Safety of the feed additive consisting of vitamin B2/riboflavin (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, EFSA was asked to deliver a scientific opinion on the characterisation and safety of vitamin B2/riboflavin produced by fermentation with a genetically modified strain of Bacillus subtilis (KCCM 10445) as a nutritional additive in feed and water for drinking for all animal species. In 2021, the FEEDAP Panel issued an opinion on the safety and efficacy of vitamin B2 98% and vitamin B2 80% in the form of riboflavin produced by B. subtilis KCCM 10445. In that assessment, the Panel could not confirm the correspondence of the deposited strain under accession number KCCM 10445 with the strain used for production owing to discrepancies in the description of the genetic modification steps. Moreover, in the absence of data, no conclusions on the skin sensitisation potential of vitamin B2 98% and vitamin B2 80% nor on the possible risk by inhalation of vitamin B2 98% could be reached. For the present assessment, the applicant provided supplementary information to partially address the limitations identified in that assessment. Based on the results of a bioinformatic analysis between the strain deposited under accession number KCCM 10445 with the strain used for production under assessment, the Panel confirmed the correspondence between the two strains. As no new data have been submitted on the safety of the additives, the conclusions from the Panel remain that the use of vitamin B2/riboflavin produced by B. subtilis KCCM 10445 is safe for the target species, the consumer and the environment. Vitamin B2 80% is not hazardous by inhalation. In the absence of data, no conclusions on the possible risk by inhalation of vitamin B2 98% could be reached. Neither the vitamin B2 98% nor the vitamin B2 80% are irritant to skin or eyes. In the absence of data, no conclusions on the skin sensitisation potential of the additives could be reached.

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

Requestor: European Commission

Question number: EFSA-Q-2022-00103

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Declarations 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.

Acknowledgements: The Panel wishes to thank the following Working Groups of the FEEDAP Panel for the support provided to this scientific output: WG Microbiology.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Fasmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Anguita M, Brozzi R, Galobart J, and Pettenati E, 2022. Scientific Opinion on the safety of the feed additive consisting of vitamin B2/riboflavin (produced by Bacillus subtilis KCCM 10445) for all animal species (Hubei Guangji Pharmaceutical Co. Ltd.). EFSA Journal 2022;20(10):7607, 7 pp. https://doi.org/10.2903/j.efsa.2022.7607

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.
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Vitamin B<sub>2</sub>/riboflavin produced by Bacillus subtilis KCCM 10445 for all animal species

www.efsa.europa.eu/efsajournal 3 EFSA Journal 2022;20(10):7607
1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003 establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 thereof defines the terms of the authorisation by the Commission.

The applicant, GUANG JI PHARMACEUTICAL BELGIUM SA NV on behalf of Hubei Guangji Pharmaceutical Co. Ltd., is seeking a Community authorisation of Vitamin B2/Riboflavin produced by Bacillus subtilis KCCM 10445 as a feed additive to be used as a nutritional additive for all animal species (Table 1).

Table 1: Description of the substances

| Category of additive | Nutritional additive |
|----------------------|----------------------|
| Functional group of additive | 3(a): vitamins, pro-vitamins and chemically well-defined substances having similar effect |
| Description | Vitamin B2/riboflavin produced by Bacillus subtilis KCCM 10445 |
| Target animal category | All animal species |
| Applicant | GUANG JI PHARMACEUTICAL BELGIUM SA NV on behalf of Hubei Guangji Pharmaceutical Co. Ltd. |
| Type of request | New opinion |

On 05.05.2021, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA), in its opinion on the safety and efficacy of the product, could not conclude on the safety/identification of Vitamin B2/Riboflavin produced by Bacillus subtilis KCCM 10445 as a feed additive for all animal species. With the data available, the Panel is not in the position to confirm the correspondence between the strain deposited under accession number KCCM 10445 and the strain under the current assessment. Also safety concerns for workers were raised. The Commission gave the possibility to the applicant to submit supplementary information and data in order to complete the assessment and to allow a revision of the EFSA's opinion. The new data have been received on 28/01/2022.

In view of the above, the Commission asks EFSA to deliver a new opinion on Vitamin B2/Riboflavin produced by Bacillus subtilis KCCM 10445 as a feed additive for all animal species based on the supplementary data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

1.2. Additional information

The additive under assessment contains riboflavin produced by fermentation with a genetically modified strain of B. subtilis (KCCM 10445).

The FEEDAP Panel issued two opinions on vitamin B2 (80%) as riboflavin produced by B. subtilis KCCM 10445 for all animal species. The first opinion dealt with the safety and efficacy of the additive (EFSA FEEDAP Panel, 2014). The second opinion covered the safety aspects linked to the presence of cells from the production strain and its recombinant DNA in the product (EFSA FEEDAP Panel, 2018b).

In 2018, the authorisation of vitamin B2 (80%) produced by B. subtilis KCCM 10445 was denied by Commission Implementing Regulation (EU) 2018/1254.1

In 2021, the Panel adopted an opinion on the safety and efficacy of vitamin B2 98% and vitamin B2 80% produced by B. subtilis KCCM 10445 (EFSA FEEDAP Panel, 2021).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information to a previous application on the same product.2

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1 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, pp. 5-8.

2 FEED dossier: FAD-2019-0053.
In accordance with Article 38 of the Regulation (EC) No 178/2002 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, a non-confidential version of the supplementary information has been published on Open.EFSA.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of vitamin B₂ 98% and vitamin B₂ 80% produced by *B. subtilis* KCCM 10445 is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012) and Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a).

3. Assessment

Riboflavin produced by *B. subtilis* KCCM 10445 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.

Riboflavin produced by *B. subtilis* KCCM 10445 is available in two formulations: vitamin B₂ 98% which is specified to contain ≥ 98% riboflavin, ≤ 1.5% loss on drying and ≤ 0.3% residue on ignition and vitamin B₂ 80% which contains ≥ 80% riboflavin, ≤ 20% maltodextrin, ≤ 3% loss on drying and ≤ 5% residue on ignition.

These products were assessed in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2021). The riboflavin is produced by a genetically modified strain of *B. subtilis* (KCCM 10445). In the context of the 2021 assessment, new whole genome sequence (WGS)-based data were submitted by the applicant to characterise the genetic modification of *B. subtilis* KCCM 10445. The data newly submitted regarding the structure of the genetic modification showed differences compared to the genetic modification steps reported in the previous opinion (EFSA FEEDAP Panel, 2014). Owing to these discrepancies, the Panel could not confirm the correspondence of the strain deposited under accession number KCCM 10445 with the strain under assessment. In that assessment, the Panel also concluded that in the absence of data, no conclusions on the skin sensitisation potential of vitamin B₂ 98% and vitamin B₂ 80% nor on the possible risk by inhalation of vitamin B₂ 98% could be reached.

The applicant has now provided supplementary information to address the limitations identified regarding the characterisation of the production strain. No additional information has been submitted regarding user safety.

3.1. Characterisation

3.1.1. Correspondence between the strain deposited under accession number KCCM 10445 and the strain under assessment

In the previous assessment (EFSA FEEDAP Panel, 2021), the Panel could not conclude on the correspondence of the strain deposited under accession number KCCM 10445 with the strain under assessment.

In the current submission, the applicant conducted a bioinformatic analysis to compare the WGS data, including the plasmid sequence, of the deposited strain and the one that is currently in use for the production of the additives 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, a sequence read-mapping analysis showed that...
98.7% of the reads of the strain currently used for production mapped to the assembled genome of the deposited strain. Further, a sequence variant analysis showed the presence of only 6 single nucleotide polymorphisms (SNPs), 1 multiple nucleotide polymorphism (MNP), 26 indels and 1 complex variant between the two genomes.

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 \emph{B. subtilis} genes was investigated: 25 out of 26 indels were ascribed to homopolymeric tracts, and might be due to errors of the sequencing/assembly technology used; the remaining indel, 1 SNP, the complex variant and the MNP found were not located in coding regions; 2 SNPs led to synonymous codon variants, and 3 SNPs led to amino acid changes, which either do not affect the active site of the corresponding protein (2 SNPs) or is located on a protein of unknown function (1 SNP).

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 additives 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.

As no new data have been submitted on the safety of the additives, the conclusions from the Panel remain that the use of vitamin B$_2$/riboflavin produced by \emph{B. subtilis} KCCM 10445 is safe for the target species, the consumer and the environment.

Vitamin B$_2$ 80% is not hazardous by inhalation. In the absence of data, no conclusions on the possible risk by inhalation of vitamin B$_2$ 98% can be reached. Neither vitamin B$_2$ 98% nor vitamin B$_2$ 80% are irritant to skin or eyes. In the absence of data, no conclusions on the skin sensitisation potential of the additives can be reached.

### 4. Conclusions

The applicant has provided new data based on the WGS which confirms the correspondence between the strain deposited under accession number KCCM 10445 and the strain used for production under assessment.

No new data have been submitted on the safety of the additives. Therefore, the conclusions from the Panel remain that the use of vitamin B$_2$/riboflavin produced by \emph{B. subtilis} KCCM 10445 is safe for the target species, the consumer and the environment. Vitamin B$_2$ 80% is not hazardous by inhalation. In the absence of data, no conclusions on the possible risk by inhalation of vitamin B$_2$ 98% can be reached. Neither the vitamin B$_2$ 98% nor the vitamin B$_2$ 80% are irritant to skin or eyes. In the absence of data, no conclusions on the skin sensitisation potential of the additives can be reached.

### 5. Documentation provided to EFSA/Chronology

| Date       | Event                                                                 |
|------------|----------------------------------------------------------------------|
| 28/01/2022 | Dossier received by EFSA. Vitamin B$_2$ (riboflavin) produced by \emph{Bacillus subtilis} KCCM 10445 for all animal species. Submitted by GUANG JI PHARMACEUTICAL BELGIUM SA NV. |
| 09/02/2022 | Reception mandate from the European Commission                         |
| 18/02/2022 | Acceptance mandate from the European Commission by EFSA               |
| 30/03/2022 | Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. \emph{Issues: characterisation} |
| 29/04/2022 | 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 |

### References

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the safety and efficacy of vitamin B$_2$ (80%) as riboflavin produced by Bacillus subtilis for all animal species, based on a dossier submitted by VITAC EEIG. EFSA Journal 2014;12(1):3531, 2 pp. https://doi.org/10.2903/j.efsa.2014.3531
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018a. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Fasimon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Gropp J, Anguita M, Galobart J, Manini P, Pizzo F, Revez J, Tarrés-Call J and Pettenati E, 2021. Scientific Opinion on the safety and efficacy of feed additives consisting of Vitamin B₂ (98%) and Vitamin B₂ (80%) as riboflavin produced by *Bacillus subtilis* KCCM 10445 for all animal species (Hubei Guangji Pharmaceutical Co. Ltd.). EFSA Journal 2021;19(6):6629, 16 pp. https://doi.org/10.2903/j.efsa.2021.6629

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Herman L, Glandorf B, Kärenlampi S, Aguilera J and Cocconcelli PS, 2018b. Scientific Opinion on the safety of vitamin B₂ (80%) as riboflavin produced by *Bacillus subtilis* KCCM-10445 for all animal species. EFSA Journal 2018;16(3):5223, 8 pp. https://doi.org/10.2903/j.efsa.2018.5223

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| FEEDAP       | EFSA Panel on Additives and Products or Substances used in Animal Feed |
| MNP          | multiple nucleotide polymorphism |
| SNP          | single nucleotide polymorphism |
| WGS          | whole genome sequence |