SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of Toyocerin® (*Bacillus cereus*) as a feed additive for sows, piglets, pigs for fattening, cattle for fattening, calves for rearing, chickens for fattening and rabbits for fattening[1, 2]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[3, 4]

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ABSTRACT

Toyocerin® is an additive containing spores of a strain of *Bacillus cereus* intended to be applied to animal feed to improve performance. On the basis of the studies provided, the Panel concludes that the additive is well tolerated by the various categories of pigs, cattle, chickens and rabbits that are the subject of this request for authorisation/re-evaluation. However, the strain shows resistance to two antibiotics, one of which at least can be ascribed to an acquired resistance. For this reason the FEEDAP Panel considers it inadvisable to introduce into target species a resistance determinant capable of transfer to other bacterial strains. Analysis of the complete genome sequence showed that the strain harbours all of the genes coding for non-haemolytic and haemolytic enterotoxins. Since the two operons present the same organisation as pathogenic *B. cereus* strains and since no mutation affecting transcription or translation has been detected, it has to be assumed that the Toyocerin® strain has the capacity to elaborate functional toxins and, thus, to pose a hazard for those exposed to the organism. This would include those handling the additive and consumers inadvertently exposed to contaminated animal products. The additive is non-irritant to eyes, and by extension, to the skin but should be treated as a sensitiser and, in particular, as hazardous to the respiratory tract. *B. cereus* is a ubiquitous soil saprophyte. Consequently, use of the strain in animal nutrition is not expected to measurably increase numbers of the organism in the environment. The addition of Toyocerin® to the feed has the potential to improve at least one aspect of production in chickens for fattening; pigs for fattening; sows, calves; cattle for fattening and rabbits for fattening. Insufficient data was available to conclude on the efficacy of Toyocerin® when used in diets for weaned piglets.

[1] On request from the European Commission, Questions No EFSA-Q-2010-01095 and EFSA-Q-2011-00832, adopted on 16 October 2012.

[2] This scientific opinion has been edited following the adoption of the Commission decision regarding certain confidentiality claims submitted by the applicant in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text.

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[4] Acknowledgement: The Panel wishes to thank the members of the Working Group on Micro-organisms, including Andrew Chesson, Pier Sandro Cocconcelli and Ingrid Halle, for the preparatory work on this scientific opinion.

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KEY WORDS

zootechnical additive, *Bacillus cereus*, safety, tetracycline, chloramphenicol, toxin, efficacy

SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the additive Toyocerin® when in diets for sows, piglets, pigs for fattening, cattle for fattening, calves for rearing, chickens for fattening and rabbits for fattening. Toyocerin® is a preparation containing spores of a strain of Bacillus cereus.

Although some of the tolerance studies submitted by the Applicant would be considered inadequate by present days standards, there are individual studies which provide sufficient assurance that the additive has no direct ill effects on the target species at the recommended dose range. Given that no adverse effects were recorded in any of the remaining studies, the FEEDAP Panel concludes that the additive is well tolerated by the target species that are the subject of this request for authorisation/re-evaluation. However, the Panel notes that the strain of *B. cereus* harbours resistance determinants to two antibiotics, one of which at least can now be ascribed to an acquired resistance. For this reason the FEEDAP Panel considers it inadvisable to introduce into target species a resistance determinant capable of transfer to other bacterial strains and adding to the pool of such determinants in the guts of livestock species.

Analysis of the complete genome sequence showed that the strain of *B. cereus* in Toyocerin® harbours all of the genes coding for the non-haemolytic and haemolytic enterotoxins. Since the two operons present the same organisation as pathogenic *B. cereus* strains and since no mutation affecting transcription or translation has been detected, it has to be assumed that the Toyocerin® strain has the capacity to elaborate functional toxins and, thus, to pose a hazard for those exposed to the organism. This would include those handling the additive and consumers inadvertently exposed to contaminated animal products.

The additive is non-irritant to eyes, and by extension, to the skin. However, given its proteinaceous nature, it should be treated as a skin and respiratory sensitiser.

B. cereus is a ubiquitous soil saprophyte with a worldwide distribution. Consequently, use of the strain in animal nutrition is not expected to measurably increase numbers of the organism in the environment.

The addition of Toyocerin® to the feed has the potential to improve at least one aspect of production in chickens for fattening at a minimum dose of 0.2×10^9 CFU/kg feed; pigs for fattening at a dose of 0.5×10^9 CFU/kg feed for the first (grower) period followed by at a minimum dose of 0.2×10^9 CFU/kg feed for the second (finisher) period; sows at a minimum dose of 0.5×10^9 CFU/kg feed for the complete cycle: calves for rearing at a minimum dose of 0.5×10^9 CFU/kg feed; cattle for fattening at a minimum dose of 0.2×10^9 CFU/kg feed and rabbits for fattening at a minimum dose of 1.0×10^9 CFU/kg feed. In the view of the Panel, insufficient data was available to conclude on the efficacy of Toyocerin® when used in diets for weaned piglets. Based on the current data, the FEEDAP Panel is unable to conclude on the compatibility of Toyocerin® with the listed coccidiostats when added to poultry and rabbit feed.

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BACKGROUND

Regulation (EC) No 1831/2003[5] 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 addition, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company Rubinum S.A.[6] for the authorisation of the product Toyocerin®, *Bacillus cereus*, when used as a feed additive for calves for rearing and for its re-evaluation when used in diets for sows, piglets, pigs for fattening, cattle for fattening, chickens for fattening and rabbits for fattening (category: zootechnical additives; functional group: gut flora stabilisers) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the applications 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 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossiers in support of these applications.[7] According to Article 8 of that Regulation, 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. The particulars and documents in support of the applications were considered valid by EFSA as of 6 December 2012 and 6 July 2011.

The additive Toyocerin® is a preparation of *Bacillus cereus* (NCIMB 40112/CNCM I-1012). This product is currently authorised for use in pigs for fattening,[8] piglets up to four months and sows from service until weaning,[9] cattle for fattening,[10] rabbits and chickens for fattening,[11] turkeys for fattening,[12] and rabbit breeding does.[13]

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the use of Toyocerin®® on toxin production and resistance to antibiotics on 5 December 2001 (EC, 2001). EFSA issued an opinion on the efficacy of this product in feeds for pigs for fattening (EFSA, 2004), one on the modification of terms of authorisation of this additive to allow its use in chicken feed with the coccidiostats diclazuril, narasin/nicarbazin and maduramycin ammonium (EFSA, 2005), another opinion on the safety and

[5] 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.
[6] Rubinum S.A., Avenida de la Llana, 123. Polígono Industrial "La Llana". P.O.B 283.
[7] EFSA Dossier references: FAD-2010-0091 and FAD-2010-0090.
[8] Commission Regulation (EC) No 1453/2004 of 16 August 2004 concerning the permanent authorisation of certain additives in feedingstuffs. OJ L 269, 17.8.2004, p. 3.
[9] Commission Regulation (EC) No 1143/2007 of 1 October 2007 amending Regulation (EC) No 256/2002 as regards the authorisation of the feed additive preparation of *Bacillus cereus* var. *toyoi*, belonging to the group of microorganisms. OJ L 256, 2.10.2007, p. 23.
[10] Commission Regulation (EC) No 255/2005 of 15 February 2005 concerning the permanent authorisations of certain additives in feedingstuffs. OJ L 45, 16.2.2005, p. 3.
[11] Commission Regulation (EC) No 1445/2006 of 29 September 2006 amending Regulation (EC) No 1200/2005 as regards the authorisation of the feed additive '*Bacillus cereus* var. *toyoi*', belonging to the group of micro-organisms. OJ L 271, 30.9.2006, p. 22.
[12] Commission Regulation (EC) No 166/2008 of 22 February 2008 concerning the authorisation of a new use of the preparation of *Bacillus cereus* var. *toyoi* (Toyocerin) as a feed additive. OJ L 50, 23.2.2008, p. 50.
[13] Commission Regulation (EC) No 378/2009 of 8 May 2009 concerning the authorisation of a new use of the preparation of *Bacillus cereus* var. *toyoi* as a feed additive for rabbits breeding does (holder of the authorisation Rubinum S.A.). OJ L 116, 9.5.2009, p. 3.

efficacy for sows from service to weaning (EFSA, 2007a). The latest EFSA opinions on the product dealt with its safety and efficacy when used with turkeys and rabbit does (EFSA, 2007b and 2008a).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 animal(s), consumer, user and the environment and the efficacy of the product Toyocerin® (*Bacillus cereus*), when used under the conditions described in Table 1.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	*Bacillus cereus* NCIMB 40112/CNCM I-1012
Registration number/EC No/No	4b1701
Category of additive	Zootechnical additives
Functional group(s) of additive	Gut flora stabilisers

Description			
Composition, description	Chemical formula	Purity criteria	Method of analysis
Bacillus cereus var. *toyoi* NCIMB 40112/CNCM I-1012	Preparation of viable spores of *Bacillus cereus* var. *toyoi* containing a minimum of 1×10^{10} CFU/g additive		Enumeration: spread plate method Identification: pulsed field gel electrophoresis (PFGE)

Trade name	TOYOCERIN®
Name of the holder of authorisation	Rubinum S.A. -Animal Health-

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		CFU/kg of complete feedingstuffs		
Cattle for fattening		0.2×10^9	0.2×10^9	
Chickens for fattening		0.2×10^9	1×10^9	
Piglets	Up to 2 months	1×10^9	1×10^9	
Piglets	Up to 4 months	0.5×10^9	1×10^9	
Pigs for fattening	Until slaughter	0.2×10^9	1×10^9	
Rabbits for fattening		0.1×10^9	5×10^9	
Sows	From service until weaning	0.5×10^9	2×10^9	
Calves for rearing	From birth up to 4	0.5×10^9	1×10^9	

	months			

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use	In the directions for use of the additive and premixture indicate the storage temperature, storage life and stability to pelleting.
Specific conditions or restrictions for handling	When handling use protective mask, gloves and goggles
Post-market monitoring	Every lot of Toyocerin®® placed onto the market is registered in order to be able to trace its destination until the final customer (feed manufacturer or premixture manufacturer)
Specific conditions for use in complementary feedingstuffs	

Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues

ASSESSMENT

1. Introduction

The additive Toyocerin® is a preparation containing spores of a single strain of *Bacillus cereus*. It was first authorised under Council Directive 70/524/EEC for piglets and pigs for fattening, sows from service to weaning, cattle for fattening, chickens for fattening, and rabbits, all without time limit. It was also provisionally authorised for use with calves.

The deliberate introduction into the food chain of *B. cereus*, a recognised human enteropathogen, has always been seen as a cause for concern. The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the presence and detection of *Bacillus* toxins in which the use of *B. cereus* in animal nutrition was, in principle, considered undesirable and to be avoided (EC, 2000). However, data produced by the applicant at that time was sufficient for SCAN to conclude that the particular strain of *B. cereus* used in Toyocerin® was a "disabled pathogen" unable to produce functional enterotoxins. Consequently, SCAN concluded that the use of this particular strain did not pose a hazard for consumers of products derived from animals given Toyocerin®.

Subsequently, EFSA produced several opinions on the use of Toyocerin®, including use with additional animal species and its compatibility with selected coccidiostats. Toyocerin® was authorised under Regulation (EC) No 1831/2003 for use with turkeys for fattening and rabbit does for breeding. These opinions essentially dealt with modifications to an existing authorisation or an extension of use. Since these extensions of use were not expected to introduce hazards not already considered, the assessments of safety were restricted to the new target species for which authorisation was sought. Consumer, environmental and user safety were not revisited.

Following the requirements of Regulation (EC) No 1831/2003, the applicant is now requesting the re-evaluation of the additive when used in feeds for sows (from service to weaning), piglets, pigs for fattening, cattle for fattening, chickens for fattening and rabbits for fattening. In addition, under a separate application, the applicant is seeking a new authorisation for use with calves for rearing. The assessment for use with this category is incorporated into this opinion.

2. Characterisation[14]

2.1. Characterisation of the active agent

The strain of *B. cereus* was isolated from soil and is deposited in three European cultures collections; the Collection Nationale de Cultures de Micro-organismes as CNCM I-1012, the National Collection of Industrial and Marine Bacteria as NCIMB 40112 and the Colección Española de Cultivos Tipo as CECT 876.[15] Identification is based on a phylogenetic analysis of 16S rRNA, 23S rRNA and *gyrB* genes.[16] The strain has not been genetically modified.

Numerous chemical, biochemical and genetic methods have been successfully examined as a means of distinguishing the additive strain from other strains of *B. cereus*, including serotyping, pyrolysis mass-spectroscopy, ribotyping and pulsed field gel electrophoresis (PGFE).[17] Genetic stability was confirmed after 180 sub-cultures *in vitro* (over a 4.5 year period), after *in vivo* transit through rats (ten serial transits) and by comparison of the plasmid profiles of the stock seed culture with the deposited strain.[18]

[14] This section has been edited following the provision of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[15] Technical dossier/Section II/Annex II_25.
[16] Technical dossier/Section II/Annex II_33.
[17] Technical dossier/Section II/Annexes II_26-35.
[18] Technical dossier/Section II/Annexes II_36 and 37.

2.1.1. Antimicrobial susceptibility

Resistance to the antibiotics tetracycline and chloramphenicol was recognised by SCAN who asked for details of the nature of the resistance (EC, 2001a). No genes known at that time to confer resistance to tetracyclines were found present in the Toyocerin® strain of *B. cereus*. PCR also failed to amplify the only *cat* gene encoding chloramphenicol resistance then known. Transposon mutagenesis produced a mutant strain with increased sensitivity to both tetracycline and chloramphenicol which, in the view of SCAN, implied a disabling insertion of the transposon at the site in chromosomal DNA conferring resistance. Cloning and sequencing of the DNA adjacent to the insertion site showed that the transposon had inserted in the flanking region of two co-regulated genes (ORF 1 and 2) located between the known chromosomal genes *gerIC* and *nucB*. ORF 1 and 2 showed no homology to any known tetracycline (or chloramphenicol) resistance gene and when cloned into a sensitive strain of *E. coli* did not confer any antibiotic resistance. From these data SCAN concluded that resistance to chloramphenicol and tetracycline are closely interrelated and may represent a multiple drug resistance mechanism possibly unique to the Toyocerin® strain. Since the two genes associated with resistance are located between recognised housekeeping genes, it indicated to SCAN that they are particular to the organism and not externally acquired. Consequently, SCAN in line with its Opinion on the criteria for assessing the safety of micro-organisms resistant to antibiotics of human clinical and veterinary importance (EC, 2001b), concluded that the probability of transfer of resistance to other organisms is not a concern.

The current EFSA guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA, 2012) has cut-off values which differ from the "breakpoints" applied by SCAN. However, a reassessment applying the EFSA values confirmed that minimum inhibitory concentrations (MICs) were lower than the cut-off values for all antibiotics except for tetracycline (> 100 mg/L) and chloramphenicol (> 100 mg/L), which were substantially higher than the respective EFSA cut-off values (8 mg/L for both).[19] Under such circumstances EFSA guidance (EFSA, 2012) requires the genetic basis for the observed resistance to be established, otherwise it is assumed that the transfer of resistance to other bacteria is probable and presents a risk.

EFSA, unlike its predecessor, had access to the bioinformatic analysis of the complete genome sequence of the *B. cereus* strain (chromosomal and plasmid) provided by the applicant.[20] This demonstrates the presence of significant matches for several genes encoding for chloramphenicol and tetracycline resistance (homology > 95%). In particular, a gene showing high homology with the *catQ* gene, encoding for a chloramphenicol acetyl-tranferase, was detected in the genome.[21] This gene is involved in chloramphenicol resistance in Gram positive pathogenic bacteria including *Clostridium perfringens*, *C. difficile*, *Streptococcus pneumoniae* and *S. pyogenes* (Del Grosso *et al*. 2001, Mingoia *et al*. 2007, Roberts and Schwarz, 2009, Rood *et al*. 1989). The presence of this gene in association with a strong resistance to chloramphenicol in the Toyocerin® strain, the susceptibility of other *B. cereus* strains to this antibiotic and the occurrence of the *catQ* gene in other bacterial genera strongly suggests that this is an acquired resistance.

The genetic basis of the resistance to tetracycline is not established. The earlier studies seen by SCAN based on insertional mutagenesis identified a genetic locus associated with tetracycline and chloramphenicol resistance.[22] The new data from the genome sequence showed that the intergenic region between the chromosomal genes *gerIC* and *nucB* contains genes coding for two proteins of unknown function and a gene having homology with integrases.[23] The role of this genetic locus in tetracycline/chloramphenicol resistance, if any, thus remains unclear. Analysis of the whole genome sequence did reveal the presence of ten genes coding for (multi-drug) efflux proteins (see Table 2), all potentially involved in tetracycline resistance. However, it is not known which of these genes are

[19] Technical dossier/Section II/Annex II_46.
[20] Technical dossier/Supplementary information February 2012.
[21] Technical dossier/Supplementary information February 2012/Techreport full genome Btoyoi Annex V.
[22] Technical dossier/Supplementary information February 2012/Annex 10.
[23] Technical dossier/Supplementary information February 2012/Annex 1/Techreport full genome Btoyoi.

transcribed and are functionally active. In the absence of data showing that resistance to tetracycline in this strain is a result of a mutational event, the FEEDAP Panel defaults to the view that the resistance to tetracycline should be treated as transferable.

Table 2: Genes coding for (multi-drug) efflux proteins found in *Bacillus cereus* CNCM I-1012

Localisation	Gene name	Gene description
scaffold00001	*ykkD*	multidrug resistance protein ykkD
scaffold00001		matE family protein
scaffold00001	*bmr3*	multidrug resistance protein 3
scaffold00001	*bmrA*	multidrug resistance ABC transporter ATP-binding/permease protein BmrA
scaffold00001 and 2	*matE*	MATE efflux family protein
scaffold00001 and 2	*norM*	MATE efflux family protein
scaffold00001 and 2	*lmrB*	drug resistance MFS transporter drug:H+ antiporter-1 family protein
scaffold00002	*yheH*	putative multidrug resistance ABC transporter ATP-binding/permease protein YheH
scaffold00002	*yheI*	putative multidrug resistance ABC transporter ATP-binding/permease protein YheI
scaffold00002	*ykkC*	multidrug resistance protein ykkC
scaffold00002	*yusP*	drug resistance MFS transporter, drug:H+ antiporter-1 family protein

2.1.2. Toxigenic potential

A first assessment of the toxigenic potential of *Bacillus cereus* CNCM I-1012 was made by SCAN based on information provided by the applicant. This included PCR experiments that failed to detect all of the components of the two *B. cereus* tripartite enterotoxins (Nhe and Hbl).[24] In particular the genes *nheB, nheC* and *hblC, hblD*, coding for structural components of the enterotoxins, were not amplified. The absence of enterotoxin production was also supported by SDS page and Western blot experiments on the culture supernatant of the production strain.[25] In addition, cytotoxicity tests with Vero cells from two different sources using concentrated supernatants of the Toyocerin® strain confirmed the lack of phenotypic expression.[26] The absence of emetic toxin cereulide was also demonstrated in a Hep-2 vacuolation test.[27] On the basis of these data, SCAN concluded that *B. cereus* lacked the potential to elaborate functional toxins.

Following the publication of the Technical Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition (EFSA, 2011), the applicant was requested to provide additional information, specifically the analysis of the whole genome sequence.

The bioinformatic analysis of the whole genome sequence, in contrast to the PCR data seen by SCAN, showed that *B. cereus* CNCM I-1012 harbours all three genes of the *nhe* operon (*nheA, nheB* and *nheC*) and all four genes (*hblA, hblB, hblC* and *hblD*) coding for the Hbl enterotoxin.[28] The two operons present the same organisation as pathogenic *B. cereus* strains and no deletion or mutation affecting their transcription, translation or secretion was found. The applicant did not provide any study supporting the lack of transcription of the operons coding for Hbl and Nhe enterotoxins. The applicant did speculate on the basis of sequence comparisons with a single *B. cereus* pathogenic strain that the minor amino acid residue substitutions in the signal sequence of NheC and HblD could affect secretion of these

[24] Technical dossier/Section II/Annex II_38.
[25] Technical dossier/Section II/Annex II_39.
[26] Technical dossier/Section II/Annexes II_37, 42 and 43.
[27] Technical dossier/Section II/Annex II_44.
[28] Technical dossier/Supplementary information February 2012/Annex 1_Technical Report full genome Btoyoi/Techreport full genome Btoyoi feb12.

enterotoxin components. However, these allelic variations appear common amongst toxigenic *B. cereus* strains, and therefore, do not provide evidence of disrupted secretion.

An important element in the SCAN conclusion was the absence of any evidence of cytotoxicity in tests with Vero cells. However, the publication of the results of another cytotoxicity assay with Vero cells in which a reduction of metabolic activity was seen in the presence of concentrated culture filtrate from the Toyocerin® strain, challenges these results (Darbouche, 2011). The more so as the methods described in the 2011 publication replicate those of the studies submitted by the applicant, including the same source of the Vero cell culture, the same positive and negative control strains and the same method of production and concentration of the culture filtrate tested.

The applicant provided PCR based information on the absence of the enterotoxin gene *cytK*.[29] However, these data were not confirmed by genome analysis. The Toyocerin® strain does not contain the cereulide synthetase gene cluster.

Overall, the evidence that the strain has the capacity to elaborate functional enteroxins and the newly reported cytotoxicty leaves the FEEDAP Panel with considerably more doubts about the safety of the Toyocerin® strain than were raised considering only the data available to SCAN.

2.2. Characterisation of the additive

2.2.1. Manufacture and characterisation

B. cereus is produced by fermentation in a typical industrial medium, concentrated and mixed with other ingredients. A detailed description of the manufacturing process of the additive is provided in the dossier. Data from five batches of the additive showed that the minimum specification was met in all cases (range 1.0 – 1.1 x 10^{10} CFU/g additive). Reference is also made to an alternative formulation containing 1 x 10^{9} CFU/g additive produced in the same manner.

The resulting additive is a white to grey powder consisting of particles with a mean diameter of ~ 50 µm as determined by laser diffraction.[30] Analysis of three batches of the final product confirmed that approximately 90% of the additive consisted of particles with diameters < 100 µm and 10% < 10 µm. A measure of dusting potential was obtained by air sampling at two points in the manufacturing plant at which the concentration of spore in the air was expected to be maximum.[31] The value obtained of approximately 5 mg dust/m^3 has significance for the manufacturing plant but its relevance to those subsequently handling the additive is less clear, but could be treated as a worst case scenario for users.

2.2.2. Quality control and impurities

Details of the quality control procedures are given for the manufacturing process.[32] The concentrated cell mass is routinely analysed for heavy metals, arsenic and microbial contamination before blending with the carrier and the final product examined for microbial contamination. Specifications are set in the final product for Cd (< 1 mg/kg), Hg (< 0.1 mg/kg), Pb (< 10 mg/kg) and As (2 mg/kg). Microbial action limits are < 3 CFU/g for coliforms including *E. coli*, < 10 CFU/g for yeasts and filamentous fungi and the absence of *Salmonella* in 25 g product. The results of the analysis of five commercial batches of the additive confirm compliance with these specifications.[33]

[29] Technical dossier/Section II/Annex II_41.
[30] Technical dossier/Section II/Annex II_22.
[31] Technical dossier/Section II.
[32] Technical dossier/Section II/Annex II_20.
[33] Technical dossier/Section II/Annex II_16.

2.3. Stability and homogeneity

2.3.1. Shelf-life

Data based on three commercial batches showed that additive is stable when stored under ambient conditions (15 – 25°C) in the packaging as supplied for at least 21 months (or six months at 30°C).[34] Removal of the product from its packaging and storing under experimental conditions (30°C/60 %RH or 44°C/80 % RH) reduced the expected shelf-life. Losses of between 20 and 30% were recorded after three months.

2.3.2. Stability in premixtures

The stability of the additive (single batch) in a typical piglet and sow vitamin-mineral premix was monitored for a period up to two months.[35] Viability was little affected in the piglet premix after six weeks storage at ambient temperature or 37°C. Similar results were obtained with the sow premix. Losses of approximately 15% were observed after two months storage at 30°C/60 %RH or 44°C/80 % RH or after four months at ambient temperature. In addition to the effects of the complete premix, the effect of individual premix components (individual minerals, fumaric and citric acids, NaCl) on the viability of *B. cereus* was monitored over a six month period.[36] Only copper and iron sulphates appeared to have a slight detrimental effect.

2.3.3. Stability in feed

Stability to pelleting was measured in two feeds for piglets, one for rabbits, one for laying hens and three for turkeys.[37] In each case the intended final concentration in feed was 1 x 10^9 CFU/kg feed with samples taken before and immediately after pelleting at temperatures between 72 – 90°C depending on the nature of the feed.[38] Loss of viability was negligible regardless of pelleting temperature.

Storage of piglet mash feeds (starter and finisher) under ambient conditions or controlled conditions (30°C) led to a small loss in viability (~20%) after three months.[39] Similar results were obtained when the feed was pelleted.[40] Even under more extreme conditions (44°C/80 %RH) loss of viability only increased to ~30%.[41] The results seen with feed for piglets were duplicated when viability was assessed in pelleted feed for chickens for fattening and rabbits stored under comparable conditions for the same period.[42] Similarly, essentially no loss of viability was found when three turkey feeds (starter, grower and finisher) were stored under monitored warehouse conditions (temperature range 6 – 22°C, humidity range 50 – 84%) for a period of three months.[43]

Three different batches of Toyocerin®, containing 1 x 10^{10} viable *B. cereus* spores/g of product, were used for the preparation of three batches of starter feeds for calves.[44] Just after mixing and before pelleting of the starter feed, each batch was split into two halves, one half was packaged in a paper bag to be used as the non-pelleted feed (i.e. mash feed) and the other half continued to the pelleting process (temperature of feed after steaming: 50-55ºC). The data provided demonstrate that Toyocerin®, when included in feeds (both pelleted and non-pelleted feed) for calves shows stability during the pelleting process and the storage for at least three months after the manufacturing date.

[34] Technical dossier/Section II/Annex II_63.
[35] Technical dossier FAD-2010-0090/Section II/Annex II_73 and 74.
[36] Technical dossier FAD-2010-0090/Section II/Annex II_67.
[37] Technical dossier FAD-2010-0090/Section II/Annex II_68.
[38] Technical dossier FAD-2010-0090/Section II/Annexes II_69-72.
[39] Technical dossier FAD-2010-0090/Section II/Annex II_75.
[40] Technical dossier FAD-2010-0090/Section II/Annex II_76.
[41] Technical dossier FAD-2010-0090/Section II/Annex II_77.
[42] Technical dossier FAD-2010-0090/Section II/Annexes II_78 and 79.
[43] Technical dossier FAD-2010-0090/Section II/Annex II_72.
[44] Technical dossier FAD-2010-0091/Supplementary information February 2012/Annex 2.

Three different batches of Toyocerin®, containing 1 x 10^{10} viable *B. cereus* spores/g of milk replacer, were used for the preparation of three batches of milk replacer in order to determine the stability of *B.* cereus in milk replacer stored for three months in ambient conditions.45 No significant differences were seen between the concentrations of B. cereus before and after three months of storage.

2.3.4. Homogeneity after mixing

Three batches of the additive were each incorporated into two tonnes of feed for chickens for fattening at an intended concentration of 1 x 10^9 CFU/kg feed.[46] After blending each feed was pelleted and then five sub-samples taken for each feed and assayed for numbers of *B. cereus*. The coefficient of variation (CV %) recorded for each batch of feed were very similar (8.0, 10.1, 7.1). These figures derived from time zero values from a stability study in which five sub-samples were also collected at monthly intervals from each feed and assayed. Although absolute numbers declined slightly during storage, the variation between samples was close to the time zero values. Taken as a whole the 20 sub-samples taken from each batch of feed provides adequate evidence of an even distribution of the additive within feeds for chickens for fattening.

One single batch of Toyocerin®, containing 1 x 10^{10} viable *B. cereus* spores/g of product, was used for the preparation of one single batch of starter feed for calves.[47] Just after manufacturing, ten sub-samples (a minimum of 200 g net weight each sub-sample) of the single starter feed batch were collected for analysing the inclusion level of *B. cereus*. The mean value of the ten sub-samples was 1.0 x 10^9 viable *B. cereus*/kg, the standard deviation was 0.07 and the CV was 7.31 %.

These data indicate that Toyocerin® is able to mix homogenously when it is included in feed.

2.4. Conditions of use

The proposed use of Toyocerin® in animal nutrition is shown in the following Table 3.

Table 3: The proposed maximum and minimum concentration of the additive in complete feeds (CFU/kg feed) for the species and categories for which authorisation is sought

Animal species/category	Minimum proposed	Maximum proposed
Cattle for fattening	0.2 x 10^9	0.2 x 10^9
Calves for rearing	0.5 x 10^9	1.0 x 10^9
Chickens for fattening	0.2 x 10^9	1.0 x 10^9
Piglets to 2 months	1.0 x 10^9	1.0 x 10^9
to 4 months	0.5 x 10^9	1.0 x 10^9
Pigs for fattening	0.2 x 10^9	1.0 x 10^9
Sows – service to weaning	0.5 x 10^9	2.0 x 10^9
Rabbits for fattening	0.1 x 10^9	5.0 x 10^9

The Panel notes that the Applicants listing of "piglets to two or to four months" does not correspond with the categories recognised in Annex IV of Regulation (EC) No 429/2008. The first would equate to weaned piglets and the second to the growing period of a fattening pig.

[45] Technical dossier FAD-2010-0091/Supplementary information February 2012/Annex 3.
[46] Technical dossier FAD-2010-0090/Section II/Annex II_78.
[47] Technical dossier/Supplementary information February 2012/Annex 4.

2.5. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

The EURL considered that the conclusions and recommendations reached previously remain valid and are applicable for the current applications.[48]

3. Safety

3.1. Safety for target species

Many of the studies made in support of target animal safety are over 30 years old and do not always meet present standards in regard to design and reporting.

3.1.1. Safety for chickens for fattening

Three separate tolerance studies are described with a common design.[49] In each trial groups of 50 one-day-old birds (equal numbers of male and females) were assigned to control group, to a group treated with the maximum recommended dose (1 x 10^9 CFU/kg feed) and to a group given 100-times the maximum dose (1 x 10^{11} CFU/kg feed). The three trials each lasted eight weeks and birds were fed an unspecified mash feed. The description of the experimental design makes no reference to replication and so it has to be assumed that birds were group housed. There was also no analytical confirmation of dose. In all trials birds were observed for signs of ill-effects and were individually weighed after four and eight weeks. Feed intake was also recorded for these periods. In one of the three trials ten birds per treatment were taken at the end of the trial for haematology (red blood cells (RBC), white blood cells (WBC), haematocrit and haemoglobin), blood chemistry (total protein (TP), albumin, albumin- globulin ratio (A/G), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), glutamate aspartate aminotransferase (ALT)) and gross pathology.

No adverse effects were seen in any of the three trials and no significant differences were seen in haematology, blood chemistry or gross pathology where these were measured.

3.1.2. Safety for pigs

3.1.2.1. Weaned piglets and pigs for fattening

A six-month study is reported covering a period from weaning at 21-days to the animals reaching slaughter weight.[50] A total of 20 piglets (Large White) were selected from three litters and assigned either to a control group or to a treatment group given the additive. Animals were individually housed throughout the trial. The treatment group was dosed with the additive according to body weight (2 x 10^9 CFU/kg body weight/day) with amounts incorporated into feed adjusted daily. On the basis of the information available, it is not possible to determine the actual dose received in terms of kg feed for any particular period. However, on average treated animals can be calculated to have received approximately 20-times the maximum recommended dose of 1 x 10^9 CFU/kg complete feed both as piglets and as fattening animals. The first six weeks of the trial in which animals reached approximately 20 kg in weight can be considered to equate to a study of piglets and the remaining 20 weeks, when animals reached ~120 kg, as the fattening period.

Animals were observed daily for clinical signs and body weight and feed intake measured at three-weekly intervals throughout the trial. Samples were taken for haematology (RBC, WBC, haematocrit and haemoglobin) and blood chemistry (TP, A/G, glucose, cholesterol, bilirubin, AST and ALT) "initially" after 103 days and at the end of the trial on day 203. No adverse effects on growth were seen

[48] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2008-0009.pdf
[49] Technical dossier FAD-2010-0090/Section III/Annex III_10.
[50] Technical dossier FAD-2010-0090/Section III/Annex III_6.

during the first six-weeks or thereafter. No significant differences were seen in blood parameters and all remained within expected levels.

3.1.2.2. Sows

A total of 14 sows of approximately eight months of age (Large White x Landrace) were assigned to two groups matched for weight. [51] The first group of seven sows was fed a basal diet and the second the basal diet supplemented with Toyocerin® at 2 x 10^9 CFU/kg body weight/day. This equates to approximately 50 times the recommended maximum dose of 2 x 10^9 CFU/kg complete feed. The trial covered one complete reproductive cycle. Observations included weight of sows, feed intake, number of piglets born alive, number of piglets born dead, number of piglets weaned, clinical observations and haematological (RBC, WBC, haematocrit and haemoglobin) and biochemical parameters (TP, albumin, globulin, glucose, cholesterol, bilirubin, alkaline phosphatase (AP), AST and ALT).

No effects on body weight of sows were seen at any period during the cycle or on reproductive parameters. The mean number of piglets born alive and numbers surviving to weaning were numerically higher in the treated group. No significant differences between treated and control groups were seen in any of the blood chemistry or haematological parameters measured

3.1.3. Safety for cattle

3.1.3.1. Calves for rearing

A study was performed to assess the efficacy and tolerance of Toyocerin® at the recommended maximum dose (1.0 x 10^9 CFU/kg feed) or at 100 times the recommended maximum dose (1.0 x 10^{11} CFU/kg feed) in 60 rearing dairy calves.[52] The duration of the trial was 42 days (weight range of about 50 to 82 kg). Within about 14 days of age (6 to 14 days of age) the calves were allotted at random to the experimental groups at a minimum of ten calves per pen in two following series according to age and weight in order to start the trial with groups as homogeneous as possible. The milk-replacer without or with Toyocerin® at the recommended maximum level or at 100 times the recommended maximum level was fed for the following 42 days in daily concentrations of 125 g milk-replacer per liter, respectively. Additionally, a complete feed for rearing calves was offered *ad libitum* without or with Toyocerin® at the recommended maximum dietary dose or at 100 times the recommended maximum dose. Hay was given *ad libitum* throughout the experimental period.

The following parameters were recorded: individual body weight and weight gain, feed intake (pen level), health status, selected blood constituents, faecal microbiota and immune response against *Mannheimia haemolytica* A1 compared to calves fed without supplementation.

With addition of Toyocerin® at the recommended maximum and at 100 times the recommended maximum dose body weight gain was significantly increased by 15.6 and 22.2 % when compared to control calves. The other zootechnical parameters were not affected by the treatments. No adverse effects on blood constituents, serum immunoglobulins were observed in any case. The microbial examination of faeces after the 42-day administration of Toyocerin® at both dose levels was similar in each experimental group.

3.1.3.2. Cattle for fattening

A trial was made with ten male calves (7 days of age) and lasted until the animals were 18 months of age.[53] Five animals were used as the control group and separately housed from the remaining five which received Toyocerin® with their feed. Animals in the treatment group were given 2 x 10^9 CFU/kg body

[51] Technical dossier FAD-2010-0090/Section III/Annex III_7.
[52] Technical dossier FAD-2010-0091/Supplementary information February 2012/Annex 5.
[53] Technical dossier FAD-2010-0090/Section III/Annex III_5.

weight/day by adjusting the weight of additive given in line with increasing body weight. On the basis of the values recorded for mean body weights and feed intake, this would appear to equate to a 500-fold overdose compared to the proposed maximum dose. Animals fed successively, milk replacer, a "milk feed" (mash diet containing milk protein), a grower feed and a fattening feed. Roughage was freely available.

Animals were weighed monthly and given a clinical examination on a daily basis. Blood was taken at the start of the trial and after 3, 6, 12 and 18 months for haematology (RBC, WBC, haematocrit and haemoglobin) and blood chemistry (TP, albumin, globulin, glucose, cholesterol, bilirubin, AP, AST and ALT).

Animals remained in good health throughout the trial with the exception of a limited outbreak of diarrhoea in both groups soon after the start of the trial. Only animals in the control group required veterinary intervention. No significant differences were seen in weight gain between the two groups, although animals in the Toyocerin® group were heavier at all stages. There were also no significant differences in haematology and blood chemistry findings between the two groups at any stage of the trial. All values remained within the normal range for beef cattle.

3.1.4. Safety for rabbits for fattening

A total of 18 five-week-old rabbits were obtained from which 12 were selected (equal number of males and females).[54] These were allocated to one of three treatments, a control group of four rabbits, four in which the feed was supplemented with Toyocerin® at the maximum recommended dose (5 x 10^9 CFU/kg feed) and four in which the feed was supplemented with x 40 times the maximum dose (2 x 10^{11} CFU/kg feed). Feed concentrations of the additive were confirmed by analysis. Animals were individually housed and monitored for general conditions throughout the eleven week trial. Weight was recorded at the start and after 4, 8 and 11 weeks and feed intake recorded. Blood samples were taken at the end of the trial for the measurement of haematology (RBC, WBC, haematocrit level, haemoglobin, differential leucocytes) and blood chemistry parameters (TP, albumin, globulin, cholesterol, bilirubin, uric acid, LDH, AP, AST and ALT).

Animals remained in good health throughout the trial. No significant differences in average body weight, weight gain or feed intake was seen between the three groups. Similarly, no significant differences in haematology or blood chemistry parameters were recorded.

3.1.5. Other tolerance trials

EFSA has assessed the safety for two other target species/categories – turkeys for fattening and rabbit breeding does. Based on the results of two tolerance studies, the first with 400 birds and the second with 80, in which turkeys tolerated up to a ten-fold overdose of the product, and the known tolerance to substantially higher doses in chickens for fattening, the FEEDAP Panel concluded that Toyocerin® is safe for turkeys at the maximum recommended dose of 1 x 10^9 CFU/kg complete feed (EFSA, 2007b).[55] The tolerance studies provided for rabbit breeding does were considered insufficient to conclude on the safety of the product for this category. Instead the FEEDAP Panel based it conclusion on the results of the more extensive study made with rabbits for fattening described above.

3.1.6. Conclusions on safety for the target species

Although some of the tolerance studies submitted by the applicant would be considered inadequate by present days standards, there are individual studies which provide adequate assurance that the additive has no direct ill effects on the target species at the recommended dose range. Given that no adverse

[54] Technical dossier FAD-2010-0090/Section III/Annex III_8.
[55] Technical dossier FAD-2010-0090/Section III/Annexes III_9 and 11.

effects were recorded in any of the remaining studies, the FEEDAP Panel concludes that the additive is well tolerated by the target species that are the subject of this assessment.

However, the Panel notes that the strain of *B. cereus* harbours resistance determinants to two antibiotics, one of which *(catQ*) at least can now be ascribed to an acquired resistance. For this reason the FEEDAP Panel considers it inadvisable to introduce into target species a resistance determinant capable of transfer to other bacterial strains and adding to the pool of such determinants in the guts of livestock species.

3.2. Safety for the consumer

The Applicant introduced a number of studies under the general heading of safety for the consumer.[56] However, the active agent belongs to a bacterial species recognised as a human enteropathogen, consequently, safety assessment should focus on its pathogenic potential. The relevance of many of the tests provided for consumer safety is questionable, particularly when they involve laboratory animals not known to be infected by *B. cereus*.

3.2.1. Genotoxicity

B. cereus cells grown in nutrient broth were harvested and then disrupted.[57] The resultant supernatant was freeze-dried and used as the test substance in two *in vitro* genotoxicity assays. No increase in revertant colonies was seen in a reverse mutation (Ames) test and no evidence of chromosomal aberrations in an assay with a Chinese hamster lung cell line (DON D-6). It was concluded that the test substance was not genotoxic under the conditions of the assays.

3.2.2. Oral toxicity studies

A 12 month chronic oral toxicity study was made with 40 male rats (Wistar-Imamichi) allocated to one of four groups, a control group of ten rats and three groups of ten rats given 2 x 10^8, 1 x 10^9 or 2 x 10^9 CFU/kg body weight/day.[58] Growth and behaviour were monitored daily, blood was sampled at three-month intervals for haematology and blood chemistry and gross pathology and histology performed at necropscopy. There were no differences between control and treated rats in any of the parameters studied, all of which remained within the normal range for the strain of rat used. No deaths occurred in any group and all rats remained clinically and pathologically normal.

A subacute oral toxicity study involved ten rats/group dosed with 0, 10^8, 10^9 or 10^{10} CFU/kg body weight for 30 days.[59] A full range of endpoints were measured including gross pathology, histology and urine analysis. No significant differences were seen in any measured parameters.

3.2.3. Toxigenic potential

In the earlier assessment, use of PCR-based methods failed to detect all components of the two tripartite enteroxins and this, taken with the lack of any phenotypic response in cytotoxicity assays, led SCAN to conclude that the strain of *B. cereus* was a disabled pathogen lacking a potential to elaborate entire toxins. However, the subsequent analysis of the complete genome sequence, revealed that *B. cereus* CNCM I-1012 harbours all three genes of the *nhe* operon and the four genes coding for the haemolytic enterotoxin. Since the two operons present the same organisation as other pathogenic *B. cereus* strains and since no mutation affecting transcription or translation has been detected, it has to be assumed that the Toyocerin® strain has the capacity to elaborate functional toxins.

[56] Technical dossier FAD-2010-0090/Section III/Annex III_12,33,36,37,38,39,40.
[57] Technical dossier FAD-2010-0090/Section III/Annex III_35.
[58] Technical dossier FAD-2010-0090/Section III/Annex III_13.
[59] Technical dossier FAD-2010-0090/Section III/Annex III_14.

3.3. Safety for the user

3.3.1. Skin and eye irritancy

A study described as a test of skin irritation is described, but is in effect a test of the capacity of the *B. cereus* strain to infect an open wound.[60] A single rabbit had two surgical incisions made. A suspension of the strain in a growth medium was applied to one incision and the growth medium alone to the second. There was no evidence of infection after five days observation.

Eye irritancy was assessed using the Draize test protocol in which 24 rabbits were assigned to one of four treatments.[61] Two groups had a suspension of the additive instilled into one eye and two groups a suspension of $CaCO_3$ (the carrier used in Toyocerin®). The eyes of the rabbits in one of each treatment group were immediately irrigated with water, while the eyes of the second group were left untreated. Rabbits were observed for seven days post-treatment. Transient inflammation was seen in both non-irrigated groups, essentially disappearing after 24 hours. No other adverse effects were noted. Consequently, the additive can be considered non-irritant to the eye.

3.3.2. Sensitisation

No studies on either skin or respiratory sensitisation were considered necessary, as the applicant describes the additive as a potential sensitiser and treats it accordingly. Information on particle size distribution shows the potential for workers to inhale dust from the additive. Thus, during the manufacture of Toyocerin® operators use a fine-dust mask guaranteed to exclude 99% of all air-borne particles. This was done following measurements of occupational exposure in the plant in which dust levels of 5 mg/m^3 were detected. These are close to the Occupational Safety and Health Association (OSHA) limit for the calcium carbonate dust (5 mg/m^3 (respirable fraction)). However, the applicant's position is not fully reflected in the proposed material safety data sheet for the product which recommends the use of a mask but not the use of gloves.[62]

3.4. Safety for the environment

B. cereus is a ubiquitous soil saprophyte with a worldwide distribution. Consequently, use of the strain in animal nutrition is not expected to measurably increase numbers of the organism in the environment.

4. Efficacy

4.1. Chickens for fattening

Four trials are presented made in three different European countries, two of which were done in research establishments and two described as farm trials. All four studies involved the comparison of a control group of birds with two or more groups given the same diet supplemented with Toyocerin®. All studies included one group given Toyocerin® at the minimum recommended dose of 0.2 x 10^9 CFU/kg complete feed and in all cases the intended dose was confirmed by analysis of the feed. Diets were typical production diets based on soybean and wheat or maize and were supplied pelleted (trial 1) or as a mash (trials 2, 3, 4). The duration of the trials was 35 days (trial 1), 60 days (trial 2), 50 days, trial 3) and 44 days (trial 4). Animals used were common commercial lines with the exception of trial 2 which used a Red Shaver (a Canadian breed) hybrid.

Birds were weighed at the start and end of each trial and variously at intermediate intervals. From these data mean weight gain was calculated. Feed intake was measured on a pen basis and feed to gain ratio calculated. Mortality was recorded in all studies. In two trials (trials 2 and 3), water consumption and in

[60] Technical dossier FAD-2010-0090/Section III/Annex III_43.
[61] Technical dossier FAD-2010-0090/Section III/Annex III_42.
[62] Technical dossier FAD-2010-0090/Section III/Annex III_41.

three trials (trials 2, 3 and 4) faecal quality (dry mattter content) were additionally measured. Data were examined by analysis of variance (Table 4).

Table 4: Summary of the effects of Toyocerin® on the performance of chickens for fattening

Trial (Total No birds)	Replicates per treatment x birds per replicate	Dose CFU/kg feed	Final weight (kg)	Weight gain (kg)	Feed intake[a] (kg)	Feed to gain ratio
1[63]		0	1.75	1.70	2.78	1.63
(1680)	15 x 28	0.2×10^9	1.77	1.73	2.60*	1.50*
		0.5×10^9	1.76	1.72	2.73	1.59
		1.0×10^9	1.78	1.74	2.75	1.58
2[64]		0	1.88	1.82	6.72	3.66
(360)	6 x 20	0.2×10^9	2.12*	2.05*	6.03*	2.90*
		1.0×10^9	2.02*	1.96*	5.56*	2.81*
3[65]		0	2.29	2.23	5.36	2.40
(3000)	20 x 50	0.2×10^9	2.45*	2.40*	5.18*	2.16*
		1×10^9	2.55*	2.50*	5.63*	2.25*
4[66]		0	2.04	2.01	4.60	2.29
(1350)	6 x 75	0.2×10^9	2.04	2.01	4.54	2.26
		1.0×10^9	2.05	2.02	4.27*	2.11*

[a]Feed intake per bird was calculated from the measured intake for the pen.
*Significantly different from the control by at least $P<0.05$.

Birds receiving Toyocerin® were heavier than control birds at the end of each trial but this difference reached significance in only two trials with the minimum recommended dose (0.2×10^9 CFU/kg feed). Similarly, feed intake was reduced in treated groups and this was significant in three trials with the minimum recommended dose. Feed to gain ratio was positively and significantly affected in these trials. In the studies where water consumption was measured, results were inconsistent. However, faecal "quality" (higher dry matter content) was significantly improved in all three trials where this parameter was measured with the minimum recommended dose. Mortality was considered normal and non treatment related.

4.2. Pigs

4.2.1. Piglets

The results of eight trials with weaned piglets, all made within Europe, were provided. However, only five trials were of a duration considered adequate.

[63] Technical dossier FAD-2010-0090/Section IV/Annex IV.25.
[64] Technical dossier FAD-2010-0090/Section IV/Annex IV.26.
[65] Technical dossier FAD-2010-0090/Section IV/Annex IV.27.
[66] Technical dossier FAD-2010-0090/Section IV/Annex IV.28.

The trials were similar in design with piglets weaned about 23 days allocated to one of two treatments. In each case a control group fed only a basal diet was compared to second group fed the diet supplemented with 1 x 10^9 CFU/kg feed (confirmed by analysis). Breeds used were typical commercial crosses, typically Landrace x Large White. A crossbreed Huelsenberger Hybridschwein was used in trial 2 and a Pietrain cross in trial 3.

Animals were weighed at the start and end of each trial and feed intake measured. Average daily gain (ADG) and feed to gain ration were then calculated. Health status was monitored and all deaths/culls recorded. The data were then treated to an analysis of variance and significance assumed at P<0.05. The results are summarised in Table 5.

Table 5: Summary of the effects of Toyocerin® on the performance of weaned piglets

Trial (Duration in days)	Total animals (Replicates x treatment X animals x replicate)	Dose CFU/kg feed	Initial weight (kg)	Final weight (kg)	Average daily gain (g)	Average daily feed intake (g)	Feed to gain ratio
1[a67]	216	0	6.32	19.73	336	618	1.84
(41)	(3 X 24)	1.0 x 10^9	6.39	21.51*	376*	585	1.55*
		1.0 x 10^9	6.30	21.12*	370*	545	1.51*
2[68]	40	0	7.89	28.45	490	830	1.70
(42)	(10 X 2)	1 x 10^9	7.90	28.71	496	820	1.67
3[69]	60	0	8.70	22.90	384	666	1.73
(37)	(3 X 10)	1.0 x 10^9	8.70	23.80	410	685	1.67*
4[70]	72	0	6.29	17.09	292	511	1.75
(37)	(6 X 6)	1.0 x 10^9	6.30	18.30	324	550	1.70
5[71]	24	0	5.46	21.72	370	677	1.84
(44)	(3 X 4)	1.0 x 10^9	5.43	22.77	394	693	1.77

[a]Part of a larger experiment in which diets differ at later stages in the production.
*Significantly different from the control by at least P<0.05.

Although there was general numerical improvement in all trials, this reached significance in at least on one parameter in only two trials (trials 1 and 3) where, in one case, final body weight, ADG and feed to gain were improved, where in the second only feed to gain improved. Mortality was considered normal and non treatment related.

[67] Technical dossier FAD-2010-0090/Section IV/Annex IV.5.
[68] Technical dossier FAD-2010-0090/Section IV/Annex IV.7.
[69] Technical dossier FAD-2010-0090/Section IV/Annex IV.8.
[70] Technical dossier FAD-2010-0090/Section IV/Annex IV.9.
[71] Technical dossier FAD-2010-0090/Section IV/Annex IV.11.

Reports of nine "field studies", only six of which had a negative control, suggested small improvements in the production of piglets given Toyocerin® at the minimum recommended dose. However, in the absence of replication no statistical analysis could be applied.

4.2.2. Pigs for fattening

A total of six trials are described which cover both the grower and finisher periods to market weight of approximately 100 kg. In each study pigs of around 25 kg were allocated to one of three treatments, a control group given basal diets and two treatment groups in which the basal diets were supplemented with the additive. In some studies a single dose was used throughout the trial while in others the dose was reduced during the finishing stage (see Table 6). There was no indication that the intended doses were confirmed by analysis of feeds. Landrace cross were used in all except trials 2 and 3 in which Bundeshybridzuchtprogramm (BHZP) pigs were used. All animas were provided *ad libitum* with typical cereal-soybean based mash diets.

Animals were weighed at the start of the trial, at the change-over (grower to finisher) stage and at the end. Feed intake was measured and average daily gain and feed conversion calculated. The data produced were in each case subject to an analysis of variance. Results are summarised in Table 6.

Table 6: Summary of the effects of Toyocerin® on the performance of pigs for fattening

Trial (Duration in days)	Total animals (Replicates x treatment X animals x replicate)	Dose[a] CFU/kg feed	Final weight (kg)	Average daily gain (g)	Average daily feed intake (kg)	Feed to gain ratio
1[72]	72	0	94.5	890	2.30	2.55
(56)	(6 X 4)	0.2×10^9	95.7	889	2.16	2.48
		$1.0/0.5 \times 10^9$	98.7*	918	2.31	2.52
2[73]	108	0	107.5	772	2.63	3.42
(105)	(18 X 2)	$0.5/0.2 \times 10^9$	107.7	815*	2.64	3.25*
		0.2×10^9	107.9	781	2.60	3.34
3[74]	48	0	108.2	744	2.10	2.82
(97)	(8 X 2)	$0.5/0.2 \times 10^9$	106.9	824*	2.14	2.59*
		$1.0/0.5 \times 10^9$	107.5	746	2.08	2.78
4[75]	216	0	89.4	710	2.08	2.93
(98)	(3 X 24)	$0.5/0.2 \times 10^9$	93.3*	730	2.02	2.77
		1.0×10^9	96.3*	767*	1.94	2.53*
5[76]	216	0	91.6	702	2.00	2.85
(98)	(4 X 18)	$0.5/0.2 \times 10^9$	94.0*	727*	2.00	2.74*
		1.0×10^9	96.0*	749*	2.00	2.64*
6[77]	36	0	97.2	844	2.17	2.56
(91)	(4 X 3)	0.5×10^9	97.8	850	2.10	2.43
		1.0×10^9	104.2*	921*	2.14	2.36

[a] Where two figures are given the first is the dose given in the grower period and the second the dose given during the finisher period.
* Significantly different from the control by at least $P<0.05$.

All trials showed at least one significant benefit in comparison to the control group. In four trials (trials 2 - 5) consistent beneficial effects on final weight, daily gain or feed to gain ratio were shown with the lowest dose regime ($0.5/0.2 \times 10^9$ CFU/kg feed). Similar effects in the other two trials required higher concentrations of the additive.

[72] Technical dossier FAD-2010-0090/Section IV/Annex IV.14.
[73] Technical dossier FAD-2010-0090/Section IV/Annex IV.16.
[74] Technical dossier FAD-2010-0090/Section IV/Annex IV.17.
[75] Technical dossier FAD-2010-0090/Section IV/Annex IV.18.
[76] Technical dossier FAD-2010-0090/Section IV/Annex IV.19.
[77] Technical dossier FAD-2010-0090/Section IV/Annex IV.20.

4.2.3. Sows

A total of four studies are described, three made over two breeding cycles and the fourth lasting 17 weeks (until 28 days post-partum). All four studies have been previously considered by the FEEDAP Panel (EFSA, 2007a).

In each case the performance of sows fed basal diets were compared with that of sows given the basal diet supplemented with Toyocerin® at the minimum recommended dose and, in the case of the first trial, at two higher doses (Table 7). The intended concentration in feed was confirmed by analysis. Where piglets had access to starter feed, this was given without Toyocerin® supplementation except trial 4 where piglets from the treatment group had access to creep and starter feeds containing 1 x 10^9 CFU/ kg feed. In all trials observations were limited to those relating to piglets other than the duration of the weaning to service interval.

Table 7: Summary of the effects of Toyocerin® on the performance of sows. Data for the first three trials are an average of the results for the two cycles

Trial (No of sows)	Dose CFU/kg feed	Live births per sow	Piglets weaned per sow	Body weight of piglets (kg)		Weaning to service (days)
				At birth	At weaning	
1[78] (80)	0	9.2	8.4	1.98	6.83	6.5
	0.5 x 10^9	9.9*	8.7*	1.99	7.34*	5.8
	1.0 x 10^9	10.4*	9.3*	1.99	7.89*	4.7*
	2.0 x 10^9	10.4*	9.4*	2.00	7.88*	4.2*
2[79] (80)	0	10.1	9.0	1.88	7.21	5.4
	0.5 x 10^9	10.5	9.4*	1.87	7.44*	4.8*
3[80] (89)	0	9.8	9.0	1.69	7.39	6.1
	0.5 x 10^9	11.2*	9.3	1.72	7.41	5.5*
4[81] (26)	0	11.8	8.9	1.75	8.06	n.a.
	0.5 x 10^9	11.6	9.7	1.54*	6.93*	n.a.

*Significantly different from the control by at least P<0.05.
n.a. not applicable

All four studies included a subjective assessment of the incidence and severity of diarrhoea during lactation (using a 0-3 score with 0 denoting absence, 1 slight, 2 middle and 3 acute diarrhoea). The diarrhoea score was significantly reduced in piglets from the treated group compared to controls in all cycles and all trials.

In 2007, the FEEDAP Panel concluded largely on the results above supported by the results of some earlier short studies not submitted with the present request, that "the use of Toyocerin® at the minimum recommended dose can increase numbers of live births, the number of piglets reaching weaning and, despite the apparently anomalous result in the fourth study, increase their bodyweight at weaning. This appears associated, in part at least, with a reduced incidence or severity of diarrhoea during lactation and subsequently during the post-weaning period" (EFSA, 2007a). No new data have been submitted which would lead the FEEDAP Panel to modify this conclusion.

[78] Technical dossier FAD-2010-0090/Section IV/Annex IV.1.
[79] Technical dossier FAD-2010-0090/Section IV/Annex IV.2.
[80] Technical dossier FAD-2010-0090/Section IV/Annex IV.3.
[81] Technical dossier FAD-2010-0090/Section IV/Annex IV.4.

4.3. Cattle

4.3.1. Calves for rearing

Six studies were performed in two different Member States but one[82] was discarded due to insufficient length. All of the remaining studies included minimum and maximum recommended doses confirmed by analyses, except for last study where no certificates of analysis were provided.

The first study included 136 calves (female, Holstein-Friesian) homogeneously distributed in three groups (of 47, 50, and 39 calves respectively, see Table 8) according to age and body weight. The additive was administered via milk replacer (fed individually, average 6.62 L/calf/day) and via concentrate, which was provided *ad libitum.* Hay and water were also freely available. The study lasted 56 days. The parameters measured were: individual body weight at the start and at the end of the experiment, individual daily intake of milk replacer. The overall intake of concentrate and hay was calculated in weekly intervals per pen. The overall feed conversion was calculated by dividing the overall feed consumption of milk replacer, concentrate and hay and the total body weight gain per calf. General health status was also monitored along the experimental period. All data were analysed by ANOVA and means compared by Scheffe test and Tukey test using the calf as the experimental unit.

Body weight and body weight gain were significantly improved in treated animals at the minimum and maximum recommended dose.

The second and third studies followed the same experimental design. They both involved 24 animals (12 female and 12 male calves, HF breed) individually reared and homogenously distributed in three groups according to body weight (see Table 8). The additive was administered through milk replacer (individually, average 5.5 kg/calf/day) and from the 10th day also via concentrate provided *ad libitum.* The trial duration was 59 days. The parameters measured along the study were: individual body weight at the start and at the end of the experiment, individual feed intake, and the average daily gain and feed efficiency calculated on that basis. All data were analysed by ANOVA and means were compared by Duncan's Multiple Range Test.

In both studies body weight and body weight gain were significantly improved in treated animals at the minimum and maximum recommended dose. In study 2, feed intake was also significantly increased in treated animals and in study 3, the feed to gain ratio was significantly improved in treated animals.

The fourth study involved 48 calves (female, Fleckvieh) homogenously distributed in four groups according to body weight (control and three Toyocerin® treated groups, Table 8). Animals were fed milk replacer with or without Toyocerin® for 56 days. The fifth study involved 36 male Braunvieh calves distributed in three groups, a control and two Toyocerin® groups (Table 8) for 70 days. Animals were housed in single pens. In both studies the parameters monitored included live weight, carcass score, status of health at intervals of 14 days and feed conversion ratio was calculated. All data were analysed by ANOVA and means compared by Duncan's Multiple Range Test.

In study 4, no significant effects were observed in treated animals. In study 5, Toyocerin® treated animals showed significant better growth parameters and a significant lower feed to gain ratio (Table 8).

[82] Technical dossier FAD-2010-0091/Section IV/Annex IV.4.

Table 8: Summary of data on the effects of Toyocerin® on the performance of calves for rearing

Trial (days of life)	Total No of animals	Dose (CFU/kg feed)	Initial weight (kg)	Final weight (kg)	Body weight gain (kg)	Total feed intake (kg)	Feed to gain ratio
1[83]* (6 to 62)	136	0	44.0	64.6[a]	20.6[a]	53.7	2.9
		0.5 x 10^9	43.6	70.6[b]	27.0[b]	56.0	2.4
		1 x 10^9	43.5	70.0[b]	26.5[b]	53.7	2.2
2[84] (5 to 64)	24	0	43.8	80.0[a]	36.2[a]	65.7[a]	1.8
		0.5 x 10^9	44.8	82.9[b]	38.1[b]	68.9[b]	1.8
		1 x 10^9	44.4	82.9[b]	38.5[b]	68.2[b]	1.8
3[85] (5 to 64)	24	0	42.3	76.6[A]	34.3[a]	67.3	2.0[a]
		0.5 x 10^9	41.5	77.0[A]	35.5[b]	66.9	1.9[b]
		1 x 10^9	42.8	78.8[B]	36.0[b]	67.7	1.9[b]
4[86] (42 to 98)	48	0	70.7	140.0	69.3	106.7	1.54
		0.25 x 10^9	70.6	139.7	69.1	105.7	1.53
		0.5 x 10^9	70.9	139.9	69.0	108.3	1.57
		1 x 10^9	70.7	142.6	71.9	107.1	1.49
5[44] (28 to 98)	36	0	64.8	150.2[a]	85.4[a]	133.2	1.6[a]
		0.5 x 10^9	64.5	156.1[b]	91.6[b]	133.7	1.5[b]
		1 x 10^9	64.5	155.3[b]	90.8[b]	133.5	1.5[b]

* Hay was not considered in the estimation of the intended dose, while hay intake was included in the measurement of the total feed intake.
Means with different superscripts within the same column differed significantly at [a, b] ($P<0.05$) and [A,B] ($P<0.1$).

4.3.2. Cattle for fattening

Four trials were made in Europe with cattle for fattening of various breeds. The trials followed a similar protocol in which animals were allocated to one of two groups with a similar average start weight. One group was given complementary feed containing Toyocerin® at 0.2 x 10^9 CFU/kg (confirmed by analysis) while the other group were given the complementary feed without the additive. Both groups had access to roughage. The weight of animals was measured at the start and end of the trial and average daily gain calculated. Feed intake was measured on a group basis and excluded from the statistical analysis. The number of animals on trial and duration are shown in Table 9. Trial 1 used only crossbred Frison x Asturin, trial 2 a mixture of Limousin, Charolais and crossbred animals and trial 3 Holstein-Friesian. Trial 4 was in effect three separate trials, 4A based on Montbeliard, 4B on Fleckvieh and Trial 4C crossbred Holstein x Limousin. The data for each sub-trial and the pooled data are given in Table 9.

[83] Technical dossier FAD-2010-0091/Section IV/Annex IV_1.
[84] Technical dossier FAD-2010-0091/Section IV/Annex IV_2.
[85] Technical dossier FAD-2010-0091/Section IV/Annex IV_3.
[86] Technical dossier FAD-2010-0091/Section IV/Annex IV_5.

Table 9: Summary of data on the effects of Toyocerin® on the performance of cattle for fattening

Trial No (No of animals)	Duration (days)	Dose (CFU/kg feed)[a]	Initial weight (kg)	Final weight (kg)	Average daily gain (kg/day)
1[87]	225	0	98	384	1.27
(80)	221	0.2 x10^9	97	408*	1.41*
2[88]	181	0	181	473	1.61
(30)	181	0.2 x10^9	180	497	1.75*
3[89]	170	0	143	392	1.47
(20)	173	0.2 x10^9	144	410	1.54
4A[90]	512	0	166	624	0.90
(60)	487	0.2 x10^9	175*	636	0.95
4B[49]	490	0	176	598	0.86
(60)	525	0.2 x10^9	177	647*	0.90
4C[49]	473	0	194	590	0.84
(50)	470	0.2 x10^9	183*	604	0.90*
4[49]	-	0	178	603	0.87
Pooled data	-	0.2 x10^9	178	630*	0.91*

[a]Roughage was not considered in the estimation of the intended dose.
*Significantly different from the control by at least P<0.05.

In all trials, animals in the Toyocerin® supplemented group reached a higher final body weight and had a greater average daily gain (ADG) than those in the non-supplemented group. This reached significance (P<0.05) for final weight in two of the individual trials and for the pooled results of trial 4. Increases in ADG were significant at P<0.05 for three individual trials and for the pooled results of trial 4 and reached P<0.1 in a further trial (trial 3). Carcass weight was also significantly increased in trial 1 (the only trial where this parameter was measured) but dressing percentage was unaffected.

4.4. Rabbits for fattening

Three trials with rabbits for fattening are described. In the first trial five consecutive fattening cycles, each of 28 days, were studied. In each cycle, kits (Prat line) were allocated to one of four experimental groups, one acting as a control and the remaining as treatment groups receiving various concentrations of the additive (see Table 10). The other two trials involved a single fattening cycle of 35 days duration. In these trials a control group was similarly compared with groups receiving Toyocerin®. Breeds used were a Hyla hybrid strain (trial 2) and Hyplus (trial 3). In all three trials feed was provided in pelleted form with or without the additive. Intended doses were confirmed by analysis of the feed. Observations made were initial and final weights and feed intake. From these data average daily gain, average daily feed intake and feed to gain ratio were calculated. Data were subjected to an analysis of variance. Finally, morbidity and mortality were monitored.

[87] Technical dossier FAD-2010-0090/Section IV/Annex IV.21.
[88] Technical dossier FAD-2010-0090/Section IV/Annex IV.22.
[89] Technical dossier FAD-2010-0090/Section IV/Annex IV.23.
[90] Technical dossier FAD-2010-0090/Section IV/Annex IV.24.

Table 10: Summary of data on the effects of Toyocerin® in rabbits for fattening

Trial No (Total number of kits)	Replicates per treatment x kits per replicate	Dose (CFU/kg feed)	Final weight (kg)	Average daily gain (g)	Average daily feed intake (g)	Feed to gain ratio (kg/kg)
		0	1.83	39.9	97.9	2.46
1[91]	18 x (6 – 9)	0.2 x 10^9	1.84	40.3	96.6	2.39*
(2271 – five cycles)	each cycle	0.5 x 10^9	1.85	40.6*	98.4	2.42*
		1.0 x 10^9	1.87*	41.1*	97.6	2.37*
2[92]		0	2.43	40.6	129	3.19
(216)	36 x 2	0.2 x 10^9	2.54*	43.6*	132	3.02*
		1.0 x 10^9	2.49*	41.9	130	3.13
3[93]		0	2.32	37.9	-	-
(1212)	68 x (5 - 6)	0.5 x 10^9	2.34	38.6	-	-
		1.0 x 10^9	2.36*	39.1*	-	-

*Significantly different from the control by at least $P<0.05$.

In the first trial no significant results were seen within cycles but the pooled data showed significant gains in terms of final body weight and average daily gain with the two highest doses. This, apparently, was a result of improved nutrient uptake/utilisation as there was no significant increase in feed intake. Similar significant responses were seen in the other two trials. A dose of 1.0 x 10^9 CFU/kg feed can be considered the minimum effective dose since it was the only dose tested in all three trials and as at least one beneficial response was seen at this dose in all trials.

4.5. Compatibility with coccidiostats

Toyocerin® is currently authorised for use in feeds for rabbits containing robenidine or salinomycin, and in feeds for chickens for fattening containing monensin sodium, lasalocid sodium, salinomycin sodium, decoquinate, robenidine, narasin, halofuginone, diclazuril, narasin/nicarbazin, or maduramycin ammonium. However, EFSA only established compatibility with diclazuril, narasin/nicarbazin and maduramycin in feed for chickens for fattening in its opinion of 2005. Subsequent to this Opinion, the FEEDAP Panel revised its guidance to take account of the mounting evidence that spores of bacilli were capable of germination and that the more sensitive vegetative state could persist (EFSA, 2008c). As a consequence, the older studies with monensin sodium, lasalocid sodium, salinomycin sodium, decoquinate, robenidine, narasin and halofuginone in feed for chickens not previously considered by EFSA but included in this application and the study considered in 2005 do not meet current requirements for the inclusion of cells in a vegetative state. No data on compatibility with coccidiostats at the maximum concentrations authorised for rabbits are presented.

[91] Technical dossier FAD-2010-0090/Section IV/Annex IV.29.
[92] Technical dossier FAD-2010-0090/Section IV/Annex IV.30.
[93] Technical dossier FAD-2010-0090/Section IV/Annex IV.31.

4.6. Conclusions

The addition of Toyocerin® to the feed of the following species/categories of animals has the potential to improve at least one aspect of production:

chickens for fattening at a minimum dose of 0.2 x 10^9 CFU/kg feed;

pigs for fattening at a dose of 0.5 x 10^9 CFU/kg feed for the first (grower) period followed by at a minimum dose of 0.2 x 10^9 CFU/kg feed for the second (finisher) period;

sows at a minimum dose of 0.5 x 10^9 CFU/kg feed for the complete cycle:

calves for rearing at a minimum dose of 0.5 x 10^9 CFU/kg feed;

cattle for fattening at a minimum dose of 0.2 x 10^9 CFU/kg feed and

rabbits for fattening at a minimum dose of 1.0 x 10^9 CFU/kg feed.

In the view of the Panel, insufficient data were available to conclude on the efficacy of Toyocerin® when used in diets for weaned piglets.

5. 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[94] and Good Manufacturing Practice.

CONCLUSIONS

Although some of the tolerance studies submitted by the Applicant would be considered inadequate by present days standards, there are individual studies which provide adequate assurance that the additive has no direct ill effects on the target species at the recommended dose range. Given that no adverse effects were recorded in any of the remaining studies, the FEEDAP Panel concludes that the additive is well tolerated by the target species that are the subject of this request for authorisation/re-evaluation. However, the Panel notes that the strain of *Bacillus cereus* harbours resistance determinants to two antibiotics, one of which at least can now be ascribed to an acquired resistance. For this reason the FEEDAP Panel considers it inadvisable to introduce into target species a resistance determinant capable of transfer to other bacterial strains and adding to the pool of such determinants in the guts of livestock species.

Analysis of the complete genome sequence showed that the strain of *B. cereus* in Toyocerin® harbours all of the genes coding for the non-haemolytic and haemolytic enterotoxins. Since the two operons present the same organisation as pathogenic *B. cereus* strains and since no mutation affecting transcription or translation has been detected, it has to be assumed that the Toyocerin® strain has the capacity to elaborate functional toxins and, thus, to pose a hazard for those exposed to the organism. This would include those handling the additive and consumers inadvertently exposed to contaminated animal products.

The additive is non-irritant to eyes, and by extension, to the skin. However, given its proteinaceous nature, it should be treated as a skin and respiratory sensitiser.

[94] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

B. cereus is a ubiquitous soil saprophyte with a worldwide distribution. Consequently, use of the Toyocerin® strain in animal nutrition is not expected to measurably increase numbers of the organism in the environment.

The addition of Toyocerin® to the feed has the potential to improve at least one aspect of production in chickens for fattening at a minimum dose of 0.2 x 10^9 CFU/kg feed; pigs for fattening at a dose of 0.5 x 10^9 CFU/kg feed for the first (grower) period followed by at a minimum dose of 0.2 x 10^9 CFU/kg feed for the second (finisher) period; sows at a minimum dose of 0.5 x 10^9 CFU/kg feed for the complete cycle: calves for rearing at a minimum dose of 0.5 x 10^9 CFU/kg feed; cattle for fattening at a minimum dose of 0.2 x 10^9 CFU/kg feed and rabbits for fattening at a minimum dose of 1.0 x 10^9 CFU/kg feed. In the view of the Panel, insufficient data was available to conclude on the efficacy of Toyocerin® when used in diets for weaned piglets. Based on the current data, the FEEDAP Panel is unable to conclude on the compatibility of Toyocerin® with the listed coccidiostats when added to poultry or rabbit feed.

DOCUMENTATION PROVIDED TO EFSA

1. Toyocerin® (*Bacillus cereus* var. *toyoi* NCIMB 40112/CNCM I-1012) for sows, piglets, pigs for fattening, cattle for fattening, chickens for fattening and rabbits for fattening. August 2010. Submitted by Rubinum S.A.

2. Toyocerin® (*Bacillus cereus* var. *toyoi*) for calves for rearing. August 2010. Submitted by Rubinum S.A.

3. Toyocerin® (*Bacillus cereus* var. *toyoi* NCIMB 40112/CNCM I-1012) for sows, piglets, pigs for fattening, cattle for fattening, chickens for fattening and rabbits for fattening. Supplementary information. March 2012. Submitted by Rubinum S.A

4. Toyocerin® (*Bacillus cereus* var. *toyoi*) for calves for rearing. Supplementary information. February 2012. Submitted by Rubinum S.A.

5. Letters from the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Toyocerin®.

6. Comments from Member States received through the ScienceNet.

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EFSA (European Food Safety Authority), 2007a. Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the safety and efficacy of the product Toyocerin® (*Bacillus cereus* var. *toyoi*) as a feed additive for sows from service to weaning, in accordance with Regulation (EC) No 1831/2003. The EFSA Journal, 458, 1-9.

EFSA (European Food Safety Authority), 2007b. Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the safety and efficacy of the product Toyocerin® (*Bacillus cereus* var. *toyoi*) as a feed additive for turkeys. The EFSA Journal, 549, 1-11.

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