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Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Disodium 5'-inosinate (IMP) produced by fermentation with *Corynebacterium ammoniagenes KCCM80161*

(FAD-2018-0094; CRL/180069)

European Union Reference Laboratory
Feed Additives

Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2018-0094 - CRL/180069**
Name of Product:	***Disodium 5'-inosinate (IMP)*** **produced by fermentation with** ***Corynebacterium ammoniagenes KCCM80161***
Active Agent (s):	**Disodium 5'-inosinate**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**Stefano Bellorini** **24/09/2019**
Report approved by: Date:	**Christoph von Holst** **24/09/2019**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *disodium 5'-inosinate (IMP)* produced by fermentation with *Corynebacterium ammoniagenes KCCM80161* 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, 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 % of *disodium 5'-inosinate (IMP)* as an active substance, which is produced by fermentation with a strain of *Corynebacterium ammoniagenes KCCM80161*.

The *feed additive* is intended to be used directly into *feedingstuffs* or through *premixtures* and in *water* for drinking with proposed maximum levels of 25 mg *IMP*/kg *feedingstuffs*.

For the identification of *IMP* in the *feed additive*, the Applicant proposed the internationally recognised FAO JECFA monograph "disodium 5'-inosinate", which is comprised of various fit-for-purpose tests that are 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 tests of the FAO JECFA monograph for the identification of *IMP* in the *feed additive*.

For the determination of *IMP* in raw materials, *flavouring premixtures* and *water* the Applicant submitted the method based on high performance liquid chromatography coupled to UV detection (HPLC-UV).

Based on the available performance characteristics, the EURL recommends the HPLC-UV method submitted by the Applicant for the determination of *disodium 5'-inosinate (IMP)* in the *feed additive, flavouring premixtures* and *water*.

As no protocol of the method or experimental data were provided by the Applicant for the determination of *IMP* in *feedingstuffs*, the EURL could not evaluate nor recommend the method for official control to determine *IMP* 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.

KEYWORDS

Disodium 5'-inosinate (IMP), sensory additives, flavouring compounds, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (new feed additive) for *disodium 5'-inosinate (IMP)* produced by fermentation with *Corynebacterium ammoniagenes KCCM80161* 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 [1,2]. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories [2,3]. *Disodium 5'-inosinate (IMP)*, produced by RNA hydrolysis, is already authorised as a sensory additive, flavouring compound under Commission Implementing Regulation (EU) 2018/238 [4].

The *feed additive* consists of a minimum of 97 % of *disodium 5'-inosinate (IMP)* as an *active substance* which is produced by fermentation with a strain of *Corynebacterium ammoniagenes KCCM80161* [3,5]. The production strain is deposited in the "Korean Culture Center of Microorganisms" (KCCM) under accession number KCCM80161 [5].

The *feed additive* is intended to be used directly into *feedingstuffs* or through *premixtures* and in *water* for drinking with proposed maximum levels of 25 mg *IMP*/kg *feedingstuffs* [3,5].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *disodium 5'-inosinate (IMP)* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the determination of *IMP* in raw materials, *flavouring premixtures* and *water* the Applicant submitted the method based on high performance liquid chromatography coupled to UV detection (HPLC-UV) [6].

The sample (50 g) is dissolved in the total volume of 100 ml of water. The aliquot of the aqueous solution is diluted 5 times before the chromatographic analysis. The analyte is

detected by UV at 254 nm wavelength. The quantification of *IMP* is performed by using an external calibration with *IMP* as a standard substance [6].

The following performance characteristics were reported in frame of the validation study for the determination of *IMP* content in the aqueous solution of the premixture ranging from 7.5 to 200 mg/L: a relative standard deviation for *repeatability* (RSD_r) ranging from 0.7 to 1.3 %; and a *recovery* rate (R_{rec}) ranging from 101 to 106 % [6]. The lowest tested level of *IMP* in water (7.5 mg/L) was assigned by the Applicant as a limit of quantification (LOQ). In addition, the Applicant demonstrated proper selectivity and specificity of the method by submitting the chromatograms of the premixture, containing *IMP* and disodium guanylate [6].

Furthermore, the above mentioned HPLC-UV method is very similar to another HPLC-UV method recommended by Commission Implementing Regulation (EU) 2018/238 [4].

Based on the available performance characteristics, the EURL recommends the HPLC-UV method submitted by the Applicant for the determination of *disodium 5'-inosinate (IMP)* in the *feed additive, flavouring premixtures* and *water*.

As no protocol of the method or experimental data were provided by the Applicant for the determination of *IMP* in *feedingstuffs*, the EURL could not evaluate nor recommend the method for official control to determine *IMP* in *feedingstuffs*.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not relevant for the present application.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the identification of *IMP* in the *feed additive*, the Applicant proposed the internationally recognised FAO JECFA monograph "disodium 5'-inosinate" [7]. The monograph specifies fit-for-purpose identification tests based on solubility, absorbance signals in spectrophotometric measurements, presence of sodium, ribose and organic phosphate [7].

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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control i) the identification tests of FAO JECFA monograph "disodium 5'-inosinate" for the identification of *disodium 5'-inosinate (IMP)* in the *feed additive*; and ii) the HPLC-UV method submitted by the Applicant for the determination of *disodium 5'-inosinate (IMP)* in the *feed additive, flavouring premixtures* and *water*.

As no protocol of the method or experimental data were provided by the Applicant for the determination of *IMP* in *feedingstuffs*, the EURL could not evaluate nor recommend the method for official control to determine *IMP* in *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the identification of *disodium 5'-inosinate (IMP)* in the *feed additive*:

- FAO JECFA monograph "disodium 5'-inosinate"

For the determination of *disodium 5'-inosinate (IMP)* in the *feed additive, flavouring premixtures* and *water*:

- High performance liquid chromatography coupled to UV detection (HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *disodium 5'-inosinate (IMP)* produced by fermentation with *Corynebacterium ammoniagenes KCCM80161* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] *Application, Reference SANTE_E5_FWD. APPL. 1831-0003-2019

[2] *Application, Application Form – Annex 1 – Subm. No. 1544783576520-2338

[3] *Application, Proposal for Register Entry – Annex A

[4] 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

[5] *Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis

[6] *Supplementary information: CJE_IMP_HPLC_190830

[7] FAO JECFA Combined Compendium of Food Additive Specifications, "Disodium 5'-inosinate", Monograph No. 1 (2006) http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/Additive-167.pdf (last visited 15/03/2019)

*Refers to Dossier no: FAD-2018-0094

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

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- RIKILT Wageningen UR, Wageningen (NL)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 — Labore Landwirtschaft, Nossen (DE)