REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of an Additive of *Bifidobacterium longum* CNCM I-5642 (PP102I) as a Feed Additive for Dogs (RP1111)

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An application was submitted to the Food Standards Agency in May 2021 from Nestle Enterprises S.A. ('the applicant') for the new of authorisation of an additive (PP102I) *Bifidobacterium longum* CNCM I-5642, under the category of 'zootechnical additives' and functional group 'physiological condition stabilisers'. The additive is proposed to be used at a recommended dose of 1 x 10^9 CFU/dog daily.

To support the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in evaluating the dossier, the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant.
The FSA/FSS concluded, based on the ACAF's advice, that the additive was correctly characterised, and that it is stable for 8 months when stored at 4°C, and in premixtures for 48 weeks at 32°C at 70% humidity, and for 88 weeks at 23°C at 60% humidity. Homogeneity in feed was also demonstrated.

As an organism suitable for the QPS approach to assessment, the additive can be considered safe for the consumer, the target animal, and the environment. The additive is not an irritant to the skin. The additive is an irritant to the eyes and should be considered a potential skin sensitiser and respiratory sensitiser.

Bifidobacterium longum CNCM I-5642 is efficacious for dogs at the proposed dose of 1 x 10^9 CFU/dog daily.

The views of ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS.

1. Introduction

The FSA and FSS have undertaken an assessment for a feed additive (PP102I) consisting of *Bifidobacterium longum* CNCM I-5642, (Nestlé Enterprises S.A., Division Nestlé Purina Petcare Europe, Entre-Deux-Villes 12, 1800 Vevey, Switzerland) under Assimilated Regulation (EC) No 1831/ 2003 (EC, 2003) under the category of 'zootechnical additives' and functional group 'physiological condition stabiliser'. The additive is proposed to be used at an inclusion rate of 1 x 10^9 CFU/dog daily.

To support the safety assessment by FSA and FSS, the ACAF provided advice to the FSA and FSS outlined in this document.

In line with Article 8 of 1831/2003, the assessment has considered whether the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by EFSA for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

The dossier was evaluated by the by ACAF at their July 2023 meeting, after which a request for further information was communicated to the applicant. The applicant provided responses to the ACAFs requests in October 2023 and January 2024.

The views of ACAF have been taken into account in this safety assessment which represents the opinion of the FSA/FSS.

Table 1. Table showing products included in this assessment

Title	Product type	Intended use/s	Intended species or categories of animals
Bifidobacterium longum CNCM I-5642	Feed additive	Zootechnical additive	Dogs

2. Assessment

2.1. Section II: Identity, characterisation and conditions of use

The additive's active substance *Bifidobacterium longum* (CNCM I-5642) has a minimum guaranteed activity of 5 x 10^{10} CFU/g. The applicant provided data from several batches supporting the identification values outlined below (Table 2):

Table 2. Identity table *Bifidobacterium longum* CNCM I-5642

Composition	
Bifidobacterium longum CNCM I-5642	7.72 x 10^{10} CFU/g
Appearance	
Dry powder	
Chemical-physical specifications	
Bulk density	0.439 – 0.493 g/ml
Dusting potential	0 mg/m^3
Particle size	<100 µm –30% <50 µm – 9% <10 µm – 1.3%
Impurities	
Bacillus cereus	<10 CFU/g
Coliforms	<10 CFU/g
Salmonella	Absent in 25g
E. coli	Absent in 25g
Enterobacteriaceae	Absent
Moulds	<10 CFU/g
Yeasts	<10 CFU/g
Arsenic	<0.017 mg/kg
Lead	0.017 – 0.019 mg/kg
Cadmium	0.058 – 0.067 mg/kg
Chromium	0.085 – 0.103 mg/kg
Sum of dioxins (WHO-PCDD/F-TEQ)	0.099 – 0.463 TEQ (pg/g)
Sum of dioxins and dl-PCBs (WHO-PCDD/F-TEQ)	0.112 – 0.505 TEQ (pg/g)

The Committee reviewed the genetic characterisation of the active ingredient and noted that a comprehensive analysis had been performed confirming the identity of the active substance as *B. longum*. Through this characterisation the applicant demonstrated that the additive meets the requirements for the qualified presumption of safety (QPS) approach to assessment. The Committee noted inconsistencies in the conditions of use of the additive and the proposed method of administration of the additive. Following a request for information, the applicant provided further material that allowed a comprehensive assessment to be performed. The additive was demonstrated to be stable for 8 months at 4°C. In feed, the additive was shown to be stable for 48 weeks at 32°C at 70% humidity, and for 88 weeks at 23°C at 60% humidity. Homogeneity in feed was also demonstrated.

The proposed conditions of use of the additive are described in Table 3:

Table 3. Proposed conditions of use of *Bifidobacterium longum* CNCM I-5642

Proposed mode of use in animal nutrition				
Additive	*Bifidobacterium longum* (CNCM I-5642)			
Registration number/ EC No/No	4exx			
Category(-ies) of additive	4. Zootechnical feed additive			
Functional group(s) of additive	e. Physiological condition stabiliser			
Description				
Description	Chemical formula	Purity criteria		Method of analysis
Preparation of live *B. longum* (CNCM I-5642)	Powder form with a min. 5×10^{10} CFU/g	Complies with EU feed hygiene regulation		LI 00.794
Trade name (if appropriate)		PP102I		
Name of the holder of authorisation (if appropriate)		Nestle Enterprises S.A (NESA)		
Proposed conditions of use				
Species or category of animal	Min-max Age	Min. content	Max. content	Withdrawal period
Dogs	--	1×10^9 CFU/dog daily	--	--

2.1.1. Conclusions on Section II

The ACAF concluded the additive was correctly characterised and is stable for 8 months at 4°C. In feed, the additive is stable for 48 weeks at 32 °C (70% humidity), and for 88 weeks at 23°C (60% humidity). Homogeneity in feed was also demonstrated.

No further concerns were raised for Section II of these dossiers.

2.2. Section III: Safety

2.2.1. Safety for the target species

The applicant demonstrated the species *B. longum* to meet the requirements for the qualified presumption of safety (QPS) approach to assessment, therefore the additive is presumed safe for the target species.

2.2.2. Safety for the consumer

The applicant demonstrated the species *B. longum* to meet the requirements for the QPS approach to assessment, therefore the additive is presumed safe for the consumer.

2.2.3. Safety for the user

The applicant presented the following tests to evaluate the safety of the additive for the user/worker:

- *In vitro* skin irritation test (EPISKIN reconstructed human epidermis model – OECD 439)

- *In vitro* eye irritation test (reconstructed human cornea-like epithelium model – OECD 492)

From the data provided the Committee concluded that the additive is not an irritant to the skin and is an irritant to the eyes. Owing to the absence of data the additive should be considered a skin sensitiser. As a microorganism product the additive should be regarded as a respiratory sensitiser. It was noted that due to the presence of particles in the smaller particle size distribution fraction, exposure through inhalation should be avoided.

2.2.4. Safety for the environment

The applicant demonstrated the species *B. longum* to meet the requirements for the QPS approach to assessment, therefore the additive is presumed safe for the environment.

2.2.5. Conclusions on safety

As the species *B. longum* was demonstrated to meet the criteria for the QPS approach to assessment, the ACAF concluded that the additive can be considered safe for the consumer, the target species, and the environment.

The ACAF concluded that the additive is not an irritant to the skin and is an irritant to the eyes. The additive should be considered a potential skin sensitiser and respiratory sensitiser.

2.3. Section IV: Efficacy

The applicant provided three long-term efficacy studies to support the efficacy claims in dogs. An overview of the trials is presented in Table 4:

Table 4. Overview of efficacy trials in dogs

Study	Replicates/Treatment	Target *B. longum*	Analysed *B. longum*	Duration
1	24 dogs; 2 treatments. 24 replicates per treatment (cross-over)	1×10^9 CFU/dog/day	2.35×10^8 CFU/dog/day	17 weeks (7+3+7)
2	20 dogs; 2 treatments. 20 replicates per treatment (cross-over)	1×10^9 CFU/dog/day	9.5×10^8 CFU/dog/day	15 weeks (6+3+6)
3	20 dogs; 2 treatments. 20 replicates per treatment (cross-over)	1×10^9 CFU/dog/day	9.83×10^8 CFU/dog/day	15 weeks (6+3+6)

The Committee reviewed each of the studies and the physiological and behavioural parameters investigated, noting the trials were performed to a high standard. The three studies performed in dogs demonstrated that the animals treated with *B. longum* were less reactive, calmer and in a better affective state when subjected to routine stressors.

The ACAF concluded that *B. longum* is efficacious in dogs when included in feed at the proposed dose of 1 x 10^9 CFU/dog daily.

2.3.1. Conclusions on efficacy

Based on the information presented in three efficacy trials, the ACAF concluded that *Bifidobacterium longum* CNCM I-5642 is efficacious in dogs when included in feed at the proposed dose of 1 x 10^9 CFU/dog daily.

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for *Bifidobacterium longum* (CNCM I-5642) (EURL-FA, 2021):

> "For the identification of Bifidobacterium longum (CNCM I-5642), the EURL recommends for the official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains. For the enumeration of Bifidobacterium longum (CNCM I-5642) in the feed additive and feedingstuffs, the Applicant submitted a single-laboratory validated and further verified pour plate count method using a reinforced clostridial agar medium. The following performance characteristics were obtained in the frame of the validation and verification studies: (i) for the average measured content of the active substance in the feed additive of 1.3 x 10^{11} CFU/g: a standard deviation for repeatability (Sr) and intermediate precision (Sip) of 0.06 $\log_{10}$ CFU/g; (ii) for the average measured content of the active substance in feedingstuffs ranging from 9.2 x 10^8 to 8.0 x 10^9 CFU/g: Sr ranging from 0.04 to 0.13 $\log_{10}$ CFU/g and Sip ranging from 0.04 to 0.17 $\log_{10}$ CFU/g. Furthermore, a limit of quantification (LOQ) of 100 CFU/g feedingstuffs can be derived following the recommendations of ISO 7218 standard. Based on the performance characteristics and the experimental data available, the EURL recommends the pour plate count method using a reinforced clostridial agar medium for the official control for the enumeration of Bifidobacterium longum (CNCM I-5642) in the feed additive and feedingstuffs."

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The ACAF concluded the additive was correctly characterised and is stable for 8 months at 4°C. In feed, the additive is stable for 48 weeks at 32°C (70% humidity), and for 88 weeks at 23°C (60% humidity). Homogeneity in feed was also demonstrated.

As the species B. longum was demonstrated to meet the criteria for the QPS approach to assessment, the ACAF concluded that the additive can be considered safe for the consumer, the target species, and the environment.

The ACAF concluded that the additive is not a skin irritant. The additive is an eye irritant and should be considered a potential skin sensitiser and respiratory sensitiser.

The ACAF concluded that Bifidobacterium longum CNCM I-5642 is efficacious when included in feed at the proposed dose of 1 x 10^9 CFU/dog daily.

The FSA/FSS agree with the conclusions reached by the ACAF. FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
CFU	Colony forming units
EFSA	European Food Safety Authority
EU	European Union
FSA	Food Standards Agency
FSS	Food Standards Scotland
QPS	Qualified presumption of safety
LOQ	Limit of quantification
PFGE	Pulsed Field Gel Electrophoresis
Sr	Standard deviation for repeatability
Sip	Intermediate precision

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References

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