

ADOPTED: 7 October 2019

doi: 10.2903/j.efsa.2019.5890

Safety of *Lactococcus lactis* NCIMB 30160 as a feed additive for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Antonio Finizio, Andreas Focks, Ivana Teodorovic, Maria Vittoria Vettori and Jordi Tarrés-Call

Abstract

Following a request from 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 for the environment of the proposed modification of the terms of the authorisation regarding the formulation of the additive *Lactococcus lactis* NCIMB 30160. The applicant has proposed to modify the manufacturing process by adding polyethylene glycol (PEG 4000) in the list of potential cryoprotectants that can be used in the freeze-drying step, up to a maximum concentration of 0.025 mg PEG 4000/kg silage. The use of PEG 4000 as an excipient in formulations with *Lactococcus lactis* NCIMB 30160 would not change the previous conclusions regarding the safety for the target animals, consumers and users. No safety concerns are expected for the environment when PEG 4000 is used as a cryoprotectant in the additive *Lactococcus lactis* NCIMB 30160 up to a maximum concentration of 0.025 mg PEG 4000/kg silage.

© 2019 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: Technological additive, silage additive, *Lactococcus lactis* NCIMB 30160, polyethylene glycol, safety

Requestor: European Commission

Question number: EFSA-Q-2019-00365

Correspondence: feedap@efsa.europa.eu

Panel members: Giovanna Azimonti, Vasileios Bampidis Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

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, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Finizio A, Focks A, Teodorovic I, Vettori MV and Tarrés-Call J, 2019. Scientific Opinion on the safety of *Lactococcus lactis* NCIMB 30160 as a feed additive for all animal species. EFSA Journal 2019;17(11):5890, 7 pp. <https://doi.org/10.2903/j.efsa.2019.5890>

ISSN: 1831-4732

© 2019 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.



Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.2. Additional information.....	4
2. Data and methodologies.....	5
2.1. Data.....	5
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Safety for the environment of PEG 4000.....	5
3.1.1. Phase I assessment.....	5
3.1.1.1. Predicted environmental concentrations.....	5
3.1.1.2. Conclusions on safety for the environment.....	6
4. Conclusions.....	6
Documentation as provided to EFSA/Chronology.....	6
References.....	7
Abbreviations.....	7

1. Introduction

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

Regulation (EC) No 1831/2003¹ establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Lactosan GmbH & Co. KG, is seeking a Community authorisation of *Lactococcus lactis* NCIMB 30160 as feed additive to be used as a silage additive for all animal species (Table 1).

Table 1: Description of the substances

Category of additive	Technological additive
Functional group of additive	Silage additive
Description	<i>Lactococcus lactis</i> NCIMB 30160
Target animal category	All animal species
Applicant	Lactosan GmbH & Co. KG
Type of request	New opinion

On 28 March 2018, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ('Authority'), in its opinion on the safety of the product, could not conclude on the safety of *Lactococcus lactis* NCIMB 30160 in all animal species, under the conditions of use as proposed by the applicant. The Panel was unable to conclude on the safety for the environment of the proposed use of PEG 4000 as excipient in formulations of the additive. After the discussion with the Member States on the last Standing Committee, it was suggested to check for the possibility to demonstrate the safety of the additive.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of Authority's opinion. The new data have been received on 13 February 2019.

In view of the above, the Commission asks the Authority to deliver a new opinion on *Lactococcus lactis* NCIMB 30160 as a feed additive for all animal species based on the additional data submitted by the applicant.

1.2. Additional information

The additive is a preparation of *Lactococcus lactis* NCIMB 30160 intended to be added to forages for all animal species to promote ensiling (technological additive, functional group: silage additive).

The European Food Safety Authority (EFSA) issued an opinion on the safety and efficacy of this additive for all animal species (EFSA FEEDAP Panel, 2011). The feed additive currently authorised is manufactured using cryoprotectants selected from a list including ascorbic acid, lactose, mannitol, monosodium glutamate, sodium citrate or whey powder. The applicant requested the modification of the authorisation to include polyethylene glycol (PEG) 4000 in that list. In its latest opinion (EFSA FEEDAP Panel, 2018), the FEEDAP Panel could not conclude on the safety for the environment of the proposed use of PEG 4000 as excipient in formulations of the additive due to the absence of data.

The Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) issued an opinion on the use of PEG as a film coating agent for use in food supplement products (EFSA, 2007).

Lactococcus lactis NCIMB 30160 is currently authorised for use in silage for all animal species.² PEG 4000 is authorised in the European Union (EU) as a food additive (E 1521).³

¹ 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.

² Commission Implementing Regulation (EU) No 1263/2011 of 5 December 2011 concerning the authorisation of *Lactobacillus buchneri* (DSM 16774), *Lactobacillus buchneri* (DSM 12856), *Lactobacillus paracasei* (DSM 16245), *Lactobacillus paracasei* (DSM 16773), *Lactobacillus plantarum* (DSM 12836), *Lactobacillus plantarum* (DSM 12837), *Lactobacillus brevis* (DSM 12835), *Lactobacillus rhamnosus* (NCIMB 30121), *Lactococcus lactis* (DSM 11037), *Lactococcus lactis* (NCIMB 30160), *Pediococcus acidilactici* (DSM 16243) and *Pediococcus pentosaceus* (DSM 12834) as feed additives for all animal species. OJ L 322, 6.12.2011, p. 3.

³ Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L 295, 12.11.2011, p. 1.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of additional information⁴ to a previous application on the same product.⁵

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of *Lactococcus lactis* NCIMB 30160 is in line with the principles laid down in Regulation (EC) No 429/2008⁶ and the relevant guidance documents: Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008).

3. Assessment

The additive is a preparation of *Lactococcus lactis* NCIMB 30160 specified to contain a minimum of 4×10^{11} CFU/g additive (EFSA FEEDAP Panel, 2018). This product is currently authorised as a technological additive (functional group: silage additive) for use in silage for all animal species. The applicant is requesting the modification of the authorisation to include PEG 4000 in the list of possible cryoprotectants used in the manufacturing process. According to the conditions of use, the recommended dose of the additive is 0.25 mg/kg forage in fresh matter.⁷ On the other hand the applicant proposes a maximum level of PEC 4000 of 0.025 mg/kg silage.⁸ Considering a water loss of 10% during the silage process (maximum level observed in the efficacy studies), this would represent a maximum content of 9.1% PEG 4000 in the additive.

The FEEDAP Panel assessed the data submitted in 2016 in its opinion adopted in 2018 (EFSA FEEDAP Panel, 2018) and could not conclude on the safety for the environment of the proposed use of PEG 4000 as excipient in formulations of the additive due to the absence of data.

In the present assessment, the additional data submitted to demonstrate the safety for the environment of PEG 4000, at the proposed maximum limit of 0.025 mg/kg silage, have been evaluated.

3.1. Safety for the environment of PEG 4000

The applicant provided supplementary information consisting on a Phase I and Phase II environmental risk assessment, safety datasheets of PEG 4000 and a review from Toxnet (Toxicology data network) database.

3.1.1. Phase I assessment

3.1.1.1. Predicted environmental concentrations

The predicted environmental concentrations (PECs) in soil (PEC_{soil}) and groundwater (PEC_{gw}) were calculated according to the criteria described in EFSA guidance on environmental risk assessment of feed additives (2008).

Physico-chemical properties

The physico-chemical properties of PEG 4000 for Phase I calculation were obtained from 3 different safety data sheets of the substance and from a review of the Toxnet database and are summarised in Table 2.⁹

⁴ FEED dossier reference: FAD-2019-0008.

⁵ FEED Dossier reference: FAD-2016-0049.

⁶ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

⁷ Technical dossier FAD-2016-0049/Section II.

⁸ Technical dossier/Annex I.

⁹ Technical dossier/Attachments 1 to 4.

Table 2: Physico-chemical properties of PEG 4000

Property	Value	Unit
Molecular weight	3,700–4,400	g/mol
Estimated $K_{oc}^{(a)}$	10	–
Water solubility	500	g/L
Vapour pressure ^(b)	< 1	Pa

K_{oc} : organic carbon-water partitioning coefficient.

(a): TOXNET Data Base of US National Library of Medicine (Technical dossier/Attachment 4).

(b): EPI Suite™, Estimation Program Interface, 2015.

The proposed maximum limit of PEG 4000 of 0.025 mg/kg silage, as a worst case, is considered as the total amount per kg feed. Dairy cows are taken as the reasonable worst case to calculate PEC values in Phase I. Results are reported in Table 3.

Table 3: PEC values for PEG 4000 in Phase I assessment

Compartment	PEC
Soil	0.33 µg/kg
Ground water	0.25 µg/L

PEC: predicted environmental concentration.

The PEC_{soil} is below the trigger value; therefore, no risk is expected for terrestrial organisms. Considering groundwater, the calculated PEC value exceeds the screening trigger of 0.1 µg/L. Nevertheless, it has to be underlined that the calculation was performed considering a very worst-case approach, since it is highly unlikely that the diet of dairy cows is composed just by silage. In a more realistic approach, silage represents only about half of the dairy cows' feed intake in a dry matter basis, which would reduce the $PEC_{groundwater}$ to 0.125 µg/L. Moreover, considering the information provided by the applicant (review of the Toxnet database),¹⁰ PEG 4000 is easily biodegradable; its biodegradability is reported as higher than 95% (23 days) (Zgoła-Grześkowiak et al., 2005).

Therefore, taking into consideration both the low amount of PEG 4000 used (maximum 0.025 mg/kg silage) and the ready biodegradability of the substance, no safety concerns are expected for groundwater despite the screening criterion for feed additives was exceeded. The environmental evaluation of PEG 4000 can stop in Phase I.

3.1.1.2. Conclusions on safety for the environment

The environmental assessment of PEG 4000 stops in Phase I. No concern is expected for the environment when the PEG 4000 component of the additive *L. lactis* NCIMB 30160 is used up to a maximum concentration of 0.025 mg PEG 4000/kg silage for all animal species.

4. Conclusions

No safety concerns are expected for the environment when PEG 4000 is used as a cryoprotectant in the additive *Lactococcus lactis* NCIMB 30160 up to a maximum concentration of 0.025 mg PEG 4000/kg silage.

Documentation as provided to EFSA/Chronology

Date	Event
04/05/2017	Dossier received by EFSA. <i>Lactococcus lactis</i> NCIMB 30160 for all animal species, submitted by Lactosan GmbH & Co. KG
27/05/2019	Reception mandate from the European Commission
04/06/2019	Application validated by EFSA – Start of the scientific assessment
07/10/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

¹⁰ Technical dossier/Attachment 4. TOXNET® (TOXicology Data NETwork), available online: <https://toxnet.nlm.nih.gov/> (see the bottom of page 39 of the attachment).

References

- EFSA (European Food Safety Authority), Aguilar F, Autrup H, Barlow S, Castle L, Crebelli R, Dekant W, Engel K-H, Gontard N, Gott D, Grilli S, Guertler R, Larsen JC, Leblanc J-C, Malcata FX, Mennes W, Milana MR, Pratt I, Rietjens I, Tobbacck P and Toldrá F, 2007. Opinion on the use of polyethylene glycol (PEG) as a film coating agent for use in food supplement products. EFSA Journal 2007;5(1):414, 22 pp. <https://doi.org/10.2903/j.efsa.2007.414>
- EFSA (European Food Safety Authority), 2008. Technical guidance of the Panel on Additives and Products or Substances used in Animal Feed for assessing the safety of feed additives for the environment. EFSA Journal 2008;842, 28 pp. <https://doi.org/10.2903/j.efsa.2008.842>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Scientific Opinion on the safety and efficacy of *Lactococcus lactis* (DSM 11037) as a silage additive for all species. EFSA Journal 2011;9(9):2374, 10 pp. <https://doi.org/10.2903/j.efsa.2011.2374>. Available online: www.efsa.europa.eu/efsajournal
- 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, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Villa RE, Wallace RJ, Wester P, Brozzi R and Saarela M, 2018. Scientific Opinion on the safety and efficacy of *Lactococcus lactis* NCIMB 30160 as a feed additive for all animal species. EFSA Journal 2018;16(3):5218, 8 pp. <https://doi.org/10.2903/j.efsa.2018.5218>
- Zgoła-Grześkowiak A, Grześkowiak T, Zembruska J and Lukaszewski Z, 2005. Comparison of biodegradation of poly(ethylene glycol)s and poly(propylene glycol)s. Chemosphere, 64, 803–809.

Abbreviations

AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
CFU	colony forming unit
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed)
K_{oc}	organic carbon-water partitioning coefficient
PEC	Predicted environmental concentration
PEG 4000	Polyethylene glycol polymer of an average molar mass of 4000 da