SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 10 February 2021

doi: 10.2903/j.efsa.2021.6470

Safety and efficacy of a feed additive consisting on *Propionibacterium freudenreichii* ssp. *shermanii* ATCC PTA-6752 for all animal species (Chr. Hansen A/S)

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the additive consisting of *Propionibacterium freudenreichii* ssp. *shermanii* ATCC PTA-6752 when used as technological additive (acidity regulator) in dry feed at a minimum inclusion level of 2×10^9 colony-forming units (CFU)/kg and in complete or complementary liquid feed for all animal species at a minimum concentration of 1×10^9 CFU/L. No minimum concentration is proposed by the applicant when used in combination with other microbial technological additives. The bacterial species *P. freudenreichii* is considered by EFSA to be eligible for the qualified presumption of safety approach to safety assessment. As the identity of the strain has been clearly established and it did not show acquired resistance to antibiotics of human and veterinary importance, the use of the strain in animal nutrition is considered safe for the target animals, consumers and the environment. No conclusions can be drawn on the skin/eye irritancy or skin sensitisation potential of the additive, but it should be considered a respiratory sensitiser. Exposure of users by inhalation is likely. No conclusions could be drawn on the efficacy of the additive when used alone as an acidity regulator in feed due to lack of data. The studies provided showed that *P. freudenreichii* ssp. *shermanii* ATCC PTA-6752 when used in combination with *Ligilactobacillus animalis* ATCC PTA-6750 has the potential to act as an acidity regulator. However, the Panel has reservations on the effects of this mixture in practical use conditions.

Keywords: Technological additive, acidity regulator, *Propionibacterium freudenreichii* ssp. *shermanii* ATCC PTA-6752, QPS, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2017-00613

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgements: The Panel wishes to acknowledge the contribution of Yolanda García Cazorla to this opinion.

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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, Fašmon Durjava M, Kouba M, López-Alonso M, López-Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Glandorf B, Herman L, Prieto Maradona M, Saarela M, Galobart J, Innocenti ML and Brozzi R, 2021. Scientific Opinion on the safety and efficacy of a feed additive consisting on *Propionibacterium freudenreichii* ssp. *shermanii* ATCC PTA-6752 for all animal species (Chr. Hansen A/S). EFSA Journal 2021;19(3):6470, 10 pp. https://doi.org/10.2903/j.efsa.2021.6470

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 as provided by the requestor

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.

The European Commission received a request from Chr. Hansen A/S[2] for authorisation of the product consisting of *Propionibacterium freudenreichii* ssp. *shermanii* ATCC PTA-6752, when used as a feed additive for all animal species (category: technological additives; functional group: acidity regulator).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 13 September 2017.

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 consisting of *P. freudenreichii* ssp. *shermanii* ATCC PTA-6752, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The product under assessment is a preparation containing viable cells of *P. freudenreichii* ssp. *shermanii* ATCC PTA-6752. It has not been previously authorised as a feed additive in the European Union.

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[3] in support of the authorisation request for the use of the product consisting of *P. freudenreichii* ssp. *shermanii* ATCC PTA-6752, as a feed additive.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agent in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the product consisting of *P. freudenreichii* ssp. *shermanii* ATCC PTA-6752, is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Guidance on technological additives (EFSA FEEDAP Panel, 2012a), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012b) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c).

[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] Chr. Hansen A/S, 10-12 Boege Allé, 2970 – Hoersholm, Denmark.
[3] FEED dossier reference: FAD-2017-0040.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0040-proprioni_freudenreichii.pdf
[5] 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

The product under assessment is based on a preparation of viable cells of a single strain of *P. freudenreichii* intended to be used as a technological additive (functional group: acidity regulator) in feed for all animal species. It will be hereafter referred to as PF24.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The strain of unknown origin is deposited in the American Type Culture Collection (ATCC) with the accession number ATCC PTA-6752.[6] It has not been genetically modified.

The taxonomical identification is based on partial sequencing (897–900 bp) of the 16S rRNA gene.[7] The strain shows a 100% sequence identity with several strains of *P. freudenreichii* ssp. *shermanii* present in the NCBI reference genomic database, including the type strain (DSM 4902), using Basic Local Alignment Search Tool (BLAST) searches which confirmed its identity as *P. freudenreichii* ssp. *shermanii.*

The susceptibility of the strain was tested against the list of antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2012b).[8] All the minimum inhibitory concentration values were equal to or below the cut-off values defined by the FEEDAP Panel for antimicrobials required for this species. Therefore, it can be concluded that the strain is susceptible to the relevant antibiotics.

3.1.2. Characterisation of the product

[redacted][9]

Analysis of five batches showed a mean value of 4.9×10^{11} CFU/g (range $4.0–7.2 \times 10^{11}$ CFU/g).[10]

Specifications are set for total coliforms (< 10 CFU/g), *Salmonella* spp. (no detection in 25 g), *Pseudomonas* spp. (< 10 CFU/g), coagulase positive *Staphylococcus* spp. (< 10 CFU/g), *Escherichia coli* (< 1 CFU/g) and total yeasts and filamentous fungi (< 10 CFU/g).[11] Analysis of three batches of the cell concentrate prior to standardisation confirmed compliance with these specifications. Other three batches of the same intermediate product were examined for the content of heavy metals (Hg, Cd and Pb), arsenic and aflatoxins B1, B2, G1 and G2.[12] In all cases, heavy metals and arsenic were found only in trace amounts (Hg < 0.01 mg/kg, Cd: 0.02–0.09 mg/kg, As: 0.03–0.04 mg/kg and Pb < 0.02 mg/kg) which do not give rise to safety concerns.[13] No aflatoxins were detected in any of the tested samples.[14]

The average bulk density is 592 kg/m^3.[15] The dusting potential of three batches measured with the Stauber-Heubach dustmeter showed a mean value of 3.8 g/m^3 (range 2.6–5.8 g/m^3).[16] The particle size distribution of the same three batches was determined using laser diffraction.[17] Results showed that approximately 30% (v/v) of the additive consists of particles with diameter lower than 50 μm and 10% lower than 10 μm.

[6] Technical dossier/Section II/Annex II.2.1.1.
[7] Technical dossier/Section II/Annex II.2.1.2.1.
[8] Technical dossier/Section II/Annex II.2.2.2.1.
[9] Technical dossier/Section II/Annexes II.3.1.1. and II.3.1.2.Conf.
[10] Technical dossier/Section II/Annex II.1.3.
[11] Technical dossier/Section II/Annexes II.1.4.1 and II.1.4.2.
[12] Technical dossier/Section II/Annex II.1.4.2.
[13] Limits of detection (LOD) for lead: 0.02 mg/kg and mercury: 0.010 mg/kg. Those for cadmium and arsenic were not specified.
[14] LOD: 0.1 μg/kg.
[15] Technical dossier/Section II/Annex II.1.5.3.
[16] Technical dossier/Section II/Annex II.1.5.2.
[17] Technical dossier/Section II/Annex II.1.5.1.

3.1.3. Stability and homogeneity

The applicant declares a minimum shelf-life of 12 months when stored in light-tight and moisture-proof packaging at –30°C or less. However, the data in support of this proposal are incomplete and unclear.[18,19] The Panel notes that the storage conditions proposed (at least –30°C) to guarantee the stability of PF24 are not compatible with standard farming conditions.

The applicant declares that PF24 should be incorporated to feed shortly before intended consumption. A short-time stability study was conducted where viability of PF24 when mixed with liquid matrices (liquid pig feed and calf milk replacer mixed in ratio 1:1 with nutrient broth to reach minimum concentration of 10^8 CFU/ml, incubated anaerobically at 37°C) was monitored after 4, 8 and 24 h. Propionic acid bacteria counts showed losses < 0.5 log at all time points.[20] However, the Panel notes that (i) the concentration tested was several orders of magnitude higher than the proposed inclusion level in feeds, (ii) the conditions of the test would not mimic the standard liquid feed preparation (i.e. sterile nutrient broth was added to the mixture) and (iii) the incubation conditions (i.e. anaerobiosis and 37°C) would not mimic standard farming conditions. No data on dry feed were supplied.

To investigate if the additive (one batch) can be homogeneously distributed into a liquid feed (reconstituted milk replacer) at an intended concentration of 10^8–10^9 CFU/mL, 10 subsamples were collected and subjected to propionic acid bacteria enumeration.[21] Counts showed a coefficient of variation < 1%. No data on dry feed were supplied.

3.1.4. Conditions of use

P. freudenreichii ssp. *shermanii* ATCC PTA-6752 is intended for use as an acidity regulator in solid feeds for all animal species at a minimum inclusion level of 2×10^9 CFU/kg complete feed and in complete or complementary liquid feed for all animal species at a minimum inclusion level of 1×10^9 CFU/L. The applicant states that this inclusion level regards its individual use. In case the additive is used in combination with other acidifying strains, no minimum recommended levels are proposed. It is to be incorporated to feed shortly before intended consumption.

3.2. Safety

3.2.1. Safety for the target species, consumers and environment

The species *P. freudenreichii* is considered to be eligible for the Qualified Presumption of Safety (QPS) approach (EFSA, 2007; EFSA BIOHAZ Panel, 2020). This approach requires the identity of the strain to be conclusively established and evidence that the strain does not show acquired resistance to antibiotics of human and veterinary importance. In the view of the FEEDAP Panel, the identity of the strain was established as *P. freudenreichii* and the antibiotic resistance qualification has been met. Consequently, *P. freudenreichii* ssp. *shermanii* ATCC PTA-6752 is presumed safe for the target species, consumers and the environment.

3.2.2. Safety for the user

No specific data on skin/eye irritation or skin sensitisation were provided for the additive under application. Therefore, no conclusions can be drawn on the skin/eye irritancy or skin sensitisation potential of the additive. The high dusting potential (3.8 g/m^3) suggests that exposure by inhalation is possible. Given the proteinaceous nature of the active agent, PF24 should be considered a respiratory sensitiser.

3.3. Efficacy

A total of nine *in vitro* studies were provided to support the efficacy of PF24 as acidity regulator. Four of these studies were not further considered because the matrices used cannot be deemed as representative of feedstuffs in which the additive is intended to be used (i.e. pig feeds and calf milk replacers diluted with a growth medium (nutrient broth) used to represent gut chime). These solutions

[18] Technical dossier/Section II/Annex II.4.1.1.
[19] Technical dossier/Section II/Annex II.4.1.2.
[20] Technical dossier/Section II/Annex II.4.1.3.
[21] Technical dossier/Section II/Annex II.4.2.

and the incubation conditions (i.e. sterilisation of the matrices, anaerobiosis, high temperature (37°C)) are not considered to represent normal feedingstuffs, nor feeding conditions.[22] A fifth study was conducted with the aim of investigating the capacity of PF24 to produce organic acids (e.g. lactic, acetic, propanoic, isobutyric, butyric, isovaleric, valeric, caproic, heptanoic and caprylic acid).[23] However, since this study showed several shortcomings (e.g. absence of replicates, anaerobic conditions, high incubation temperature (37°C), not reflecting practical conditions), similarly to the above, this study was not further considered.

In the four *in vitro* studies considered PF24 was used in combination with another additive (*Ligilactobacillus animalis* ATCC PTA-6750,[24] LA51 in Table 1). In order to inoculate the feeds with the additives, a premix containing both additives at a concentration of 6.3–8.4 × 10^{13} total CFU/kg with lactose (39.5%) and silicon dioxide (1%) was prepared. In study 1,[25] this premix and the control (prepared with equivalent amounts of lactose) were mixed with water in a 60:40 ratio to simulate water uptake during transport. In studies 2,[26] 3[27] and 4,[28] this premix was further diluted with lactose before being used in the treated feeds (study 2: commercial milk for cats, study 3: complete feed (25% wheat, 25% barley, 25% maize, 25% soybean), representing dry feeds for pigs, ruminants and poultry and study 4: high fibre dry feed mix, representing total mixed ration for ruminants). In these studies, the control feeds were prepared with equivalent amounts of lactose.

In all studies at time zero and different subsequent time points, samples from six replicates were taken for measuring the pH, except in study 2 where only one replicate was analysed at time zero. In studies 1, 3 and 4, the samples were diluted with water in a ratio 1:2 to measure the pH. The data were analysed with an analysis of variance (ANOVA) with the replicate as experimental unit. Differences were considered significant at a level of at least $p < 0.05$. The details of the experimental design and results are shown in Table 1.

Table 1: Summary of the *in vitro* trials of different feed matrices treated with PF24 and LA51

Study	Matrix	No replicates Aerobic incubation conditions	Treatment PF24/LA51 (CFU/l or kg)	pH at the time points				
				0 h	8 h	24 h	48 h	72 h
1	Lactose-based complementary feed	6 72 h, 30°C	Control	6.64	5.48[a]	5.30[a]	–	5.23[a]
			7.3 × 10^{13}/3.9 × 10^{13}	6.65	5.35[b]	5.13[b]	–	4.98[b]
2	Commercial milk for cats	6 24 h, 35°C	Control	6.47	6.48[a]	6.42[a]	–	–
			7.1 × 10^{7}/3.5 × 10^{7}	6.49	6.45[b]	6.25[b]	–	–
3	Complete feed	6 24 h, 35°C	Control	6.21	6.28[a]	6.58[a]	–	–
			7.1 × 10^{11}/3.5 × 10^{11}	6.18	5.38[b]	4.71[b]	–	–
4	High fibre dry mix	6 48 h, 30°C	Control	5.65	–	5.60	5.50[a]	–
			7.1 × 10^{7}/3.5 × 10^{7}	5.47	–	5.59	4.75[b]	–

PF24: *P. freudenreichii* ssp. *shermanii* ATCC PTA-6752.
LA51: *L. animalis* ATCC PTA-6750.
a, b: Means in the same column within a study are significantly different compared to control at $p \leq 0.05$.
–: not measured.

In the four studies, PF24 was used in combination with another additive showing a significant reduction of pH in all treated samples compared to the controls, starting from 8 h after preparation. No data were provided to support the efficacy of PF24 when used alone.

Considering the conditions in which the effects were observed (e.g. high incubation temperature (35°C), composition of the feeds chosen to represent liquid feedingstuffs for all animal species) and the conditions of use proposed (to be stored at –30°C and added just before consumption), the FEEDAP Panel has reservations on the effects of the simultaneous use of PF24 and LA51 in practical conditions of use.

[22] Technical dossier/Section IV/Annexes IV.1.2-IV.5.
[23] Technical dossier/Section IV/Annex IV.1.
[24] Currently under assessment (FAD-2016-0068).
[25] Technical dossier/Supplementary information May 2019/Applicant Reply of Sin May 2019 and Annex IV.1.9.
[26] Technical dossier/Supplementary information May 2019/Applicant Reply of Sin May 2019 and Annex IV.1.7.
[27] Technical dossier/Supplementary information May 2019/Applicant Reply of Sin May 2019 and Annex IV.1.8.
[28] Technical dossier/Supplementary information May 2019/Applicant Reply of Sin May 2019 and Annex IV.1.10.

3.3.1. Conclusions on efficacy

The Panel is not in the position to conclude on the efficacy of PF24 when used alone as an acidity regulator in feed. The studies provided showed that PF24 when used in combination with *L. animalis* ATCC PTA-6750 has the potential to act as an acidity regulator in feeds. However, the Panel has reservations on the effects of this mixture in practical use conditions.

4. Conclusions

Based on the QPS approach to safety assessment, *P. freudenreichii* ssp. *shermanii* ATCC PTA-6752 is presumed safe for the target species, consumers and the environment.

No conclusions can be drawn on the skin/eye irritancy or skin sensitisation potential of the product under assessment. Owing to the proteinaceous nature of the active agent, PF24 should be considered a respiratory sensitiser. Exposure of users by inhalation is likely.

The Panel is not in the position to conclude on the efficacy of the additive when used alone as an acidity regulator in feed due to lack of data. The studies provided showed that *P. freudenreichii* ssp. *shermanii* ATCC PTA-6752 when used in combination with *Ligilactobacillus animalis* ATCC PTA-6750 has the potential to act as an acidity regulator. However, the Panel has reservations on the effects of this mixture in practical use conditions.

5. Documentation as provided to EFSA/Chronology

Date	Event
07/07/2017	Dossier received by EFSA. *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752) for all animal species. Submitted by Chr. Hansen A/S.
01/08/2017	Reception mandate from the European Commission
13/09/2017	Application validated by EFSA – Start of the scientific assessment
30/10/2017	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: method of analysis*
13/12/2017	Comments received from Member States
12/11/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: efficacy*
14/12/2018	Reception of supplementary information from the applicant
17/12/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
07/05/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
10/02/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;587, 16 pp. https://doi.org/10.2903/j.efsa.2007.587

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernández Escámez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Correia S and Herman L, 2020. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). EFSA Journal 2020;18(2):5966, 56 pp. https://doi.org/10.2903/j.efsa.2020.5966

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: Tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. https://doi.org/10.2903/j.efsa.2011.2175

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for technological additives. EFSA Journal 2012;10(1):2528, 23 pp. https://doi.org/10.2903/j.efsa.2012.2528

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. https://doi.org/10.2903/j.efsa.2012.2740

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

Abbreviations

CFU colony-forming unit
EURL European Union Reference Laboratory
PFGE Pulsed Field Gel Electrophoresis

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Propionibacterium freudenreichii* ssp. *shermanii* ATCC PTA-6752

In the current application, authorisation is sought under Article 4(1) for *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752), under the category 1 'technological additives' and the functional groups (a) 'preservatives', (j) 'acidity regulators' and (n) 'hygiene condition enhancers', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species.

According to the Applicant, the *feed additive* contains as active substance viable cells of the non-genetically modified strain *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752). The *feed additive* is to be marketed as a powder containing a minimum *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752) content of 2×10^{11} colony-forming unit (CFU)/g. The *feed additive* is intended to be used in *water* and liquid *feedingstuffs* at a minimum dose of 1×10^{11} CFU/l, and in dry *feedingstuffs* at a minimum dose of 2×10^{11} CFU/kg.

For the identification of *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752), the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for genetic identification of bacterial strains.

For the enumeration of *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752) in *feed additive*, *feedingstuffs* and *water,* the Applicant submitted the ring-trial validated spread plate method EN 15787. Based on the performance characteristics available, the EURL recommends this method for official control.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.