21}\)

#### 3.1.5.2. Stability

The stability of the additive (1 batch, 3 subsamples) in a vitamin/mineral premixture for laying hens (containing 4% choline chloride) was studied when supplemented at 0.082% (820 mg/kg, corresponding to 607 mg riboflavin/kg) and stored at 25 ± 2°C in tightly sealed containers and protected from light for 6 months. At the end of the storage period, the average loss of the 3 subsamples was 6%.\(^\text{22}\)

The stability of the additive (one batch) in compound feed for laying hens (presumably meal, basal diet consisting of maize and bean pulp, containing also 3% fish meal) when supplemented at 8.2 mg additive/kg feed (with 1% of the premixture described above, corresponding to 6 mg riboflavin/kg feed) was studied in 3 subsamples. Samples were stored at 25 ± 2°C in sealed containers protected from light for 3 months. The average loss at the end of the storage period was 2.5%.\(^\text{23}\)

The stability of the additive (1 batch, 3 sub-samples) in water for drinking was studied when supplemented at 0.1 g/L. Samples were stored at 25°C in tightly sealed containers for 7 days. Losses at the end of the storage period was 0.3%.\(^\text{24}\)

#### 3.1.5.3. Homogeneity

The capacity for homogeneous distribution of the additive in feed was studied in 10 subsamples of a compound feed (for laying hens) with the additive at concentration of 8.2 mg/kg. The coefficient of variation of the additive levels was 3.85%.\(^\text{25}\)

Since the additive is highly soluble in water (81.1 g/l), a demonstration of homogeneity is not necessary in water for drinking.

### 3.1.6. Conditions of use

The additive vitamin B\textsubscript{2} in the form of riboflavin-5’-phosphate ester monosodium salt is intended to be added to feed or water for drinking of all animal species and categories. It can be incorporated into feed directly or via premixtures with no maximum content. The supplementation rate is directed to meet the animal's need.

### 3.2. Safety

#### 3.2.1. Safety of the riboflavin production organism

The production strain of the riboflavin (98%) present in the additive belongs to B. subtilis, which is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2021). The identity of the production strain and the qualification for the QPS status were confirmed in a previous evaluation (EFSA FEEDAP Panel, 2021). The genetic modification introduced acquired antimicrobial resistance genes; however, viable cells and recombinant DNA of this production strain were not detected in the riboflavin 98% used for the

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19 Technical dossier/Section II/Annex_II_12.
20 Technical dossier/Section II/Annex_II_9.
21 Technical dossier/Section II/Annex_II_38.
22 Technical dossier/Section II/Annex_II_39.
23 Technical dossier/Section II/Annex_II_40.
24 Technical dossier/Section II/Annex_II_41.
25 Technical dossier/Section II/Annex_II_40.
formulation of the final additive. Therefore, the use of *B. subtilis* KCCM 10445 in the production of riboflavin contained in the final product does not raise safety concerns as regards the genetic modification of the production strain. In addition, the applicant, in the context of the current assessment, submitted evidence in support of the correspondence of the deposited strain and the strain used currently for production.

### 3.2.2. Safety for the target species, consumer and environment

Safety concerns from the additive may derive either from vitamin B<sub>2</sub> or from the residues of the production process. The active substance is produced by a genetically modified microorganism for which the recipient strain is considered by EFSA to qualify for the QPS approach to safety assessment and for which the safety aspects of the genetic modification were previously assessed (EFSA FEEDAP Panel, 2021).

Since the additive under assessment contains the phosphate ester monosodium salt of a riboflavin (98%) preparation already considered safe (EFSA FEEDAP Panel, 2021, 2022), the FEEDAP Panel concluded that the addition of the salt does not add any toxicological property to the final additive.

For nutritional additives not already authorised, safety for the target animals can be presumed without the need for additional studies if sufficient purity of the additive can be demonstrated. As default value for an additive not exceeding an inclusion rate of 1,000 mg/kg complete feed, the unidentified fraction should not contribute to more than 1%.

The nutrient requirements/recommendations of the target species for vitamin B<sub>2</sub>, the background levels of vitamin B<sub>2</sub> in feed materials and the tolerance to overdoses of vitamin B<sub>2</sub> were reviewed by the FEEDAP Panel in previous opinions (EFSA FEEDAP Panel, 2014, 2016, 2018b). The Panel concluded that the use levels based on the requirement/background levels would pose no safety concerns to the target species. The Panel is not aware of any more recent findings which would modify the above conclusion. The inclusion rate of riboflavin would usually not exceed 10 mg/kg complete feed, corresponding to 10.2 mg vitamin B<sub>2</sub> 98%.

Moreover, the production strain is considered safe from the toxicological point of view. In support of the safety for the target species and for the consumer, despite not needed, the applicant has submitted genotoxicity studies (an Ames test<sup>26</sup> and an *in vivo* chromosomal aberration test)<sup>27</sup> which demonstrated that the additive under assessment is neither mutagenic nor clastogenic.

In addition, the applicant submitted a 90-day study<sup>28</sup> and a prenatal developmental toxicity study<sup>29</sup> conducted in rats. However, due to the several severe limitations (90-day study: low numbers of rats for the terminal observations – gross observations, haematology, clinical chemistry, haematology, organ weight; prenatal developmental toxicity study: no indication on purity of the test item, results reported in the summary/abstract are not confirmed with data present in the tables to a large extent, number of animals used was borderline, no information on administration to control group, no individual values were given for dams and fetuses, except for body weight and feed intake for dams and developmental effects for fetuses; results on dams: no information on body weight gain, very few details for mortality, morbidity, pertinent behavioural changes and clinical signs were provided, details on implantations, resorptions and corpora lutea in dams are completely missing; results foetuses: for developmental effects such as visceral and skeletal effects no details were provided on the type of malformations; no information given about external malformations) and the total lack of any histopathological examination presented, the FEEDAP Panel considered that these studies cannot be further used in the assessment.

The active substance riboflavin-5′-phosphate ester monosodium salt occurs in nature. Its use in animal nutrition is not expected to substantially increase the concentration in the environment. Considering that viable cells and recombinant DNA of the riboflavin production strain *B. subtilis* KCCM 10445 were not detected in the final product (EFSA FEEDAP Panel, 2021), a risk for the environment resulting from the use of riboflavin in animal nutrition is not foreseen.

Therefore, the FEEDAP Panel concluded that riboflavin 5′-phosphate ester monosodium salt is safe for the target species, the consumer and the environment.

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<sup>26</sup> Technical dossier/Section III/Annex III_3.
<sup>27</sup> Technical dossier/Section III/Annex III_1.
<sup>28</sup> Technical dossier/Section III/Annex III_4.
<sup>29</sup> Technical dossier/Section III/Annex III_5.
3.2.3. Safety for the user

Effects in the respiratory system

The highest dusting potential of the additive is 16,537 mg/m³ and, therefore, the exposure by inhalation is very likely.

An acute inhalation toxicity (non-GLP-compliant) study with the additive was performed according to Organisation for Economic Co-operation and Development (OECD) TG 403. Exposure of Sprague–Dawley rats to an aerosol with 2,000 mg riboflavin 5’ phosphate sodium salt/m³ for 4 h (limit test) did not cause mortality, significant effects on body weight or signs of toxicity. No gross changes were observed at autopsy. The additive did not induce acute inhalation toxicity under the experimental conditions of the study.

Effects on skin and eyes

An acute dermal irritation/corrosion study (non-GLP compliant) and an acute eye irritation/corrosion study (non-GLP compliant) were done in rabbits following OECD TG 404 and OECD 405, respectively. Riboflavin 5’ phosphate sodium did not induce irritation or corrosive effects in the skin or in the eyes, respectively.

No skin sensitisation data were submitted by the applicant. Riboflavin is a known photosensitiser which may elicit skin and eye photoallergic reactions.

3.2.3.1. Conclusions on the safety for the users

The additive is not a skin/eye irritant and it is not considered a respiratory sensitiser. Riboflavin is a known photosensitiser which may elicit skin and eye photoallergic reactions.

3.3. Efficacy

Riboflavin (vitamin B₂) has been used world-wide in animal nutrition for decades. Owing to the long history of use and its established nutritional role in domestic animals, riboflavin is regarded as effective in covering the animal’s requirement. Data on requirement, allowances and recommendations for feed supplementation are easily accessible in the standard literature on animal nutrition.

The FEEDAP Panel considers that the additive under assessment is effective in covering the animal’s requirement when administered via feed and/or water for drinking.

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 and Good Manufacturing Practice.

4. Conclusions

The production strain and its recombinant DNA were not detected in riboflavin 98%. Therefore, the riboflavin 98% used to produce the final additive, does not pose any safety concern associated with the genetic modification of the production strain.

The use of riboflavin-5’-phosphate ester monosodium salt (vitamin B₂) is safe for the target species, the consumer and the environment.

The additive is not a skin/eye irritant, and it is not considered a respiratory sensitiser. Riboflavin is a known photosensitiser which may elicit skin and eye photoallergic reactions.

The additive under assessment is effective in covering the animals’ requirements for vitamin B2 when administered via feed and/or water for drinking.

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30 Technical dossier/Section III/Annex_III_6.
31 Technical dossier/Section III/Annex_III_8.
32 Technical dossier/Section III/Annex_III_7.
33 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.
5. Documentation provided to EFSA/Chronology

| Date       | Event                                                                 |
|------------|----------------------------------------------------------------------|
| 18/11/2020 | Dossier received by EFSA. Riboflavin 5’-phosphate ester monosodium salt (solid form produced after phosphorylation of riboflavin 98%) produced by Bacillus subtilis KCCM-10445 for all animal species. Submitted by GUANG JI PHARMACEUTICAL BELGIUM SA NV on behalf of Hubei Guangji Pharmaceutical Co. Ltd. |
| 26/11/2020 | Reception mandate from the European Commission                       |
| 31/03/2021 | Application validated by EFSA – Start of the scientific assessment    |
| 30/06/2021 | Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives |
| 01/07/2021 | Comments received from Member States                                 |
| 05/07/2021 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: genetic modification/production process |
| 18/02/2022 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 18/03/2022 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: characterisation of the production strain/purity |
| 13/04/2022 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 27/09/2022 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment |

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Abbreviations

CAS Chemical Abstracts Service
CFU colony-forming unit
EC European Commission
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
GLP Good laboratory practice
IUPAC International Union of Pure and Applied Chemistry
LOQ limit of quantification
MNPs Multiple nucleotide polymorphisms
OECD Organisation for Economic Co-operation and Development
PhEur European Pharmacopoeia
QPS Qualified presumption of safety
SNPs Single nucleotide polymorphisms
Appendix A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for vitamin B2/riboflavin 5’-phosphate ester monosodium salt (solid form produced after phosphorylation of riboflavin 98% produced by Bacillus subtilis KCCM10445)

In the current application an authorisation is sought under Article 4 for vitamin B2 (riboflavin 5’-phosphate ester monosodium salt) as feed additive under the category/functional group 3(a) “nutritional additive”/“vitamins, provitamins and chemically well-defined substances having a similar effect” according to the classification system of Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the feed additive for all animal species. The product presented by the Applicant contains riboflavin 5’-phosphate ester monosodium salt as active substance with a purity (mass fraction) of 73 to 79% riboflavin based on dry substance. The feed additive is intended to be added directly into feedingstuffs (or through premixtures) and water for drinking. The Applicant recommended inclusion levels of the active substance ranging from 4 to 110 mg/kg complete compound feed and specifically for ruminants and horses between 27 and 116 mg/head/day. For the determination of riboflavin 5’-phosphate ester monosodium salt in the feed additive the Applicant proposed the methods presented within the European Pharmacopoeia riboflavin sodium phosphate monograph where the quantification is based on spectrophotometry at 444 nm. The EURL recommends this method for official control to quantify riboflavin 5’-phosphate ester monosodium salt in the feed additive. For the determination of the riboflavin in premixtures, the Applicant proposed the ring-trial validated method by the Association of German Agricultural Analytical Research Institutes (VDLUFA - Bd. III, 13.9.1) based on ion-pair reversed phase High Performance Liquid Chromatography coupled to UV detection (HPLC-UV). The method proposed is not intended for the determination of riboflavin 5’-phosphate ester monosodium salt in premixtures and the EURL cannot recommend this method for official control for the determination of riboflavin 5’-phosphate ester monosodium salt in premixtures. For the determination of riboflavin 5’-phosphate ester monosodium salt (as total riboflavin/vitamin B2) in feedingstuffs and water the Applicant proposed a ring-trial validated CEN method intended for foodstuffs (EN 14152). The analytical method is based on acidic hydrolysis followed by enzymatic dephosphorylation and further analysis using HPLC coupled to fluorescence detection (FLD). The CEN method was ring-trial validated using milk powder and pig liver certified reference materials (CRM). The following performance characteristics for the determination of the total vitamin B2 content ranging from 145 to 1,055 mg/kg were reported: a relative standard deviation for repeatability (RSDr) ranging from 1.7 to 3.2%; a relative standard deviation for reproducibility (RSDR) ranging from 7.3 to 7.9%; EURL Evaluation Report “Vitamin B2/riboflavin 5’-phosphate ester monosodium salt” 2/7 and a recovery rate (Rrec) of ca. 100%. Furthermore, as described in a former EURL report, similar performance characteristics have been obtained by applying the CEN method for the analysis of total vitamin B2 in samples of feedingstuffs and water thus confirming the extension of scope of the CEN method to these matrices. Based on these performance characteristics, the EURL recommends for official control the ring-trial validated CEN method (EN 14152:2003) to determine riboflavin 5’-phosphate ester monosodium salt (as total riboflavin/vitamin B2) in feedingstuffs and water. The same method could be used for the determination of riboflavin 5’-phosphate ester monosodium salt in premixtures by applying a proper dilution. However, as no experimental data were provided to confirm this, the EURL cannot evaluate or recommend this method for official control for the determination of riboflavin 5’-phosphate ester monosodium salt in premixtures. Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.