SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 5 May 2021

doi: 10.2903/j.efsa.2021.6619

Safety and efficacy of a feed additive consisting of disodium 5′-guanylate produced with *Corynebacterium stationis* KCCM 10530 and *Escherichia coli* K-12 KFCC 11067 for all animal species (CJ Europe GmbH)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of disodium 5′-guanylate produced by fermentation with *Corynebacterium stationis* KCCM 10530 and *Escherichia coli* K-12 KFCC 11067 when used as a sensory additive (flavouring compound) in feed and water for drinking for all animal species. The additive does not raise safety concerns under the proposed conditions of use for the target species, consumers the users and the environment. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) expressed reservations on the use of the additive in water for drinking due to concerns on its impact on hygienic conditions of the water. The Panel concluded that the additive is efficacious to contribute to the flavour of feed.

Keywords: Sensory, flavouring compounds, disodium 5′-guanylate, safety, efficacy, all animal species

Requestor: European Commission

Question number: EFSA-Q-2020-00267

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

Acknowledgments: The Panel wishes to thank the following for the support provided to this scientific output: The Working Group on Animal Nutrition, the Working Group on Microbiology and the Working Group on Toxicology.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Fašmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Dierick N, Martelli G, Galobart J, Manini P, Pettenati E, Tarrés-Call J and Anguita M, 2021. Scientific Opinion on the safety and efficacy of a feed additive consisting of disodium 5'-guanylate produced with *Corynebacterium stationis* KCCM 10530 and *Escherichia coli* K-12 KFCC 11067 for all animal species (CJ Europe GmbH). EFSA Journal 2021;19(6):6619, 15 pp. https://doi.org/10.2903/j.efsa.2021.6619

ISSN: 1831-4732

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from CJ Europe GmbH[2] for authorisation of disodium 5′-guanylate produced with *Corynebacterium stationis* KCCM 10530 and *Escherichia coli* K-12 KFCC 11067, when used as a feed additive for all animal species (category: sensory additives; functional group: flavouring compounds).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 17 June 2020.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product disodium 5′-guanylate produced by *Corynebacterium stationis* KCCM 10530 and *Escherichia coli* K-12 KFCC 11067, when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The product under assessment is based on disodium 5′-guanylate produced by fermentation using *C. stationis* KCCM 10530 and *E. coli* K-12 KFCC 11067. This product is not authorised as a feed additive in the European Union.

The European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has issued an opinion on the safety and efficacy of disodium 5′-ribonucleotides, disodium 5′-guanylate, disodium 5′-inosinate for all animal species (EFSA FEEDAP Panel, 2014). Disodium 5′-guanylate produced by RNA hydrolysis is currently authorised as a sensory additive for use in all animal species in accordance with Regulation (EU) 2018/238[3].

Disodium 5′-guanylate has been evaluated by the Scientific Committee for Food (SCF; European Commission, 1991) and by the Joint WHO/FAO Expert Committee on Food (JECFA; WHO, 1974, 1993) and is currently authorised as a food additive (E 627) ('additives other than colours and sweeteners', 'group I-with a maximum of 500 mg/kg', 'other additives that may be regulated combined').[4]

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[5] in support of the authorisation request for the use of disodium 5′-guanylate as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] CJ Europe GmbH, Unterschweinstiege 2-14, 60549, Frankfurt am Main, Germany.
[3] Commission Implementing Regulation (EU) 2018/238 of 15 February 2018 concerning the authorisation of disodium 5′-ribonucleotides, disodium 5′-guanylate and disodium 5′-inosinate as feed additives for all animal species. OJ L 53, 23.2.2018, p. 1.
[4] Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives Text with EEA relevance OJ L 295, 12.11.2011, p. 1.
[5] FEED dossier reference: FAD-2019-0085.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the disodium 5′-guanylate in animal feed. The Executive Summary of the EURL report can be found in Annex A.[6]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of disodium 5′-guanylate produced by fermentation using *C. stationis* KCCM 10530 and *E. coli* K-12 KFCC 11067 is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

This opinion assesses the safety and efficacy of disodium 5′-guanylate (hydrated form) produced with two non-genetically modified strains *C. stationis* KCCM 10530 and *E. coli* K-12 KFCC 11067 when used as a sensory additive (functional group: flavouring compounds) in feed and water for drinking for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the production strains

The salt disodium 5′-guanylate under assessment is produced from 5′-guanylic acid (synonym: guanosine monophosphate (GMP)) by the combined action of two non-genetically modified strains, *C. stationis* KCCM 10530 and *E. coli* KFCC 11067.

3.1.1.1. *Corynebacterium stationis* KCCM 10530

[redacted] non-genetically modified strain of *C. stationis* which is deposited at the Korean Culture Center of Microorganisms (KCCM) with the accession number KCCM 10530.[8]

[redacted] The taxonomic identification of KCCM 10530 as *C. stationis* [redacted][11]

The susceptibility of the production strain to antibiotics was tested [redacted][12] All the minimum inhibitory concentration (MIC) values were equal or lower than the cut-off values established for *Corynebacterium* in the referred Guidance. [redacted]

[6] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2019-0085-gmp.pdf
[7] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
[8] Technical dossier/Section II/Confidential Annex/Annex CONFID II.2.01, deposited as *C. ammoniagenes.*
[9] Technical dossier/Section II/Ref II.2.02.
[10] Technical dossier/Supplementary information October 2020/General answer.
[11] Technical dossier/Section II/Confidential Annex/Annex CONFID II.2.03.
[12] Technical dossier/Section II and Confidential Annex/Annex CONFID II.2.06 and II.2.07.

The WGS of the production strain [redacted] were interrogated for the presence of antimicrobial resistance [redacted].[13] No relevant hits were identified.

The WGS of the production strain was also interrogated for the presence of toxin and virulence factor genes [redacted] No relevant hits were identified.[16]

3.1.1.2. *Escherichia coli* K-12 KFCC 11067

[redacted] non-genetically modified strain of *Escherichia coli* which is deposited at the KCCM under the accession number KFCC 11067.[17] The production strain KFCC 11067 [redacted]

The taxonomic identification of the production strain as *E. coli* was done [redacted].[18]

[redacted].[19]

The susceptibility of the production strain to the eight antibiotics recommended for Enterobacteriaceae in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms was tested [redacted] (EFSA FEEDAP Panel, 2018b).[20] All the MIC values were equal or lower than the cut-off values established for Enterobacteriaceae in the referred guidance. [redacted]

The WGS of the production strain [redacted] were interrogated for the presence of AMR genes [redacted].[13] No relevant hits were identified in the genome of the production strain.

3.1.2. Manufacturing process[21]

[redacted]

The applicant declares that no antibiotics are used during the manufacturing process.[22]

3.1.3. Characterisation of the additive

The additive is the hydrated form of disodium-GMP (International Union of Pure and Applied Chemistry (IUPAC) name: disodium [(2R,3S,4R,5R)-5-(2-amino-6-oxo-1H-purin-9yl)-3,4-dihydroxyoxolan-2-yl] methyl phosphate (synonyms: guanosine-5'-monophosphate disodium salt; 5'-GMP 2Na; 5'-guanylic acid disodium salt), a compound identified with the Chemical Abstracts Service (CAS) No 5550-12-9 and the

[13] Technical dossier/Section II.

[redacted]

[16] Technical dossier/Supplementary information October 2020/Confidential/Annex CONFID_01.
[17] Technical dossier/Section II/Confidential Annex/Annex CONFID II.2.02.
[18] Technical dossier/Section II and Confidential Annex/Annex CONFID II.2.04.
[19] Technical dossier/Section II/Confidential Annex/Annex CONFID II.2.07 and CONFID II.2.09.
[20] Technical dossier/Section II/Confidential Annex/Annex CONFID II.2.06.
[21] Technical dossier/Section II and Supplementary information October 2020 and Supplementary information March 2021.
[22] Technical dossier/Section II/Confidential Annex/Annex CONFID II.3.37.

European Inventory of Existing Commercial chemical Substances (EINECS) No 226-914-1). The molecular weight of the anhydrous form is 407.19 g/mol and the molecular formula is $C_{10}H_{12}N_5Na_2O_8P$. The molecular structure of the anhydrous form is given in Figure 1.

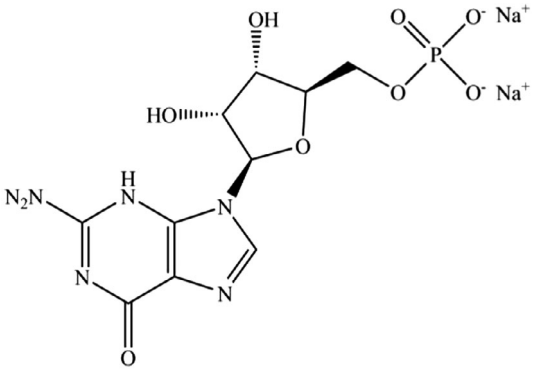

Figure 1: Molecular structure of disodium 5'-guanylate (anhydrous form)

The additive contains by specification ≥ 97% disodium-GMP (on dry matter basis) and ≤ 25% water (corresponding to the hydrated form of disodium-GMP, similar to heptahydrate).[23] The analysis of five batches showed an average water content of 19.5% (range 19.1–19.9%, and an average value of disodium-GMP 98.7% on dry matter basis (range 98.2–99.1%), measured by UV spectroscopy.[24] The applicant provided, upon request, the analysis of the disodium-GMP in the same batches by HPLC coupled with UV detector and the results showed a mean value of 101% of disodium-GMP on dry matter basis (ranging from 100.9% to 101.9%) and the content of water at the time of analysis was 20.1%. (19.9–20.4%).[25]

In another analytical report[26] the applicant analysed five additional batches for disodium-GMP (average 78.46% (range 78.3–79.1%) on as is basis); water (21.1%); nitrogen containing components (ammonium, nitrates and nitrites, not detected); free amino acids (not detected); nucleoside, nucleotide and base (not detected); organic acids (formic, acetic, citric, malic, succinic, lactic, not detected) and some other elements (not detected, except for sodium 9.2% (9.15–9.21%) on 'as is' basis).

Three batches of the additive were analysed for chemical and microbiological impurities. Heavy metals (lead, cadmium and mercury) and arsenic were below the limits of quantification (LOQs).[27] Aflatoxins (B1, B2, G1, G2), ochratoxin A, zearalenone and deoxynivalenol were below the corresponding LOQs.[28] Dioxins (polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F)), dioxin-like polychlorinated biphenyls (DL-PCBs) and non-dioxin-like PCBs were analysed and were below the corresponding LOQ. The following calculated values were reported for dioxins: 0.07 ng WHO-PCDD/F-TEQ/kg; for the sum of dioxins and DL-PCBs: 0.14 ng WHO-PCDD/F-DL-PCB-TEQ/kg; and non-dioxin-like PCBs: 0.6 μg/kg. *Salmonella* spp. was not detected in 25 g, yeasts < 100 colony forming units (CFU)/g, moulds < 100 CFU/g, Enterobacteriaceae < 10 CFU/g and *Escherichia coli* < 10 CFU/g.

Three batches of the product were tested [redacted] for the presence of viable cells of the production strains [redacted] Viable cells were not detected in the product.

[23] Technical dossier/Section II/Annex II.1.01.
[24] Technical dossier/Section II/Annex II.1.02.
[25] Technical dossier/Supplementary information March 2021/Annex SIN.01.
[26] Technical dossier(Section II/Annex II.1.03. Limit of detection for ammonium, nitrates, nitrites and elements (sodium, magnesium, calcium, fluoride, bromide, chloride, phosphate and sulphate) was 0.01 mg/kg, free amino acids 0.5 mg/kg, for nucleoside, nucleotide, bases and organic acids 1 mg/kg.
[27] Technical dossier/Section II/Annex II.1.04. Limit of quantification in mg/kg were 0.015 for lead, 0.01 for cadmium, 0.01 for mercury and 0.04 for arsenic.
[28] Technical dossier/Section II/Annex II.1.04. Limit of quantification in μg/kg was 0.2 for aflatoxins (B1, B2, G1 and G2), 0.5 for ochratoxin A, 10 for zearalenone and 50 for deoxynivalenol.
[29] Teechnical dossier/Section II/Confidential Annex/CONFID_II.2.05.

The content of lipopolysaccharides from the *E. coli* was measured in three batches using the method of analysis EP2.6.15.[30] The results showed all batches were below the limit of detection of 0.5 EU/g.

The additive is a white crystalline powder with a solubility in water of 50 g/L and a bulk density of 500–750 kg/m^3. The dusting potential of the additive measured in three batches following the Stauber–Heubach method gave results ranging from 2.1 to 3.1 g/m^3.[31] The particle size distribution was measured by sieving method, particles below 105 and 44 μm diameter were 78 and 27.5% (w/w), respectively.[32]

3.1.4. Stability and homogeneity

The shelf-life of the additive was determined by monitoring three batches stored in bags corresponding to the commercial packaging at 25°C for 36 months.[33] No losses of GMP were observed up to 36 months.

The stability of the additive (three batches) in a vitamin and mineral premixture for chickens for fattening (without choline chloride) was studied when added at 5% and stored at 25°C, 60% relative humidity (RH) for 6 months. At the end of 6 months, no losses were observed in the content of GMP in the premixture.[34]

The stability of the additive (three batches) was evaluated when added at 0.4% to a mash[35] and pelleted[36] feed for chickens for fattening (details on the feed not given) after storage at 25°C, 60% RH for 3 months. Pelleting temperature was 72°C. The pelleting did not affect the content of the active substance. At the end of the 3 months, no losses of GMP were observed in mash and pelleted feed.

The stability of the additive in water was studied by suspending 1.0 or 0.025 g of the additive in 1 L of water and samples stored at 25 or 40°C for up to 48 h.[37] No losses were observed in GMP content after 48 h at 25 or 40°C at either concentration.

No data were provided to demonstrate the capacity of the additive to homogeneously distribute in feed.

3.1.5. Conditions of use

Disodium-GMP is intended to be used in feed or water for drinking in all animal species as a flavouring compound. The applicant proposes a maximum use level of 50 mg additive/kg complete feed. For its use in water the applicant proposed that the levels of the additive should be two to three times lower for poultry, porcine species and rabbits, for other species or when used in feed and water concomitantly the daily amount that would be consumed via feed should not be exceeded.[38] If used in feed and water concomitantly the amount should not exceed the daily intake that would result from the addition in feed at 50 mg/kg.

3.2. Safety

Safety concerns from the additive may derive either from the active substance or from the residues of the fermentation process/production strains remaining in the final product. The production strains *C. stationis* KCCM 10530 and *E. coli* K-12 KFCC 11067 are not genetically modified and did not show acquired resistance to antimicrobials. Based on the WGS data provided, *C. stationis* KCCM 10530 is not expected to produce any toxic compound during fermentation. *E. coli* K-12 KFCC 11067 is a derivative of *E. coli* K-12 which is well-characterised and its safety (non-pathogenicity) has been documented (Gorbach, 1978). The strain has been shown to be ineffective in colonising the human gut (Smith, 1975) and its genome (MG1655 and W3110) has been fully sequenced (Hayashi et al., 2006). In addition, no viable cells of the production strains were detected in the final product. No safety

[30] Technical dossier/Supplementary information October 2020/Annex SIN.04.
[31] Technical dossier/Section II/Annex II.1.06.
[32] Technical dossier/Section II/Annex II.1.05.
[33] Technical dossier/Section II/Annex II.4.01.
[34] Technical dossier/Section II/Annex II.4.04.
[35] Technical dossier/Section II/Annex II.4.02.
[36] Tchnical dossier/Section II/Annex II.4.03.
[37] Technical dossier/Section II/Annex II.4.05 and supplementary information October 2020/Annex SIN-05.
[38] Calculated values would equate (in mg/day) to 100 for veal calf, 454 for cattle for fattening, 1,136 for dairy cows, 68 for sheep/goat, 454 for horse, 14.2 for dog and 3.4 for cats.

concerns deriving from the production strains used to produce the product are expected for the target species, consumers and the environment.

Regarding the safety for the target species, GMP is widely distributed in all tissues of animals and plants. Its role in purine metabolism as well as its breakdown to uric acid and to allantoin (in mammals except for primates), is well known. In respect to the recommended levels of use disodium-GMP in feed (50 mg/kg feed), the FEEDAP Panel notes that the GMP and 5'-inosinate content of plant-derived feeding materials has been reported as being in the range 1–10 mg/kg (Mateo and Stein, 2004). Higher levels of total nucleotides have been measured in some feed ingredients (i.e. 38 mg/kg in soybean meal, 75 mg/kg in fish meal and 294 mg/kg in dried whey (Mateo and Stein, 2004). Particularly, in milk from sows (first week of lactation) the levels of GMP are approximately 51 mg/L (Mateo and Stein, 2004). The FEEDAP Panel reiterates its conclusion that the disodium-5'-ribonucleotides, per se, at the proposed use level of 50 mg/kg feed as of no concern for the target species (EFSA FEEDAP Panel, 2014).

Considering all the above, no concerns for the target animals would arise from the supplementation of the diets with disodium-GMP produced with *C. stationis* KCCM 10530 and *E. coli* K-12 KFCC 11067 at 50 mg/kg complete feed or when used in combination with other ribonucleotides up to the same level. The applicant established conditions of use in water, as well as for concomitant use in feed and water, that would mirror the intakes resulting from the supplementation in feed, which are considered safe. However, the FEEDAP Panel has reservations on the use of the additive via water due to hygienic reasons (EFSA FEEDAP Panel, 2010).

Regarding the safety for consumers, GMP is metabolised and excreted efficiently by the target animals. It is not expected that the composition of tissues and products of animal origin will be affected by the use of disodium-GMP as a feed additive, at the proposed conditions of use. The FEEDAP Panel also notes that the same substance is authorised as an additive in food at levels up to 500 mg/kg.

GMP is naturally present in tissues of animals and plants. The use of disodium-GMP as a feed additive at the levels proposed is not expected to increase its concentration in the environment and, therefore, it is of no safety concern for the environment.

Overall, the FEEDAP Panel concludes that disodium-GMP produced with *C. stationis* KCCM 10530 and *E. coli* K-12 KFCC 11067 is safe under the proposed conditions of use for the target species, for the consumer and for the environment. However, the Panel has reservations on the use of the additive in water for drinking of the target animals due to concerns on its impact on the hygienic conditions of the water.

3.2.1. Safety for the user

3.2.1.1. Effects on the respiratory system

The additive has a dusting potential up to 3.1 g/m^3 and the particle size distribution shows a proportion of particles having diameters below 105 μm of up to 78% with particles below 44 μm being 28%. Thus, the users/workers can be exposed by inhalation.

A valid acute inhalation test in laboratory animals, performed according to the Organisation for Economic Co-operation and Development (OECD) Guideline 403, showed an LC_{50} greater than 4.28 mg/L in male and female rats.[39]

The bacterial endotoxin activity (analysed in three batches) was below the limit of detection of 0.5 IU/g. The scenario used to estimate the exposure of persons handling the additive to endotoxins in the dust, based on the EFSA guidance on user safety (EFSA FEEDAP Panel, 2012), is described in Appendix A. The threshold for the quantity of inhaled endotoxins per working day is 900 IU, derived from the provisional occupational exposure limits given by the Dutch Expert Committee on Occupational Safety (Health Council of the Netherlands, 2010) and the UK Health and Safety Executive (HSE, 2013). Based on calculations of the content of endotoxins in dust considering an endotoxin activity of 0.5 IU/g (using the limit of detection of the method of analysis), the estimated exposure would be 0.86 IU per 8-h working day, indicating no risk by inhalation due to exposure to endotoxins for people handling the additive.

[39] Technical dossier/Section III/Annex III.3.03 and Supplementary information October 2020/Annex 07 and 11.

3.2.1.2. Effect on eyes and skin

The skin irritation potential of the additive was tested in a study performed according to OECD guideline 439.[40] The results of the study indicate that the additive should not be considered as a skin irritant.

The eye irritation potential of the additive was tested in a valid study performed according to OECD guideline 437.[41] The results of the study indicate that the additive should not be considered as an eye irritant.

In a valid dermal sensitisation study following OECD guideline 429 (local lymph-node assay) and Method B42 Skin Sensitization (local lymph node assay) of Commission Regulation (EC) No 440/2008, the additive did not show any skin sensitisation potential.[42]

3.2.1.3. Conclusions on safety for the user

Based on the data/studies provided, the Panel concludes that the additive is considered safe for the users.

3.3. Efficacy

GMP is mentioned in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009), by the Flavour and Extract Manufactures Association (FEMA) as a flavour enhancer (FEMA 3668), i.e. a substance with no specific taste on its own but which has an ability to enhance existing flavours. Further, disodium-GMP is authorised under Commission Regulation (EU) No 1129/2011 on food additives.

The FEEDAP Panel considers that the effect of disodium-GMP to increase the taste of food is well documented and, therefore, no further demonstration of efficacy when used in feed or water for drinking is necessary.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[43] and Good Manufacturing Practice.

4. Conclusions

The additive, disodium-GMP in its hydrated form produced with *C. stationis* KCCM 10530 and *E.coli* K-12 KFCC 11067, is safe for the target species at 50 mg/kg complete feed or when used in combination with other ribonucleotides up to the same level. The applicant established conditions of use in water that would mirror the intakes resulting from the supplementation in feed; however, the FEEDAP Panel has reservations on the use of the additive via water due to hygienic reasons.

The use of the additive is also considered safe for the consumers, users and the environment.

The additive is efficacious to contribute to the flavour of feed and water for drinking.

5. Documentation provided to EFSA/Chronology

Date	Event
12/12/2019	Dossier received by EFSA. Disodium 5'-guanylate produced by *Corynebacterium ammoniagenes* KCCM 10530 and *Escherichia coli* K-12 KFCC 11067 for all animal species. Submitted by CJ Europe GmbH
01/04/2020	Reception mandate from the European Commission
17/06/2020	Application validated by EFSA – Start of the scientific assessment
24/07/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for the target species and safety for the user*

[40] Technical dossier/Section III/Annex III.3.05 and Supplementary information October 2020/Annex 08 and 11.
[41] Technical dossier/Section III/Annex III.3.04 and Supplementary information October 2020/Annex 09 and 11 and Supplementary information Annex SIN_03.
[42] Technical dossier/Section III/Annex III.3.06 and Supplementary information October 2020/Annex 10 and 11.
[43] 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.

Date	Event
21/10/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
04/09/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
08/01/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Characterisation*
02/03/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
05/05/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

CAS	Chemical Abstracts Service
DL-PCBs	dioxin-like polychlorinated biphenyl
EINECS	European Inventory of Existing Commercial chemical Substances
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavour and Extract Manufactures Association
GMP	guanosine monophosphate
IUPAC	International Union of Pure and Applied Chemistry
KCCM	Korean Culture Center of Microorganisms
LC_{50}	lethal concentration, median
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
PCDD/F	polychlorinated dibenzo-*p*-dioxins and dibenzofurans
RH	relative humidity
WGS	whole genome sequence
WHO	World Health Organization
XMP	Xanthosine monophosphate

Appendix A – Safety for the user

The effects of endotoxin inhalation and the exposure limits have been described in a previous opinion (EFSA FEEDAP Panel, 2015).

Calculation of maximum acceptable levels of exposure from feed additives

The probable exposure time according to EFSA guidance (EFSA FEEDAP Panel, 2012) for additives added in premixtures assumes a maximum of 40 periods of exposure per day, each comprising 20 s = 40 × 20 = 800 s/day. With an uncertainty factor of 2, maximum inhalation exposure would occur for 2 × 800 = 1,600 s = 0.444 hday. Again, assuming a respiration volume of 1.25 m^3/h, the inhalation volume providing exposure to potentially endotoxin-containing dust would be 0.444 × 1.25 = 0.556 m^3/day. This volume should contain no more than 900 IU endotoxin, so the dust formed from the product should contain no more than 900/0.556 = 1,619 IU/m^3.

Calculation of endotoxin content of dust

Two key measurements are required to evaluate the potential respiratory hazard associated with the endotoxin content of the product (the dusting potential of the product, expressed in g/m3, and the endotoxin activity of the dust, determined by the Limulus amoebocyte lysate assay (expressed in IU/g)). If data for the dust are not available, the content of endotoxins of the product can be taken instead. If the content of endotoxins of the relevant additive is a IU/g and the dusting potential is b g/m^3, then the content of endotoxins of the dust, c IU/m^3, is obtained by simple multiplication, a × b. This resulting value is further used for calculation of the potential inhalatory exposure of users to endotoxins from the additive under assessment (Table A.1) (EFSA FEEDAP Panel, 2012).

The content of endotoxins in the product was found to be below the limit of detection of 0.5 IU/g, this value was considered for the calculation described in Table A.1.

Table A.1: Estimation of user exposure to endotoxins from the additive disodium-GMP produced with *Corynebacterium stationis* KKCCM 10530 *and Escherichia coli K-12* KFCC 11067, including consideration of using a filter mask FFP2 or FFP3 as a preventative measure

Calculation	Identifier	Description	Amount	Source
	a	Endotoxin content IU/g product	0.5	Technical dossier
	b	Dusting potential (g/m^3)	3.1	Technical dossier
a × *b*	*c*	Endotoxin content in the air (IU/m^3)	1.55	
	d	No of premixture batches made/ working day	40	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
	e	Time of exposure (s) per production of one batch	20	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
d × *e*	*f*	Total duration of daily exposure/ worker (s)	800	
	g	Uncertainty factor	2	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
f × *g*	*h*	Refined total duration of daily exposure/worker (s)	1,600	
h/3,600	*i*	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m^3) per eight-h working day	10	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
j/8 × *i*	*k*	Inhaled air during exposure (m^3)	0.56	
c × *k*	*l*	**Endotoxin inhaled (IU) during exposure per eight-h working day**	**0.86**	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3) per eight-h working day	90	Health Council of the Netherlands, 2010

Calculation	Identifier	Description	Amount	Source
$m \times j$	n	**Health-based recommended exposure limit of total endotoxin exposure (IU) per eight-h working day**	**900**	
$l/10$		Endotoxins inhaled (IU) per eight-h working day reduced by filter mask FF P2 (reduction factor 10)	–	
$l/20$		Endotoxins inhaled (IU) per eight-h working day reduced by filter mask FF P3 (reduction factor 20)	–	

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Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for Disodium 5'-guanylate (GMP) produced by fermentation with *Corynebacterium ammoniagenes*[44] KCCM 10530 and *Escherichia coli* K-12 KFCC 11067

In the current application an authorisation is sought under Article 4(1) for disodium 5'-guanylate (GMP) produced by fermentation with Corynebacterium ammoniagenes KCCM 10530 and Escherichia coli K12 KFCC 11067 under the category/functional group 2(b) 'Sensory additives'/'flavouring compounds' according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the feed additive for all animal species and categories.

The feed additive consists of a minimum of 97% (w/w) of disodium 5'-guanylate (GMP) as an active substance, which is produced by fermentation with the strains of Corynebacterium ammoniagenes KCCM 10530 and Escherichia coli K12 KFCC 11067.

The feed additive is intended to be used directly into feedingstuffs or through premixtures and in water for drinking with proposed maximum levels of 50 mg GMP/kg feedingstuffs.

For the identification of GMP in the feed additive, the Applicant proposed the internationally recognised FAO JECFA monograph "disodium 5'-guanylate", which is comprised of various fit-for-purpose tests based on measuring solubility, absorbance signals in spectrophotometric measurements, presence of sodium, ribose and organic phosphate.

The EURL recommends for official control the above mentioned FAO JECFA monograph for the identification of GMP in the feed additive.

For the determination of GMP in the feed additive, flavouring premixtures and water the Applicant submitted a single-laboratory validated method based on high performance liquid chromatography coupled to UV detection (HPLC-UV).

The following performance characteristics were reported in frame of the validation study for the determination of GMP content in the aqueous solution of the premixtures ranging from 7.8 to 182 mg/L: a relative standard deviation for repeatability (RSDr) ranging from 0.3 to 0.6%; and a recovery rate (Rrec) of 100%. The lowest tested level of GMP in water (7.8 mg/L) was assigned by the Applicant as a limit of quantification (LOQ). In addition, the Applicant demonstrated proper selectivity of the method by submitting the chromatograms of flavouring premixtures, containing GMP and disodium 5'-inosinate.

Based on the available performance characteristics, the EURL recommends for official control the single-laboratory validated HPLC-UV method submitted by the Applicant for the determination of disodium 5'-guanylate (GMP) in the feed additive, flavouring premixtures and water.

The Applicant did not provide any method for the determination of *GMP* in *feedingstuffs*, therefore the EURL could not evaluate nor recommend any method for official control to determine *GMP* in *feedingstuffs*. Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

[44] Formerly taxonomically classified as Corynebacterium ammoniagenes and classified afterwards as Corynebacterium stationis.