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Feed Additives

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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 stationis* KCCM 80235

(FAD-2020-0098; CRL/190046)

European Union Reference Laboratory
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Dossier related to:	**FAD-2020-0098 - CRL/190046**
Name of Product / Feed Additive:	***Disodium 5'-inosinate (IMP)*** **produced by fermentation with** ***Corynebacterium stationis*** **KCCM 80235**
Active Agent (s):	**Disodium 5'-inosinate**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**María José González de la Huebra** **25/06/2021**
Report approved by: Date:	**Christoph von Holst** **25/06/2021**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *disodium 5'-inosinate (IMP)* produced by fermentation with *Corynebacterium stationis* KCCM 80235 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. The *feed additive* consists of a minimum of 97 % (w/w of dry matter) of *disodium 5'-inosinate (IMP)* as an *active substance* which is produced by fermentation with a strain of *Corynebacterium stationis* KCCM 80235. The *feed additive* is intended to be used directly into *feedingstuffs* or through *premixtures* with proposed maximum levels of 50 mg *IMP* / kg *feedingstuffs* and in *water* for drinking.

For the determination of *IMP* in the *feed additive*, flavouring *premixtures* and *water*, the EURL previously recommended for official control in the frame of the dossier FAD-2018-0094 the internationally recognised FAO JECFA monograph "disodium 5'-inosinate" and a method based on high performance liquid chromatography coupled with UV detection (HPLC-UV). The EURL considers that these recommendations are also valid in the frame of the current application.

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 or recommend a 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 an authorisation is sought under Article 4(1) (new feed additive) for *disodium 5'-inosinate (IMP)* produced by fermentation with *Corynebacterium stationis* KCCM 80235 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, the authorisation is sought for the use of the *feed additive* for all animal species [2]. The two products of *disodium 5'-inosinate (IMP)*, produced by RNA hydrolysis and by fermentation, are already authorised as a sensory additives, flavouring

compounds under Commission Implementing Regulations (EU) 2018/238 [3] and (EU) 2020/1764 [4].

The *feed additive* consists of a minimum of 97 % (w/w of dry matter) of *disodium 5'-inosinate (IMP)* as an *active substance* which is produced by fermentation with a strain of *Corynebacterium stationis* KCCM 80235 [5]. The *feed additive* is intended to be used directly into *feedingstuffs* or through *premixtures* with proposed maximum levels of 50 mg *IMP* / kg *feedingstuffs* and in *water* for drinking [2,6].

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 the *feed additive* the Applicant proposed [7] the internationally recognised FAO JECFA monograph "disodium 5'-inosinate" [8] and a Chinese national standard method [9], which is based on the mentioned monograph.

However, the EURL previously evaluated and recommended for official control for the determination of *IMP* in the *feed additive*, *flavouring premixtures* and *water* [10] a method based on high performance liquid chromatography coupled to UV detection (HPLC-UV) [11], which was submitted by the same Applicant in the frame of the dossier FAD-2018-0094 for a very similar product. Based on the overall available information, the EURL considers that these recommendations are also valid in the frame of the current application.

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 or recommend a 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 EURL previously recommended for official control for a very similar product [10] the internationally recognised FAO JECFA monograph "disodium 5'-inosinate", where the fit-for-purpose identification tests based on solubility, absorbance signals in spectrophotometric measurements, presence of sodium, ribose and organic phosphate are specified [8]. The EURL considers that these recommendations are also valid in the frame of the current application.

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 the FAO JECFA monograph "disodium 5'-inosinate" for the identification of *disodium 5'-inosinate (IMP)* in the *feed additive*; and (ii) the HPLC-UV method 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 or 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)* 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. 1831-0086-2020

[2] *Application, Annex 1 – Submission Number 1605705556016-2740

[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*

[4] Commission Implementing Regulation (EU) 2020/1764 of 25 November 2020 concerning the authorisation of disodium 5'-inosinate produced by fermentation with *Corynebacterium stationis* KCCM 80161 as a feed additive for all animal species, *OJ L 397, 26.11.2020, p. 21–23*

[5] *Technical dossier, Section II: II.1.3 Qualitative and quantitative composition (active substance, other components, impurities, batch to batch variation)

[6] *Technical dossier, Section II: II.5.1 Proposed mode of use in animal nutrition

[7] *Technical dossier, Section II: II.6.1 Methods of analysis for the active substance

[8] 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

[9] *Technical dossier, Section II – Ref_II_6_02

[10] EURL evaluation report : FAD-2018-0094, Disodium 5'-inosinate (IMP) https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad2018-0094-imp.pdf

[11] +Supplementary information: CJE_IMP_HPLC_190830

*Refers to Dossier no: FAD-2020-0098
+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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