SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 27 September 2023

doi: 10.2903/j.efsa.2023.8347

Safety and efficacy of the feed additive consisting of *Enterococcus lactis* NCIMB 10415 (Cylactin®) for all poultry for fattening or reared for laying/breeding and ornamental birds, all Suidae, calves and other ruminant species for fattening or rearing (DSM Nutritional products Ltd)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the assessment of Cylactin® as zootechnical additive. The active agent of the additive is *Enterococcus lactis* NCIMB 10415, and three additive formulations currently authorised: Cylactin® LBC G35, Cylactin® LBC ME10 and Cylactin® LBC ME20 plus. The additive is currently authorised in the EU for use in poultry (chickens and minor poultry species for fattening, chickens and minor species reared for laying), calves and kids for rearing and for fattening, sows, suckling and weaned piglets and pigs for fattening. The applicant is now seeking the renewal of its authorisation and the extension of use for chickens and minor poultry species reared for breeding, turkeys for fattening and reared for breeding, ornamental birds, lambs for rearing and for fattening, minor or other ruminants' species for rearing and fattening, minor suckling and weaned Suidae species, pigs and minor Suidae species for fattening, rearing or reproduction. In addition, the applicant is seeking authorisation for use in water for drinking for all above-mentioned target species and categories. The applicant has provided evidence that the additive currently on the market complies with the conditions of authorisation. The FEEDAP Panel concludes that the additive is safe for the target animals, consumers and the environment under the authorised/new proposed conditions of use. The Cylactin® LBC ME10 and LBC ME20 plus are not skin and eye irritants, but no conclusion could be drawn on the potential of Cylactin® LBC G35 to be skin and eye irritant. Moreover, no conclusions could be drawn on the additive skin sensitisation potential. The additive is considered a potential respiratory sensitiser. The efficacy for the new target species/categories as well its use in water was extrapolated from the previous efficacy studies.

Keywords: zootechnical additive, gut flora stabiliser, Cylactin®, *Enterococcus lactis* NCIMB 10415, safety, efficacy, renewal

Requestor: European Commission

Question number: EFSA-Q-2020-00391

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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 acknowledge the contribution to this opinion of Nicole Bozzi, Qasim Chaudhry, Jan Mast and Eveline Verleysen, the cross-cutting Working Group nanotechnologies of the Scientific Committee and following Working Groups of the FEEDAP Panel: WG Environment, WG Microbiology and WG Toxicology.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Durjava, M., Dusemund, B., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Villa, R. E., Woutersen, R., Cocconcelli, P. S., … Revez, J. (2023). Safety and efficacy of the feed additive consisting of *Enterococcus lactis* NCIMB 10415 (Cylactin®) for all poultry for fattening or reared for laying/breeding and ornamental birds, all Suidae, calves and other ruminant species for fattening or rearing (DSM Nutritional products Ltd). *EFSA Journal*, *21*(10), 1–19. https://doi.org/10.2903/j.efsa.2023.8347

ISSN: 1831-4732

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

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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 a feed additive shall submit an application in accordance with Article 7. In particular, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest 1 year before the expiry date of the authorisation.

The European Commission received a request from the company DSM Nutritional Products Ltd represented in the EU by DSM Nutritional Products Sp. Z o.o.[2] for renewal of the authorisation of the product Cylactin® (*Enterococcus lactis*[3] NCIMB 10415), when used as a feed additive (category: zootechnical additive; functional group: gut flora stabiliser) in feed for chickens and minor poultry species for fattening, chickens and minor species reared for laying, calves and kids for rearing and fattening, sows, suckling and weaned piglets and pigs for fattening. A new authorisation of the product is requested for chickens and minor poultry species reared for breeding, for turkeys for fattening and reared for breeding, ornamental birds, lambs and minor ruminant species for rearing and fattening, pigs for rearing and for reproduction, minor suckling and weaned Suidae species, minor Suidae species for rearing, fattening and reproduction and the use in water for drinking.

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) and under Article 14(1) (renewal of the authorisation). The dossier was received on 8 May 2020 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2020-00391. The particulars and documents in support of the application were considered valid by EFSA as of 31 July 2020.

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 product Cylactin® (*Enterococcus lactis* NCIMB 10415), when used under the proposed conditions of use (see **Section 3.1.4**).

1.2. Additional information

The additive Cylactin® is a preparation of viable cells of *Enterococcus lactis* NCIMB 10415. EFSA has issued the following opinions on Cylactin®: two on the safety for dogs and cats (EFSA, 2004; EFSA FEEDAP Panel, 2013a), one on the safety and efficacy for calves, lambs and kids for rearing and for fattening (EFSA FEEDAP Panel, 2013b), three on the safety and efficacy for chickens for fattening (EFSA FEEDAP Panel, 2010a, 2010b, 2014), one on the safety and efficacy for minor poultry species for fattening and minor poultry species reared for laying (EFSA FEEDAP Panel, 2014), one on the efficacy for pigs for fattening (EFSA FEEDAP Panel, 2018a) and one re-evaluation regarding the safety and efficacy for pigs for fattening, piglets and sows (EFSA FEEDAP Panel, 2015). Additionally, EFSA has issued one opinion on the preparation of viable cells of *Enterococcus lactis* NCIMB 10415 as silage additive for all animal species (EFSA FEEDAP Panel, 2013c).

The additive Cylactin® is authorised in different formulations, and herein three formulations are assessed: one non-coated (Cylactin® LBC G35) and two micro-encapsulated (Cylactin® LBC ME10 and Cylactin® LBC ME20 plus). The additive is authorised for use in feed for chickens for fattening (formulations Cylactin® LBC ME10 and Cylactin® LBC ME20 plus),[4] chickens reared for laying, minor poultry species for fattening and minor poultry species reared for laying (formulations Cylactin® LBC

[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] DSM Nutritional Products Ltd, Switzerland represented by DSM Nutritional Products Sp z o.o., UI Tarcynska 113, PL 96–320, Mszczonow, Poland.
[3] Previously identified as *Enterococcus faecium*.
[4] Commission Implementing Regulation (EU) No 361/2011 of 13 April 2011 concerning the authorisation of *Enterococcus faecium* NCIMB 10415 as a feed additive for chickens for fattening (holder of authorisation DSM Nutritional products Ltd represented by DSM Nutritional Products Sp. Z.o.o) and amending Regulation (EC) No 943/2005. OJ L 100, 14.4.2011, p. 22.

ME10 and Cylactin® LBC ME20 plus),[5] sows, suckling piglets, weaned piglets and pigs for fattening (all three formulations)[6] and calves (all three formulations),[7] cats and dogs (other micro-encapsulated formulation with shellac not herein under assessment)[7] (4b1705).

In addition, the additive (corresponding to the formulation Cylactin® LBC ME10) is authorised for use for all animal species as a technological additive (functional group: silage additives) (1k20601).[8]

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[9] in support of the authorisation request for the use of Cylactin® (*Enterococcus lactis* NCIMB 10415) as a feed additive.

The confidential version of the technical dossier was subjected to target consultation of the interested Member States from 3 August 2020 to 3 November 2020 for which received comments that were considered for the assessment.

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 to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the *Enterococcus lactis* NCIMB 10415 in animal feed are valid and applicable for the current application.[10]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Cylactin® (*Enterococcus lactis* NCIMB 10415) is in line with the principles laid down in Regulation (EC) No 429/2008[11] and the relevant guidance documents: Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013d), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), 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 consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018c) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

[5] Commission Implementing Regulation (EU) 2015/518 of 26 March 2015 concerning the authorisation of the preparation of *Enterococcus faecium* NCIMB 10415 as a feed additive for chickens reared for laying, minor poultry species for fattening and minor poultry species reared for laying and amending Implementing Regulation (EU) No 361/2011 as regards the compatibility with coccidiostats (holder of the authorisation DSM Nutritional Products Ltd represented by DSM Nutritional products Sp. z o.o). OJ L 82, 27.3.2015, p. 75.

[6] Commission Implementing Regulation (EU) 2019/11 of 3 January 2019 concerning the authorisation of the preparation of *Enterococcus faecium* NCIMB 10415 as a feed additive for sows, suckling piglets, weaned piglets, pigs for fattening and amending Regulations (EC) No 252/2006, (EC) No 943/2005 and (EC) No 1200/2005 (holder of authorisation DSM Nutritional products Ltd, represented by DSM Nutritional Products Sp. z o.o.). OJ L 2, 04.01.2019, p. 17.

[7] Commission Implementing Regulation (EU) No 1061/2013 of 29 October 2013 concerning the authorisation of a preparation of *Enterococcus faecium* NCIMB 10415 as a feed additive for calves, kids, cats and dogs and amending Regulation (EC) No 1288/2004 (holder of the authorisation DSM Nutritional Products Ltd represented by DSM Nutritional products Sp. Z o.o). OJ L 289, 30.10.2013, p. 38.

[8] Commission Implementing Regulation (EU) No 304/2014 of 25 March 2014 concerning the authorisation of the preparations of *Enterococcus faecium* NCIMB 10415, *Enterococcus faecium* DSM 22502 and *Pediococcus acidilactici* CNCM I-3237 as feed additives for all animal species. OJ L 90, 26.3.2014, pp. 8–11.

[9] FEED dossier reference: FAD-2020-0034.

[10] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0269%2B0294.pdf

[11] 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.

3. Assessment

Cylactin®[12] is a zootechnical additive consisting of dehydrated cells of *Enterococcus lactis* NCIMB 10415 intended to be used in feed and/or water for drinking for several animal species. Among those species, the additive is currently authorised for use in feed for chickens and minor poultry species for fattening and reared for laying, for suckling and weaned piglets and pigs for fattening and sows and for calves and kids for rearing and fattening. With the present application, the applicant seeks:

- the renewal of the authorisation of the additive for the species for which it is currently authorised;
- the extension of use of the additive in feed for chickens and minor poultry species reared for breeding, turkeys for fattening and reared for breeding, ornamental birds, pigs for rearing and reproduction, minor Suidae species for rearing, fattening and reproduction, minor suckling and weaned Suidae species, for lambs for rearing and fattening, and minor or other ruminant species for rearing and fattening;
- the authorisation of use of the additive in water for drinking for all the animal species and categories above mentioned, already authorised and/or applied for authorisation with the present application.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The active agent was isolated from the faeces of a healthy infant. It is deposited in the National Collection of Industrial, Marine and Food Bacteria (NCIMB) under the accession number NCIMB 10415.[13,14] The strain has not been genetically modified.

The strain was originally identified as *E. faecium* by phenotypic tests, it was characterised with the molecular typing methods (RAPD and PFGE), and shown to harbour one plasmid.[15] Recently the strains of *E. faecium* clade B have been reassigned to *E. lactis* species based on genomic studies that showed genetic and evolutionary differences between clade A and the intertwined clade B and *E. lactis* (Belloso Daza et al., 2021). The description of the species *Enterococcus lactis* demonstrated that it is a closely related to *Enterococcus faecium* (Morandi et al., 2012).

In the context of the current application, the active agent was identified as *E. lactis* by digital DNA–DNA hybridisation (dDDH) and average nucleotide identity (ANI) based on the whole genome sequence (WGS). The dDDH values of the strain NCIMB 10415 were of 90.30% and 60.10% with *E. lactis* KCTC 21015^T and *E. faecium* DSM 20477^T, respectively. Similarly, the strain NCIMB 10415 showed a higher value of ANI with *E. lactis* than with *E. faecium* type strains: 98.90% with *E. lactis* KCTC 21015^T and 94.8% with *E. faecium* DSM 20477^T.[16]

The susceptibility of *E. lactis* NCIMB 10415 to the list of antimicrobials recommended by the FEEDAP Panel (EFSA FEEDAP Panel, 2018b) was tested using broth serial twofold microdilution procedure.[17] The minimum inhibitory concentrations (MICs) of the strain compared with the defined EFSA cut-off values for the closest related species *Enterococcus faecium*. The MIC values for all antibiotics tested were below the EFSA cut-off values except for erythromycin and clindamycin (8 mg/L) and kanamycin (2048 mg/L), that exceeded the cut-off values by one dilution. Exceedance of the cut-off value by one dilution is considered to be within the variation of the method, and thus, not considered of concern. Therefore, the strain is considered to be susceptible to all the relevant antibiotics.

The WGS of the strain, including plasmid, was interrogated for the presence of antimicrobial resistance (AMR) genes against CARD (thresholds of 80% identity and 70% coverage at amino acid level) and ResFinder (thresholds of 80% identity and 60% coverage at amino acid level) databases.[18] The search in CARD identified four hits (*aac(6′)-Ii, eatA, msrC* and *efrA/B*), confirmed to be in the chromosome,[19] whereas no hits were obtained with the other database search. The hits are

[12] The additive has also the trade name of Cernivet®.
[13] Technical dossier/Section II/Annex II.32.
[14] Technical dossier/Supplementary Information August 2021/Appendix 0–3.
[15] Technical dossier/Section II/Annex II.34, Annex II.35, Annex II.37 and Annex II.43.
[16] Technical dossier/Supplementary Information August 2021/Appendix 0–5.
[17] Technical dossier/Section II/Annex II.47.
[18] Technical dossier/Supplementary information August 2021/Appendix 0–6.
[19] Technical dossier/Supplementary information August 2021/Appendix 0–6 and Appendix 0–8.

considered intrinsic of *E. lactis* and *E. faecium* clade B genomes. Therefore, no hits of concern were identified.

According to guidance (EFSA FEEDAP Panel, 2018b), the *E. faecium* safety should be assessed demonstrating the absence of genetic markers typical of the clinical isolates *E. faecium* clade A (IS*16*, *esp*, *hyl*) and the susceptibility to ampicillin. Taking in consideration the allocation of *E. faecium* clade B strains to *E. lactis* species (Belloso Daza et al., 2021), the FEEDAP Panel considers these criteria applicable also to *E. lactis* strains. *E. lactis* NCIMB 10415 is susceptible to ampicillin (MIC < 2 mg/L) and the WGS analysis demonstrated the absence of IS*16*, *hyl*-like sequences and *esp* gene.

3.1.2. Characterisation of the additive

The additive Cylactin® is authorised in three formulations: one non-coated (Cylactin® LBC G35) and two micro-encapsulated (Cylactin® LBC ME10 and Cylactin® LBC ME20 plus). The formulations Cylactin® LBC ME10 and Cylactin® LBC ME20 plus are intended for all types of feed, whereas Cylactin® LBC G35 is suitable for mash meal and liquid feeds. In addition, the formulations Cylactin® LBC G35 and Cylactin® LBC ME10 are suitable for use in water. The formulation Cylactin® LBC G35 is currently not authorised for use in poultry.

The active agent of Cylactin®, pure dehydrated culture of live *E. lactis* NCIMB 10415, is obtained by [redacted][20] The three formulations have the following guaranteed minimum concentrations of the active agent and carriers/encapsulating agents:

- Cylactin® LBC G35: 3.5×10^{10} CFU/g additive, with 93% (w/w) lactose as carrier.
- Cylactin® LBC ME10: 1.0×10^{10} CFU/g additive, with 88% (w/w) saccharose as carrier and 10% (w/w) hydroxypropyl methylcellulose[21] as encapsulating agent.
- Cylactin® LBC ME20 plus: 2.0×10^{10} CFU/g additive, with 76% (w/w) saccharose as carrier, 10% (w/w) hydroxypropyl methylcellulose as an encapsulating agent and 10% (w/w) of a coating agent defined by the applicant as [redacted] shellac.[22]

Compliance with the above-mentioned specifications was demonstrated by the analysis of recent batches of each formulation with the mean values of 1.2×10^{11} CFU/g (range $1.0–1.5 \times 10^{11}$ CFU/g), 5.4×10^{10} CFU/g (range $4.3–6.6 \times 10^{10}$ CFU/g) and 6.6×10^{10} CFU/g (range $5.8–8.3 \times 10^{10}$ CFU/g) for Cylactin® LBC G35 (seven batches), Cylactin® LBC ME10 (eight batches) and Cylactin® LBC ME20 plus (eight batches), respectively.[23]

Specifications are set for total aerobic microbial contamination ($\leq 10^3$ CFU/g), *Escherichia coli* (no detection in 1 g), *Salmonella* spp. (no detection in 25 g), *Pseudomonas aeruginosa* (no detection in 1 g), coagulase-positive staphylococci (in particular *Staphylococcus aureus*) (no detection in 0.1 g) and total filamentous fungi and yeasts ($\leq 10^2$ CFU/g), and analysis of the above-mentioned batches confirmed compliance with the established specifications.[23] No data on *Enterobacteriaceae* concentration was provided.

A total of three batches for each formulation were analysed for cadmium, lead, mercury and arsenic concentrations and mycotoxins.[24] Cadmium, mercury, aflatoxins, zearalenone, ochratoxin A and fumonisins B1 and B2 were not detected in the batches tested.[25] Arsenic and lead were not detected in the batches of Cylactin® LBC G35 formulation.[26] In the Cylactin® LBC ME10 formulation, arsenic was detected in two batches (0.012 and 0.013 mg/kg) and lead was detected in the three batches tested (mean 0.0097 mg/kg, range 0.009–0.01 mg/kg).[27] In the Cylactin® LBC ME20

[20] Technical dossier/Section II/Annex II.49 and Annex II.50.
[21] Currently under re-evaluation according to art. 10 of Regulation EC No 1831/2003 by the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP).
[22] The FEEDAP Panel notes that shellac is currently under re-evaluation by the Panel on Food Additives and Flavourings (FAF) as a food additive.
[23] Technical dossier/Supplementary Information August 2021/Appendix 0–22 – Appendix 0–27.
[24] Technical dossier/Supplementary Information August 2021/Appendix 0–25 – Appendix 0–28.
[25] Limit of detection (LOD) for cadmium and mercury 0.0017 mg/kg, sum of aflatoxins B1, B2, G1, G2 1 μg/kg, zearalenone 10 μg/kg, ochratoxin A 0.5 μg/kg and fumonisin B1 and B2 20 μg/kg.
[26] Technical dossier/Supplementary Information August 2021/Appendix 0–25.
[27] Technical dossier/Supplementary Information August 2021/Appendix 0–26.

formulation, averages of 0.017 mg As/kg (range 0.015–0.019 mg/kg) and 0.017 mg Pb/kg (range 0.011–0.022 mg/kg) were found.[28]

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

The applicant declared that the manufacturing process has not been significantly modified since the original authorisation, but the current formulation Cylactin® LBC ME20 plus has been subject to minor variations in the percentages of carrier and coating materials for technical reasons. In the previous formulation, the additive contained 84.6% (w/w) saccharose, 2.3% (w/w) hydroxypropyl methylcellulose and 9.1% (w/w) shellac, while in the current one, these proportions are 76%, 10% and 10%, respectively.[29] In order to establish the impact of these changes on the physico-chemical properties of the additive, the applicant has provided new data, as described below.

Two batches of the current formulation Cylactin® LBC ME20 plus were analysed for bulk and tapped density, and results showed an average of 820 kg/m^3 (range 810–830 kg/m^3) and 945 kg/m^3 (range 930–960 kg/m^3), respectively.[30]

The dusting potential of the additive, assessed with the Heubach I test, showed mean values of 200 mg/m^3 air (range 150–290 mg/m^3 air), 37 mg/m^3 air (range 10–70 mg/m^3 air) and 3 mg/m^3 air (range 1–4 mg/m^3 air) for Cylactin® LBC G35, Cylactin® LBC ME10 and the current Cylactin® LBC ME20 plus, respectively.[31] Moreover, the particle size distribution analysed in three batches of the current Cylactin® LBC ME20 plus formulation by laser diffraction method showed that all the particles were greater than 250 μm.[31]

3.1.3. Stability and homogeneity

The stability and homogeneity of the additive were assessed in the previous opinions (EFSA FEEDAP Panel, 2010a, 2013b). New data regarding the stability and homogeneity in water were submitted. The stability of three batches each of Cylactin® LBC G35 and Cylactin® LBC ME10, suspended in water for drinking at 20°C, was tested after 24 and 48 h. Losses of *E. lactis* counts after the testing periods were negligible (< 0.5 log).[32] The capacity of these formulations to distribute homogeneously in water for drinking was evaluated in the stability study based on the analysis of 10 subsamples and the coefficient of variation (CV) ranged 5–7% for Cylactin® LBC G35 and 7–8% for Cylactin® LBC ME10.[32]

The shelf-life of Cylactin® LBC ME20 plus was determined by monitoring three batches stored in high-density polyethylene containers with seal stopper at 2–8°C for 24 months and 25°C/60% relative humidity for 6 months. Negligible reduction of total enterococcal count (< 0.5 log) was observed at the end of both experimental periods.[33] The capacity for homogeneous distribution of Cylactin® LBC ME20 plus was tested when mixed in one batch of a complete feed for chickens (pelleted at 85°C) at 2.0 × 10^9 CFU/kg feed. Bacterial counts measured in 15 subsamples showed a CV of 11.8%.[34]

3.1.4. Conditions of use

Different formulations of Cylactin® [micro-encapsulated form with shellac (LBC ME20 plus), other micro-encapsulated form (LBC ME10) and non-coated granulated form (LBC G35)] are currently authorised as a zootechnical additive in feed for pigs, several poultry and ruminant species and categories as shown in Table 1.

[28] Technical dossier/Supplementary Information August 2021/Appendix 0–27.
[29] Technical dossier/Supplementary Information August 2021/DSM_Cylactin renewal SIN_reply_EFSA-Q-2020-00391.
[30] Technical dossier/Supplementary Information August 2021/Appendix 0–11.
[31] Technical dossier/Supplementary Information August 2021/Appendix 0–13.
[32] Technical dossier/Section II/Annex II.46.
[33] Technical dossier/Supplementary Information August 2021/Appendix 0–14.
[34] Technical dossier/Supplementary Information August 2021/Appendix 0–15.

Table 1: Current authorisations of Cylactin® for target species

Target species	Cylactin® LBC Formulations[35]	Use level in feed CFU/kg	Other provisions of the authorisations
Chickens for fattening	ME10 ME20 plus	3×10^8	1. In the directions for use of the additive and premixture, indicate the storage temperature, storage life and stability to pelleting. 2. The use is permitted in feed containing the authorised coccidiostats: decoquinate, monensin sodium, robenidine hydrochloride, diclazuril or semduramycin.
Chickens reared for laying	ME10 ME20 plus		1. In the directions for use of the additive and premixture, indicate the storage conditions and stability to pelleting. 2. The use is permitted in feed containing the authorised coccidiostats: monensin sodium, diclazuril, lasalocid A sodium or salinomycin sodium.
Minor poultry species for fattening and reared for laying	ME10 ME20 plus		1. In the directions for use of the additive and premixture, indicate the storage conditions and stability to pelleting. 2. The use is permitted in feed containing the authorised coccidiostats: diclazuril or lasalocid A sodium.
Suckling piglets	G35 ME10 ME20 plus	1×10^9	1. In the directions for use of the additive and premixtures, the storage conditions and stability to heat treatment shall be indicated. 2. The additive shall be administered to pregnant and lactating sows and simultaneously to the suckling piglets 3. For use in weaned piglets until approximately 35 kg. 4. For users of the additive and premixtures, feed business operators shall establish operational procedures and organisational measures to address potential risks resulting from their use. Where those risks cannot be eliminated or reduced to a minimum by such procedures and measures, the additive and premixtures shall be used with personal protective equipment.
Weaned piglets		3.5×10^8	
Pigs for fattening			
Sows		7×10^8	
Calves for fattening and rearing		1×10^9	In the directions for use of the additive and premixture, indicate the storage conditions and stability to pelleting.
Kids for fattening and rearing			

The applicant does not request to modify the conditions of the current authorisations.
In addition, the applicant requests the following:

1) authorisation of the Cylactin® LBC G35 formulation in feed for chickens for fattening, chickens reared for laying, minor poultry species for fattening or reared for laying at the same minimum use level as currently authorised.
2) authorisation of all formulations of the additive for use in feed for chickens and minor poultry species reared for breeding, turkeys for fattening or reared for breeding, ornamental birds, pigs and minor pigs species for reproduction, pigs for rearing, minor suckling and weaned Suidae species, minor Suidae species for rearing and fattening, lambs for rearing and fattening, minor or other ruminant species for rearing and fattening, at the minimum use levels detailed in Table 2.
3) authorisation of the additive LBC G35 and LBC ME 10 formulations in water for drinking for all the animal species and categories above mentioned, already authorised and/or applied for authorisation with the present application. The applicant proposed for the use of Cylactin® in water for drinking that the concentrations in water can be derived from the proposed use level in feed, considering that for poultry, Suidae and ruminant species for rearing and for fattening, the water intake would be two to three times higher than feed intake (in dry matter).

[35] Refered to in the regulation as micro-encapsulated form with shellac (Cylactin® LBC ME20 plus), other micro-encapsulated forms (Cylactin® LBC ME10) and non-coated granulated form (Cylactin® LBC G35).

Table 2: Request for extension to new target species

Target species	Use level in feed CFU/kg
Chickens and minor poultry species reared for breeding, turkeys for fattening or reared for breeding and ornamental birds	3×10^8
Minor suckling Suidae species	1×10^9
Pigs for rearing, minor weaned Suidae species and minor pig species for rearing and fattening	3.5×10^8
Pigs for reproduction and minor Suidae species for reproduction	7×10^8
Lambs for rearing and fattening, minor or other ruminants' species for rearing and fattening	1×10^9

3.2. Safety

3.2.1. Safety of the additive

In the previous opinions, the Panel concluded that the herein under assessment Cylactin® formulations (Cylactin® LBC G35, Cylactin® LBC ME10 and Cylactin® LBC ME20 plus) were considered safe for target species, consumers and the environment (EFSA FEEDAP Panel, 2010a, 2013b, 2014, 2015). In the context of the current application, the identity of the strain NCIMB 10415 was reassigned to *E. lactis*, and evidence that the strain does not harbour acquired AMR genes or virulence was provided. The FEEDAP Panel considers the criteria to access the safety of *E. faecium* applicable also to *E. lactis* strains. In addition, the manufacturing process of the additive, its composition and the conditions of use for the target species for which an authorisation exists have not been substantially modified. Consequently, the conclusions already reached are still deemed valid, and the Panel considers that under assessment Cylactin® formulation remains safe for the target species, consumers and the environment. The Panel also considers that the extension of use of the additive in feed for all poultry for fattening or reared for laying/breeding and ornamental birds, calves and other ruminants for fattening or reared for milk production at the same physiological stage and all Suidae and in water for drinking for these species and the species for which an authorisation exists would not introduce hazards not already assessed. Therefore, the proposed extensions of use are considered of no concern for the target species, consumers and environment.

As regards user safety, in a previous opinion (EFSA FEEDAP Panel, 2010a), the Panel concluded that Cylactin® is not a skin or eye irritant based on the data provided for Cylactin® LBC ME20 plus. These conclusions can be extrapolated to the other micro-encapsulated formulation. However, no new data was provided in the current application for the non-encapsulated formulation (G35), and therefore, the Panel cannot conclude on the potential of this formulation to be skin and eye irritant.

Moreover, the data on the skin sensitisation test performed with the Cylactin® LBC ME20 plus formulation were previously submitted and assessed (EFSA FEEDAP Panel, 2010a); however, the Panel notes that the OECD test guidelines available at present are designed to assess the skin sensitisation potential of chemical substances only and that currently no validated assays for assessing the sensitisation potential of microorganisms are available.[36]

The applicant conducted an extensive literature search (ELS) on the safety of the additive covering the period from 1 January 2010 and 19 April 2021 in the databases Scopus and PubMed, including the search terms 'cylactin' and 'NCIMB 10415'.[37] The search identified a total of 82 hits in Scopus and 61 hits in PubMed, from which after deduplication (56 hits in common in both databases), a total of 27 hits were identified as potentially relevant and screened for safety for the target species, consumers, users and environment. Thereof, two hits did not regard the additive and 25 focused on efficacy. None of the publications reported adverse effects, toxicological concerns or any other safety-related negative observations of the additive under assessment on the target species, consumers, users or the environment.

The formulations Cylactin® LBC G35 and LBC ME10 of the additive do not contain excipients of concern. The formulation of Cylactin® LBC ME20 plus includes [redacted] shellac as a coating agent, which is not currently authorised as a feed additive. The applicant provided data that allowed the Panel to characterise and assess the safety of this coating agent (see Appendix A for the complete assessment). Based on the data assessed, the FEEDAP Panel concludes that the use of

[36] https://www.efsa.europa.eu/sites/default/files/2022-07/feedap20220629-30_m.pdf
[37] Technical dossier/Supplementary Information August 2021/Appendix 0–29.

this excipient as a coating agent in one of the formulations of the additive under assessment (Cylactin® LBC ME20 plus) does not raise safety concerns for the target species, consumers and environment.

3.2.1.1. Conclusions on safety

Considering all the above and the fact that the composition and the manufacturing process of the different formulations of Cylactin® have not been substantially modified, the FEEDAP Panel concludes that the additive remains safe for target species, consumers and the environment under the authorised conditions of use.

The Panel also concludes that the proposed extension of use of the additive for the new target species and use in water would not add hazards not already considered in the previous opinions. Therefore, the use of the Cylactin® formulations (LBC G35, LBC ME10 and LBC ME20 plus) in feed for chicken and minor poultry species reared for breeding, turkeys for fattening and reared for breeding, ornamental birds, lambs and minor or other ruminant species for fattening and rearing, pigs and minor pig species for rearing and fattening, minor pig species (suckling and weaning), pigs and minor pig species for reproduction, and the use in water for drinking for all the target species for which application is made is safe for the target species, consumers and the environment.

The micro-encapsulated Cylactin® formulations (LBC ME10 and LBC ME20 plus) are not skin and eye irritants, but the Panel cannot conclude on the potential of non-encapsulated formulation to be skin and eye irritant. Moreover, no conclusions can be drawn on the additive skin sensitisation potential.

3.3. Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of use for those species/categories for which there is an authorisation. Therefore, there is no need to assess the efficacy of the additive in the context of the renewal of the authorisation.

The current application includes the extension of use:

- in the Cylactin® LBC G35 formulation in feed for chickens for fattening, chickens reared for laying, minor poultry species for fattening or reared for laying at the same minimum use level as currently authorised;
- of all Cylactin® formulations in feed for chickens and minor poultry species for breeding, turkeys for fattening or reared for breeding, ornamental birds, pigs and minor pigs species for reproduction, pigs for rearing, minor suckling and weaned pig species, minor pig species for rearing and fattening, lambs for rearing and fattening, minor or other ruminant species for rearing and fattening, at the minimum use levels detailed in Table 2;
- of Cylactin® G35 and ME 10 formulations in water for drinking for all the animal species and categories above mentioned, already authorised and/or applied for authorisation with the present application.

The different formulations of the additive are considered equivalent in terms of efficacy.

The FEEDAP Panel in accordance with the provisions of the FEEDAP Panel Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018c) considers that the conclusions already reached:

- in chickens for fattening (EFSA FEEDAP Panel, 2010a) can be extended/extrapolated to chickens and minor poultry species reared for breeding, turkeys for fattening and reared for breeding and ornamental birds at the corresponding use level (3×10^8 CFU/kg complete feed);
- in sucking and weaned piglets (EFSA FEEDAP Panel, 2015) and pigs for fattening (EFSA FEEDAP Panel, 2018a) can be extrapolated to minor suckling Suidae species (1×10^9 CFU/kg complete feed), minor weaned Suidae species and minor Suidae species for rearing and fattening (3.5×10^8 CFU/kg complete feed) at the corresponding use levels;
- in sows (EFSA FEEDAP Panel, 2015) can be extrapolated to pigs and minor pig species for reproduction at the corresponding use level (7×10^8 CFU/kg complete feed);
- in calves for fattening and calves for rearing (EFSA FEEDAP Panel, 2013b) can be extrapolated to lambs for fattening and rearing and minor or other ruminants' species for rearing and fattening at the same corresponding use level (1×10^9 CFU/kg complete feed).

The applicant proposed that the concentrations in water can be derived from the use levels in feed, considering that for poultry, pigs and ruminant species for rearing and for fattening, the water intake would be two to three times higher than feed intake (in dry matter). The FEEDAP Panel concludes that the additive is efficacious when used in water provided that the exposure of the animals is similar to that resulting of the use of the additive in feed.

Regarding the compatibility with coccidiostats, the FEEDAP Panel considers that the conclusions already reached previously (EFSA FEEDAP Panel, 2010a, 2014) can be extrapolated for the other poultry species at the relevant physiological stage and for the coccidiostats for which an authorisation exists.

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[38] and good manufacturing practice.

4. Conclusions

The applicant has provided evidence that the additive currently on the market complies with the existing conditions of authorisation.

The FEEDAP Panel concludes that the use of Cylactin® formulations Cylactin® LBC G35, Cylactin® LBC ME10 and Cylactin® LBC ME20 plus under the current authorised conditions of use remains safe for all animal species, consumers and the environment. The proposed new uses in target species and water are considered safe.

Regarding the user safety, the micro-encapsulated Cylactin® formulations (LBC ME10 and LBC ME20 plus) are not skin and eye irritants, but the Panel cannot conclude on the potential of non-encapsulated formulation to be skin and eye irritant. Moreover, no conclusions can be drawn on the additive skin sensitisation potential. The additive is considered a potential respiratory sensitiser.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation. The FEEDAP Panel concludes that all Cylactin® formulations have the potential to be efficacious in feed for chickens and minor poultry species for breeding, turkeys for fattening or reared for breeding, ornamental birds (3×10^8 CFU/kg complete feed), in minor suckling Suidae species (1×10^9 CFU/kg complete feed), pigs for rearing, minor weaned Suidae species and minor Suidae species for rearing and fattening (3.5×10^8 CFU/kg complete feed), pigs and minor Suidae species for reproduction (7×10^8 CFU/kg complete feed), and lambs for rearing and fattening, minor or other ruminant species for rearing and fattening (1×10^9 CFU/kg complete feed).

Similarly, the Panel concludes that the use of Cylactin® G35 and ME 10 formulations in water for drinking for all the animal species and categories above mentioned has the potential to be efficacious provided that the exposure of the animals is equivalent to that as when used in feed.

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Abbreviations

ANS	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
CFU	colony-forming unit
CV	coefficient of variation
EURL	European Union Reference Laboratory
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
NCIMB	National Collection of Industrial, Marine and Food Bacteria
WGS	Whole genome sequencing

Appendix A – Assessment of [redacted] shellac as coating agent

The formulation of Cylactin® LBC ME20 plus includes as a coating agent [redacted] shellac, claimed by the applicant to be used as food additive (E 904),[39] which is not currently authorised as a feed additive. EFSA published a statement on the safety and efficacy of shellac for all animal species (EFSA FEEDAP Panel, 2020), in which the Panel could not deliver an opinion on the safety and efficacy due to the lack of adequate data. The applicant has provided new data to support the safety of the above-mentioned coating agent for the target species, consumer and environment, namely, data on characterisation,[40] physico-chemical properties following the Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA SC, 2021),[41] genotoxicity,[42] subchronic oral toxicity,[43] carcinogenicity[44] and reproduction toxicity.[45,46] No information was made available on the toxicokinetics [absorption, distribution, metabolism, excretion (ADME)] nor any information on possible deposition of shellac or its metabolites in tissues and/or products.

A.1. Characterisation and physico-chemical properties of Shellac

According to the European Pharmacopeia 10.0, shellac is defined as '*purified material obtained from the resinous secretion of the female insect Kerria lacca (Kerr) Lindinger (Laccifer lacca Kerr)*'[47] and is considered the general term for the refined form of lac. This polyester resin is a complex mixture of aliphatic and sesquiterpenic acids, with the following major components: aleuritic acid (ca. 45%), jalaric acid (ca. 27%), shellolic acid (ca. 2–8%), butolic acid (5–8%) and kerrolic acid (1%).[48] Shellac contains a variable amounts of wax depending on the treatment applied to the crude secretion (seedlac),[49] and the European Pharmacopeia 10.0 considers four types based on the refining process: wax-containing shellac (obtained from seedlac and purified by filtration of the molten substance and/or by hot extraction using a suitable solvent), bleached shellac (treatment with sodium hypochlorite after dissolution in a suitable alkaline solution), bleached dewaxed shellac (treatment with sodium hypochlorite after dissolution in a suitable alkaline solution and removal of the insoluble wax by filtering) and dewaxed shellac (treatment with a suitable solvent and removal of the insoluble wax by filtering).[47] From these, only two types of shellac are authorised as food additive (E 904): bleached shellac and wax-free bleached shellac.[50]

The type of shellac used in one formulation of the additive under assessment is [redacted].[51,52] The applicant provided data on the manufacturing of the shellac used in the additive,[53] certificates of analyses on five batches of the product used for coating the additive under assessment, for physico-chemical parameters and microbiological contamination[54] and data for three batches for chemical contaminants (including cadmium, lead, mercury and arsenic) of

[39] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354. and Technical dossier/Supplementary Information August 2021/Appendices 0/Section II_Identity Shellac.
[40] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendix 0–30.
[41] Technical dossier/Supplementary Information February 2023/Appendix 12 and Appendix 14.
[42] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_III_AnimConsUser/3–23 and 3–25.
[43] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_III_AnimConsUser/3–26 and 3–27.
[44] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_III_AnimConsUser/Appendices 3–28, 3–29, 3–30, 3–31, 3–31, 3–32 and 3–33.
[45] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_III_AnimConsUser/Appendices 3–13, 3–14, 3–15, 3–16 and 3–17.
[46] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendix 0–31 Sect_III_AnimConsUser_Shellac.
[47] Technical dossier/Supplementary Information February 2023/Appendix 5.
[48] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_II_Identity/Appendix 2–32 and Annexes_Sect_III_ERA/Annex_III_ERA_12_CIR_1986.
[49] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_II_Identity/Appendix 2–22.
[50] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012.
[51] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_II_Identity/Appendix 2–9 PDS.
[52] [redacted]
[53] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_II_Identity/Appendix 0–30.
[54] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_II_Identity/Appendix 2–23.

the product used as raw material to produce the [redacted][55] and stability studies.[53] All these analyses showed compliance with the specifications according to the current pharmacopoeias (Ph.Eur. and USP).[56]

The applicant provided data generated by various analytical techniques [redacted] to demonstrate the absence of small/nano particles in [redacted].[41] The FEEDAP Panel notes that the results could not be used to unequivocally exclude the presence of small/nano particles and that the characterisation of the particulate nature of a polymeric material is inherently difficult. Considering that (i) shellac is soluble in alkaline aqueous media and ethanol, (ii) shellac forms continuous polymeric films/sheets and not particles when in solid form and (iii) the test items [redacted] used in the toxicological studies can be considered representative for the shellac under assessment and are considered to cover any potential hazard which may be related to the presence of potentially present small/nano particles, the FEEDAP Panel concludes that the safety of shellac tested can be adequately covered by the conventional risk assessment.

A.2. Safety

A.2.1. Toxicological studies including genotoxicity

A.2.1.1. Genotoxicity

Bacterial reverse mutation test

[redacted] shellac was evaluated for the induction of reverse mutations in Salmonella Typhimurium tester strains (TA1537, TA1535, TA98, TA100 and TA102) according to the OECD TG 471, in a study claimed to be GLP compliant.[57] [redacted] No increase in the number of revertant colonies was observed with any of the tester strains in either the presence or absence of metabolic activation.

In vitro *mammalian cell micronucleus test*

[redacted] shellac was evaluated in an *in vitro* micronucleus assay performed in human peripheral blood lymphocytes for its ability to induce structural chromosomal damage and aneuploidy according to the OECD TG 487 (2016) and claimed to be GLP compliant.[58] [redacted] The frequencies of micronuclei in binucleated cells were comparable between treated and vehicle control cultures both in the presence and absence of metabolic activation.

A.2.1.2. General Toxicology

Subchronic repeated dose toxicity study

[redacted] shellac was evaluated in a 90-day repeated-dose oral toxicity study in rats, conducted according to the OECD TG 408 and claimed to be GLP compliant.[43] [redacted] the FEEDAP Panel considered 20,000 mg [redacted] shellac/kg diet (corresponding to 1,384

[55] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_II_Identity/Appendix 2–28.
[56] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendix 0–30 and Appendices_Sect_II_Identity/Appendix 2–9 PDS.
[57] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_III_AnimConsUser/3–23.
[58] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_III_AnimConsUser/3–25.

and 1,569 mg/kg bw per day for males and females, respectively), the highest level tested, as the no observed adverse effect level (NOAEL).

Carcinogenicity studies

[redacted] shellac was evaluated in a combined chronic toxicity/carcinogenicity study in rats, following prolonged and repeated dietary exposure of Wistar rats.[44] [redacted] Based on these results, the FEEDAP Panel considered 10,000 mg [redacted] shellac/kg diet (corresponding to 452 mg/kg bw per day for male rats and 569 mg/kg bw per day for female rats, respectively), the highest level tested, as the NOAEL.

Reproduction toxicity study

The potential adverse effects on reproduction and fertility arising as a result of repeated daily dietary administration of [redacted] were investigated in two successive generations i.e. Parent (P) and First Filial (F1) of Wistar rats, following the OECD TG 416.[59] [redacted]

An oral dietary reproduction/developmental toxicity screening test in Wistar rats (n = 12/sex per group) was performed according to OECD TG 421.[60] [redacted] The Panel considered the low-dose group (300 mg [redacted] shellac/kg bw per day) as the NOAEL for maternal toxicity and the high-dose (1,200 mg [redacted] shellac/kg bw per day) as the NOAEL for reproductive and developmental toxicity.

Oral prenatal developmental toxicity studies

An oral prenatal developmental toxicity test was performed according to OECD TG 414 and claimed to be GLP compliant.[61] [redacted]

[59] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_III_AnimConsUser/Appendices 3–13, 3–14, 3–15, 3–16 and 3–17.
[60] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendix 0–33 LoA_TESA Data_FEEDAP and 10858 glp.
[61] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_III_AnimConsUser/Appendix 3–34.

[redacted] Based on the results, the NOAEL of [redacted] shellac for maternal and developmental toxicity is 1,000 mg [redacted] shellac/kg bw per day (highest dose tested).

A.2.1.3. Conclusion on the toxicological studies

Based on the toxicological studies performed with the test items representative of the shellac under assessment [redacted], the FEEDAP Panel concluded that [redacted] shellac is not genotoxic. The toxicological data set available allowed to identify the lowest NOAEL of 300 mg/kg bw per day, based on reproduction/developmental toxicity study in rats.

A.2.2. Safety of [redacted] shellac for the target species

To evaluate the safety of [redacted] shellac for the target species, the maximum safe concentration of [redacted] shellac in complete feed for the target species was calculated as described in the guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP, 2017a),[62] using the lowest NOAEL of 300 mg/kg bw per day identified in a reproduction/developmental toxicity study in rats and based on the proposed maximum inclusion level of the additive in feed and results reported in Table A.1.[46]

Table A.1: Maximum safe concentration of [redacted] shellac in feed for target species

Animal category	Default values for daily feed intake (g dry matter/kg)	Safe concentration in feed (mg/kg complete feed)**	Maximum inclusion level of [redacted] shellac in complete feed (mg/kg complete feed)
Chicken for fattening	79	33	1.5
Laying hen	53	50	1.5
Turkey for fattening	59	45	1.5
Piglet	44	60	1.75
Pig for fattening	37	72	1.75
Sow lactating	30	88	3.5
Veal calf (milk replacer)	19	140	5
Cattle for fattening	20	132	5
Dairy cow	31	86	5
Sheep/goat	20	132	5

**: Complete feed DM = 88%, milk replacer DM = 94.5%.

The results in Table A.1 show that the exposure of target animals to [redacted] shellac resulting from the use of Cylactin® LBC ME20 plus at the highest proposed use level (1.5–5 mg [redacted] shellac/kg complete feed) is far below its maximum safe concentration in feed.[46] Therefore, the use of shellac as a coating agent in Cylactin® LBC ME20 plus is of no concern under the proposed (authorised) conditions of use.

A.2.3. Safety of [redacted] shellac for the consumer

The FEEDAP Panel identified a NOAEL of 300 mg [redacted] shellac/kg bw per day, based on a decrease of body weight gain in dams at highest dose levels tested in a reproductive toxicity study. No data on residues in products of animal origin were made available for any of the constituents of the shellac; therefore, an exposure of the consumer could not be estimated. The few

[62] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendices_Sect_III_AnimConsUser/3–19 FACTS_ Shellac NOAEL.

studies identified in the extensive literature review (Kunkel and Seo, 1994; Kunkel et al., 1995),[63] even if not designed to study the ADME of shellac, suggest a low digestibility of shellac in the stomach and small intestine but a possible degradation by the colonic microbiota into component acids that might be metabolised and excreted in the target species.

Shellac is currently authorised as a food additive (E 904), to be used *quantum satis* in a wide range of foods and food supplements.[64] Considering the low concentration in feed (1.5–5 mg [redacted] shellac/kg complete feed) resulting from the inclusion of the additive according to the proposed conditions of use, the Panel does not consider that the use of [redacted] shellac would contribute substantially to the consumers exposure. Therefore, no concern is expected for the safety for the consumer from the use of [redacted] shellac in the formulation of the additive Cylactin® LBC ME20 plus.

A.2.4. Safety of shellac for the environment

To support the safety of shellac for the environment, the applicant conducted an ELS without time restriction on 11 May 2021.[65] The core question focused on retrieving information about natural occurrence in Europe, degradation, biodegradation, the occurrence of constituents in materials other than shellac. The ELS included in the search the constituent's monomers of the resin, namely aleuritic acid (45%), butolic acid (5–8%), kerrolic acid (1%) and two sesquiterpenoid acids (i.e. jalaric (27%) and shelloic acids 2–8%).[66] The search was performed via four cumulative databases, 14 single databases, 12 publisher databases. A total of 87 database papers were selected among nearly 400 scores. In addition, 40 references were selected among 140 hits in other bibliographic sources, yielding 127 scientific reference units. In the final review, additional 12 publications were excluded, resulting in 115 relevant publications.[67] All the constituents of shellac or substances chemically closely related to them are present in the European environment in considerably higher concentrations than in feed containing the additive (maximum 5 mg/kg complete feed). The ELS has not identified any data to suggest that shellac is an environmental toxin or pollutant. The decomposition of shellac constituents is likely to occur by the micro- and macro-decomposers present in the environmental compartments.

A.2.5. Conclusions on the safety of [redacted] shellac

[redacted] shellac is not genotoxic nor carcinogenic. The lowest NOAEL identified from the available toxicological studies corresponded to 300 mg/kg bw per day. The use of [redacted] shellac in one of the formulations of the additive under assessment (Cylactin® LBC ME20 plus) does not raise concern for the target species, consumer and environment.

A.3. References

Kunkel ME and Seo A, 1994. *In vitro* digestibility of selected polymers. Journal of Environmental Polymer Degradation, 2:245–251. https://doi.org/10.1007/BF02071972

Kunkel ME, Seo A and Shallo HE, 1995. Digestibility of selected carbohydrate and lipid based polymers. Journal of the American Dietetic Association, 95:A21. https://doi.org/10.1016/S0002-8223(95)00420-3

[63] Technical dossier/Supplementary Information August 2021/Appendices 0/Annexes Sect III ERA/Annex_III_ELS_115_297_Kunkel_and_Seo_1994 and Annex_III_ELS_113_292_Kunkel_et_al_1995.

[64] 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; Commission Regulation (EU) 675/2012 of 23/07/2012 amending Annex II to Regulation (EC) 1333/2008 of the European Parliament and of the Council as regards the use of Talc (E 553b) and Carnauba wax (E903) on unpeeled coloured boiled eggs and the use of Shellac (E904) on unpeeled boiled eggs; and Commission Regulation (EU) 1147/2012 OF 04/12/2012 amending Annex II to Regulation (EC) 1333/2008 of the European Parliament and of the Council as regards the use of beeswax (E901), carnauba wax (E903),shellac (E904), microcrystalline wax (E905) on certain fruits.

[65] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendix 0–32 Sect III ERA Shellac and Annexes_Sect_III_ERA/Annex_III_ERA_4_Shellac_ELS.

[66] Technical dossier/Supplementary Information August 2021/Appendices 0/Appendix 0–32 Sect III ERA Shellac and Annexes_Sect_III_ERA/Annex_III_ERA_12_CIR_1986.

[67] Technical dossier/Supplementary Information August 2021/Appendices 0/Annexes_Sect_III_ERA/Annex_III_ERA_4_Shellac_ELS.