



*Ministero dell' Ambiente  
e della Tutela del Territorio e del Mare*

DIREZIONE GENERALE PER LE VALUTAZIONI  
E LE AUTORIZZAZIONI AMBIENTALI

IL DIRETTORE GENERALE

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**Oggetto: Informativa della Commissione europea sulla possibile contaminazione da OGM di integratori alimentari ad uso veterinario.**

La Commissione europea, con messaggio del 22 marzo 2018, ha trasmesso alle autorità competenti ai sensi del regolamento (CE) n. 1829/2003 e della direttiva 2001/18/CE un'informativa in merito alla possibile presenza in integratori alimentari ad uso veterinario (Riboflavina all'80%) di *Bacillus subtilis* geneticamente modificato e del suo DNA ricombinante.

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In tale informativa la Commissione precisa che, il laboratorio tedesco di riferimento dell'UE sugli additivi per mangimi ha riscontrato la presenza di cellule vitali di *Bacillus subtilis* geneticamente modificato e del suo DNA ricombinante in campioni di riferimento nell'ambito dell'esame della domanda di rivalutazione di un integratore alimentare di vitamine del gruppo B ad uso veterinario presentata dalla GEIE VITAC ai sensi del Regolamento (CE) n. 1831/2003.

A seguito di tale segnalazione la Commissione europea ha richiesto all'EFSA di formulare un nuovo parere sulla sicurezza del prodotto. Sulla base dei nuovi dati, l'EFSA ha adottato, il 7 marzo 2018, un nuovo parere in cui si conclude che la presenza di cellule vitali e/o DNA del ceppo di produzione GM che ospita marcatori di resistenza agli antimicrobici di importanza umana e veterinaria rappresenta un rischio per le specie bersaglio, i consumatori, gli utilizzatori e l'ambiente (all. 1).

In seguito al parere dell'EFSA, la Commissione europea sta valutando le azioni da intraprendere per quanto riguarda la domanda di rivalutazione presentata dalla GEIE VITAC.

Attualmente non vi sono ulteriori prove della presenza di cellule vitali del ceppo di produzione geneticamente modificato o del suo DNA in prodotti a base di Riboflavina all'80% autorizzati all'immissione in commercio ai sensi della direttiva 70/524/CEE, abrogata dal regolamento (CE) n. 1831/2003.

Tuttavia, alla luce delle conclusioni del parere dell'EFSA, la Commissione europea raccomanda agli Stati membri di continuare a svolgere controlli ufficiali per garantire che i prodotti commercializzati sul proprio territorio soddisfino tutti i requisiti di sicurezza, incoraggiando le autorità competenti ai sensi del regolamento (CE) n. 1829/2003, della direttiva 2001/18/CE e per la nutrizione animale a coordinare le loro azioni.

Si chiede pertanto alle Amministrazioni in indirizzo di voler predisporre specifiche azioni di controllo per verificare l'eventuale presenza di contaminazione nell'integratore alimentare ad uso veterinario commercializzato sul territorio nazionale, sulla base della metodologia analitica descritta nel parere EFSA in allegato.

Si rimane in attesa di conoscere le eventuali iniziative intraprese.

**Il Direttore Generale**

Giuseppe Lo Presti

(documento informatico firmato digitalmente  
ai sensi dell'art. 24 D.Lgs. 82/2005 e ss.mm.ii)

All.:c.s.

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## Safety of vitamin B<sub>2</sub> (80%) as riboflavin produced by *Bacillus subtilis* KCCM-10445 for all animal species

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### Abstract

Riboflavin (80%) is a feed additive produced by fermentation of a genetically modified *Bacillus subtilis* strain. In 2014, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of riboflavin (80%) based on a dossier supplied by the applicant. The Panel concluded that neither the production strain nor its recombinant DNA was detected in the final product, and therefore, the additive does not give rise to any safety concern with regard to the genetic modification of the production strain.

The Community Reference Laboratory on Feed additives, in the context of an official control, reported on the presence of recombinant DNA in samples of the additive. The European Commission asked EFSA to deliver a new opinion on the safety of Vitamin B<sub>2</sub> (80%) based on the new data, complementing the former one. The analysed samples contained DNA belonging to the production strain, including the genetic modification. Moreover, one of the samples contained viable cells from the production strain. Because the production strain carries antimicrobial resistance genes introduced by the genetic modification, the FEEDAP Panel considers that Riboflavin (80%) poses a risk for the spread of viable cells and DNA of a genetically modified strain-harboring genes coding for resistance to antimicrobials.

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**Keywords:** vitamin B<sub>2</sub>, riboflavin, nutritional additive, safety, genetically modified microorganism

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## Summary<sup>1</sup>

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 of vitamin B<sub>2</sub> in the form of riboflavin produced by *Bacillus subtilis* as an additive to feed and water for drinking for all animal species and categories.

The additive riboflavin (80%) is produced by fermentation of the genetically modified *B. subtilis* strain KCCM-10445. The FEEDAP Panel issued an opinion on the safety and efficacy of the additive (EFSA FEEDAP Panel, 2014) based on a dossier supplied by the applicant. The Panel concluded that neither the production strain nor its recombinant DNA was detected in the final product, and therefore, the additive does not give rise to any safety concern with regard to the genetic modification of the production strain.

The European Commission informed EFSA that the Community Reference Laboratory on Feed additives, in the context of an official control, reported on the presence of recombinant DNA in samples of the additive. The European Commission asked EFSA to deliver a new opinion on the safety of Vitamin B<sub>2</sub> (80%) as riboflavin produced by *B. subtilis* based on the new data indicating the presence of recombinant DNA, complementing the former one.

The strain *B. subtilis* KCCM-10445 was previously characterised by the FEEDAP Panel (EFSA FEEDAP Panel, 2014). Owing to the genetic modification, the strain harbours genes conferring resistance to antimicrobials.

Analyses of two batches of the product performed by the German official laboratory revealed the presence of viable cells in both of them (550 CFU/g and  $2.2 \times 10^5$  CFU/g, respectively). The viable cells were identified as *B. subtilis* by analysis of the 16S rRNA gene sequence. DNA isolated from colonies of both batches was further analysed targeting fragments specific to the production strain *B. subtilis* KCCM-10445. The results indicate that the colonies found in one of the batches tested belong to the production strain. These data are in contrast with the data previously provided by the applicant (EFSA FEEDAP Panel, 2014), according to which the production strain could not be detected in the product. The batches analysed by the applicant were different from those analysed by the German official laboratory.

The same two batches of the product were tested for the presence of recombinant DNA from the production strain. The data suggests that remnants of the intact antimicrobial resistance gene could remain in the product. These data are in contrast with the data previously provided by the applicant (EFSA FEEDAP Panel, 2014), according to which no recombinant DNA was detected in the product.

The product contains viable cells and DNA of the genetically modified *B. subtilis* used as production strain. Therefore, the FEEDAP Panel considers that the additive vitamin B<sub>2</sub> (80%) poses a risk for the spread of viable cells and DNA of a genetically modified strain-harbouring genes coding for resistance to antimicrobials of human and veterinary importance.

<sup>1</sup> This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

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## 1. Introduction

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

Regulation (EC) No 1831/2003<sup>2</sup> establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 defined the terms of the authorisation by the Commission.

The applicant, VITAC EEIG, is seeking a Community authorisation of Vitamin B<sub>2</sub> (80%) as riboflavin produced by *Bacillus subtilis* for all animal species, as a nutritional additive. (Table 1)

**Table 1:** Description of the substance

<b>Category of additive</b>	Nutritional additive
<b>Functional group of additive</b>	vitamins, pro-vitamins and chemically well-defined substances having similar effect
<b>Description</b>	Vitamin B <sub>2</sub> (80%) as riboflavin produced by <i>Bacillus subtilis</i>
<b>Target animal category</b>	For all animal species
<b>Applicant</b>	VITAC EEIG
<b>Type of request</b>	New opinion

The Community Reference Laboratory on Feed additives informed the Commission that, in the context of the official controls, one German official laboratory had analysed the reference samples stored in the Community Reference Laboratory of Feed additives. The samples analysed corresponded to all the vitamin B<sub>2</sub> dossiers that were under re-evaluation procedure by Article 10(2) to Regulation 1831/2003 on feed additives. The laboratory found recombinant DNA from the production strain in the sample of VITAC EEIG. The other samples were clean.

The applicant indicated in the dossier that the additive did not contain recombinant DNA from the production strain that was genetically modified, while the analysis performed demonstrated the presence of this recombinant DNA, therefore, non-compliance has been identified. The information provided by the applicant is not correct as recombinant DNA is present in the additive.

These new facts that come into knowledge would impact the assessment of this product as EFSA has based its opinion on the information provided by the applicant and on the assumption that the additive is free from recombinant DNA, in accordance with the information provided by the applicant.

In view of the above, the Commission asks the Authority to deliver a new opinion on the safety of Vitamin B<sub>2</sub> (80%) as riboflavin produced by *Bacillus subtilis* based on the new data indicating the presence of recombinant DNA.

### 1.2. Additional information

The additive under assessment consists in vitamin B<sub>2</sub> in the form of riboflavin produced by the genetically modified *Bacillus subtilis* strain KCCM-10445. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of this additive for all animal species and categories (EFSA FEEDAP Panel, 2014). In its opinion, the Panel concluded that neither the production strain nor its recombinant DNA were detected in the final product, and therefore, the additive does not give rise to any safety concern with regard to the genetic modification of the production strain.

In the context of official controls, one German official laboratory analysed the reference samples of the additive and found viable cells and recombinant DNA from the production strain in them. The samples analysed were from different production batches from those used to produce the data included in the dossier submitted to EFSA.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data provided by the Community Reference Laboratory on Feed additives,<sup>3</sup> plus new data requested to the applicant in support of the authorisation request for

<sup>2</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

<sup>3</sup> FEED dossier reference: FAD-2016-0046.

the use of vitamin B<sub>2</sub> (80%) as riboflavin produced by *B. subtilis* for all animal species as a feed additive. The FEEDAP Panel also used the data from its previous risk assessment of the additive to deliver the present output.

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of vitamin B<sub>2</sub> (80%) as riboflavin produced by *B. subtilis* is in line with the principles laid down in Regulation (EC) No 429/2008 and the Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011).

## 3. Assessment

The present assessment is based on the report by the Community Reference Laboratory on Feed Additives about the presence of recombinant DNA from the production strain in samples of the product,<sup>4</sup> and therefore, it covers the safety aspects linked to the possible presence of cells from the production strain and its recombinant DNA in the product. Other aspects of the safety and efficacy of the product have been previously addressed by the FEEDAP Panel (EFSA FEEDAP Panel, 2014).

### 3.1. Characterisation<sup>5</sup>

#### 3.1.1. Characterisation of the production strain

Riboflavin (80%) is produced by fermentation of the genetically modified *B. subtilis* strain KCCM-10445. The strain, as previously characterised by the FEEDAP Panel (EFSA FEEDAP Panel, 2014), carries four antimicrobial resistance genes, three of them artificially inserted.

#### 3.1.2. Presence of cells of the production strain in the product

Analyses of two batches of the product performed by the German official laboratory revealed the presence of viable cells in both of them (550 CFU/g and  $2.2 \times 10^5$  CFU/g, respectively).<sup>6</sup> Tests were done by plating on non-selective medium and incubation at 37°C for 18–24 h. Different dilutions of the product were plated to reach a theoretical limit of detection of up to 50 CFU/g.<sup>7</sup> The viable cells were identified as *B. subtilis* by analysis of the 16S rRNA gene sequence. DNA isolated from colonies of both batches was further analysed targeting fragments specific to the production strain *B. subtilis* KCCM-10445 as follows:

- By real-time polymerase chain reaction (PCR).
- By conventional PCR.

Amplification was found in four of five colonies analysed for the batch containing 550 CFU/g. The results were confirmed by analysing the DNA of the only colony isolated from a second sample of the same batch. No amplification was found in the colonies isolated from the batch containing  $2.2 \times 10^5$  CFU/g.<sup>8</sup> The results indicate that the colonies found in one of the samples tested belong to the production strain.

These data are in contrast with the data previously provided by the applicant in the framework of the risk assessment by EFSA (EFSA FEEDAP Panel, 2014). According to the applicant's data, the production strain could not be detected in three batches of the product. The batches analysed by the applicant were different from those analysed by the German official laboratory.

#### 3.1.3. Presence of DNA from the production strain in the product

The German official laboratory further analysed the same two batches of the product for the presence of recombinant DNA from the production strain. PCR was performed using the same primer pairs as detailed above. Amplification was found by conventional PCR (465-bp fragment) in three of

<sup>4</sup> Technical dossier/Report: Detection of recombinant DNA in reference samples. Vitac request for the evaluation of vitamin B<sub>2</sub> (riboflavin). July 2016.

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

<sup>6</sup> Technical dossier/supplementary information August 2016.

<sup>7</sup> Technical dossier/supplementary information October 2016.

<sup>8</sup> Technical dossier/Supplementary information March 2017.

four samples of one batch and in the only sample tested of the other.<sup>6</sup> The limits of detection, in terms of amount of DNA per weight of product, were not calculated. The results indicate that recombinant DNA from the production strain remains in the tested batches of the product.

These data are in contrast with the data previously provided by the applicant in the framework of the risk assessment by EFSA (EFSA FEEDAP Panel, 2014). According to the applicant's data, no recombinant DNA was detected above the limits of detection provided in three batches of the additive by conventional PCR. Limits of detection were calculated following the recommendations of the relevant guidance (EFSA GMO Panel, 2011).

### 3.2. Safety<sup>9</sup>

The production strain *B. subtilis* KCCM-10445 carries several antimicrobial resistance genes.

Although the set of data provided by the applicant in the frame of the risk assessment by EFSA indicated that the production strain and its DNA are absent from the product, the analyses conducted by the German official control laboratory on two reference samples deposited at the European Union Reference Laboratories (EURL) demonstrated the presence of viable cells of the production strain in one sample and of its DNA in both of them. The fragments found suggest that, apart from the production strain, remnants of the intact antimicrobial resistance gene could remain free in the product.

## 4. Conclusions<sup>10</sup>

The production strain *B. subtilis* KCCM-10445 carries four antimicrobial resistance genes, three of them introduced by genetic modifications. In a previous assessment (EFSA FEEDAP Panel, 2014), the Panel concluded that neither the production strain nor its recombinant DNA was detected in the final product, and therefore, the additive does not give rise to any safety concern with regard to the genetic modification of the production strain.

New data provided by the German official control laboratory on product batches different from those analysed by the applicant show that the product contains viable cells and/or DNA of the genetically modified production strain. Therefore, the FEEDAP Panel concludes that the product vitamin B<sub>2</sub> (80%) as riboflavin poses a risk for the target species, consumers, users and the environment due to the spread of viable cells and DNA of a genetically modified strain-harboring genes coding for resistance to antimicrobials of human and veterinary importance.

### Documentation provided to EFSA

- 1) Report. Detection of recombinant DNA in reference samples. VITAC request for the evaluation of vitamin B<sub>2</sub> (riboflavin). July 2016. Submitted by the European Commission
- 2) Safety of vitamin B<sub>2</sub> (80%) as riboflavin produced by *Bacillus subtilis* for all animal species. Supplementary information. August 2016. Submitted by the Joint Research Centre
- 3) Safety of vitamin B<sub>2</sub> (80%) as riboflavin produced by *Bacillus subtilis* for all animal species. Supplementary information. October 2016. Submitted by the Joint Research Centre and the Center for Agricultural Technology Augustenberg (Germany)
- 4) Safety of vitamin B<sub>2</sub> (80%) as riboflavin produced by *Bacillus subtilis* for all animal species. Supplementary information. March 2017. Submitted by the Joint Research Centre
- 5) Safety of vitamin B<sub>2</sub> (80%) as riboflavin produced by *Bacillus subtilis* for all animal species. Supplementary information. April 2017. Submitted by VITAC

### References

- 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<sub>2</sub> (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, 22 pp. <https://doi.org/10.2903/j.efsa.2014.3531>
- EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA Journal 2011;9(6):2193, 54 pp. <https://doi.org/10.2903/j.efsa.2011.2193>

<sup>9</sup> This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

<sup>10</sup> This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

## Abbreviations

FEEDAP	The Panel on Additives and Products or Substances used in Animal Feed
PCR	polymerase chain reaction
EURL	European Union Reference Laboratories

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