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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

Microcrystalline Cellulose
(FAD-2016-0062; CRL/100321)

Sodium Carboxymethyl Cellulose
FAD-2016-0064; CRL/100321)

European Union Reference Laboratory
Feed Additives

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Dossier related to:	**FAD-2016-0062 - CRL/100321** **FAD-2016-0064 - CRL/100321**
Name of Feed Additive:	***Microcrystalline Cellulose*** ***Sodium Carboxymethyl Cellulose***
Active Agent (s):	**-**
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** **18/07/2019**
Report approved by: Date:	**Christoph von Holst** **18/07/2019**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 10 for *microcrystalline cellulose* and *sodium carboxymethyl cellulose* under the category / functional group 1 (c,d,e,f) "technological additives" / "emulsifiers, stabilisers, thickeners and gelling agents" according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additives* for all animal species.

Microcrystalline cellulose is a material of white to off-white hygroscopic granules or powder of fine fibers. *Sodium carboxymethyl cellulose* is a white to off-white hygroscopic powder or granules. The Applicant states that the specific purity criteria set in Commission Regulation (EU) 231/2012 for the use of *microcrystalline cellulose* and *sodium carboxymethyl cellulose* as the food additives are also applicable when using them as the *feed additives*.

The *feed additives* are intended to be included into *feedingstuffs* through *premixtures* with no minimum or maximum dose indicated by the Applicant.

For the identification/characterisation of the *feed additives*, the Applicant referred to Commission Regulation (EU) 231/2012, where the criteria and specific qualitative and quantitative tests/methods are indicated for checking the compliance with the criteria specified for *microcrystalline cellulose* and *sodium carboxymethyl cellulose*.

For *microcrystalline cellulose* the identity tests for solubility and suspension, including colour reaction and analysis by infrared spectrometry, have to be performed. In addition, the methods for purity measurements include the determination of the loss on drying, water-soluble matter, sulfated ash, pH of a 10 % suspension and the presence/absence of starch. Finally, the determination of the cellulose content is specified by the above mentioned Regulation.

For *sodium carboxymethyl cellulose* the following tests for the identity check are outlined: solubility, foam and precipitate formation together with a colour reaction. The methods for purity checking include measurements of the degree of substitution, loss on drying, total glycolate and sodium contents. Finally, the determination of the *sodium carboxymethyl cellulose* content is required according to the above mentioned Regulation.

All of the above mentioned tests/methods are described in the FAO JECFA *'microcrystalline cellulose'* and *'sodium carboxymethyl cellulose'* monographs and the *'volume 4'* of the FAO JECFA combined compendium for food additives specifications.

The EURL recommends for the identification/characterisation of the *feed additives* the above mentioned methods described in the FAO JECFA *'microcrystalline cellulose'* and *'sodium carboxymethyl cellulose'* monographs and the *'volume 4'* of FAO JECFA combined compendium for food additives specifications.

As the accurate quantification of *microcrystalline cellulose* and *sodium carboxymethyl cellulose* added to *premixtures* or *feedingstuffs* is not achievable experimentally the EURL cannot evaluate nor recommend any method for official control to quantify *microcrystalline cellulose* and *sodium carboxymethyl cellulose* in *premixtures* or *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

Microcrystalline cellulose, *sodium carboxymethyl cellulose*, technological additives, emulsifiers, stabilisers, thickeners, gelling agents, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 10 (re-authorisation of an existing feed additives) for *microcrystalline cellulose* and *sodium carboxymethyl cellulose* under the category / functional group 1 (c,d,e,f) "technological additives" / "emulsifiers, stabilisers, thickeners and gelling agents" according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of the *feed additives* for all animal species [2,3].

Microcrystalline cellulose is a material of white to off-white hygroscopic granules or powder of fine fibers [4]. *Sodium carboxymethyl cellulose* is a white to off-white hygroscopic powder or granules [5]. The Applicant states [4,5] that the specific purity criteria set in the Commission Regulation (EU) 231/2012 [6] for the use of *microcrystalline cellulose* and *sodium carboxymethyl cellulose* as the food additives are also applicable when using them as the *feed additives*.

The *feed additives* are intended to be included into *feedingstuffs* through *premixtures* with no minimum or maximum dose indicated by the Applicant [2,3,4,5].

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 *microcrystalline cellulose* and *sodium carboxymethyl cellulose* 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)

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

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/characterisation of the *feed additives*, the Applicant referred to Commission Regulation (EU) 231/2012 [6], where the criteria and specific qualitative and quantitative tests/methods are indicated for checking the compliance with the criteria specified for *microcrystalline cellulose* and *sodium carboxymethyl cellulose*.

For *microcrystalline cellulose* the identity tests for solubility and suspension, including colour reaction and analysis by infrared spectrometry, have to be performed. In addition, the methods for purity measurements include the determination of the loss on drying, water-soluble matter, sulfated ash, pH of a 10 % suspension and the presence/absence of starch. Finally, the determination of cellulose content is specified by the above mentioned Regulation.

For *sodium carboxymethyl cellulose* the following tests for the identity check are outlined: solubility, foam and precipitate formation together with a colour reaction. The methods for purity checking include measurements of the degree of substitution, loss on drying, total glycolate and sodium contents. Finally, the determination of the *sodium carboxymethyl cellulose* content is required according to the above mentioned Regulation.

These tests/methods are described in the FAO JECFA *'microcrystalline cellulose'* [7] and *'sodium carboxymethyl cellulose'* [8] monographs, and the *'volume 4'* of the FAO JECFA combined compendium for food additives specifications [9].

In the frame of the identification/characterisation of *microcrystalline cellulose* when performing a solubility test, water, ethanol, ether, diluted mineral acids and aqueous sodium hydroxide solutions are used [6,7,9].

For a suspension test, the sample is mixed with water, blended at high speed and allowed to stand for 1 to 3 h [6,7].

When performing a colour reaction, the sample is mixed with phosphoric acid and heated in a water bath for 30 min. Then, a solution of pyrocatechol in phosphoric acid is added to the sample solution and heated for another 30 min [6].

The infrared absorption measurement is performed with the sample dispersed in potassium bromide [6,7,9]. The obtained spectrum is compared with the reference spectrum from the monograph [7].

For the determination of the loss on drying, an accurate amount of sample (1 to 2 g) is placed in an oven at 105 °C and kept for 3 h. After cooling down to room temperature the sample is weighed again, and the difference of these masses is defined as loss on drying [6,7,9].

For the determination of sulfated ash, a diluted sulfuric acid is added to the sample (10 g). The sample is then gently heated until most of it is volatilised. The insoluble matter is ignited at 800 ± 25 ^{o}C for 15 min. The residue is weighed after the cooling down to determine the amount of sulfated ash [6,7,9].

For the determination of water soluble matter, the sample is shaken with water for 10 min, the solution is filtered, the filtrate is evaporated until dryness and the residue is weighed after cooling down to calculate the relative water soluble matter content [7].

For pH measurements, the sample is shaken with water for 20 min, centrifuged and the pH of the supernatant is determined [6,7].

For the determination of the presence/absence of starch, the suspension of the sample is treated with iodine [6].

For the determination of the content of cellulose, the sample (125 mg) is mixed with water and a potassium dichromate solution is added to the sample. After the mixing, sulfuric acid is added and the reaction mixture is heated up to the boiling point. Then, the mixture is cooled down, diluted with water and an aliquot is titrated with ferrous ammonium sulfate solution using *ortho*-phenanthroline as an indicator. The same procedure is followed for the blank sample. The relative amount of cellulose is calculated on anhydrous basis and after blank correction [7].

In the frame of the identification/characteristation of *sodium carboxymethyl cellulose*, water and ethanol are used when performing a solubility test [6,8,9].

When performing the foam test, 0.1 % of aqueous sample solution is vigorously shaken [6,8].

For the precipitate formation, the solution of the sample is mixed with the solutions of copper or aluminium sulfates [6,8].

For the colour reaction, the sample (0.5 g) is dispersed in water and stirred until it is completely dissolved. 1-Naphthol and sulfuric acid are added to the aliquot of the solution for producing a colour [6,8].

For the determination of the degree of substitution, the sample (5 g) is suspended in methanol or ethanol and then shaken for 30 min and decanted. The extract is treated with silver nitrate several times until it shows no presence of chloride anymore. Then, the solvent is evaporated until constant weight of the solid substance. Afterwards, an aliquot of the substance (2 g) is charred in the flame, cooled down, moistened with concentrated sulfuric acid and heated until the fuming is finished. Then, ammonium carbonate is added to the residue, the mixture is heated and the procedure is repeated with the addition of sulfuric acid in case the residue still contains some carbon. The carbon-free residue of formed sodium sulfate is weighed and the sodium content is calculated. The known content of sodium is used to calculate the degree of substitution [8].

For the determination of the loss on drying, an accurate amount of sample (1 to 2 g) is placed in an oven at 105 °C and kept until constant weight, cooled down to room temperature and then weighed [6,8,9].

For the determination of sodium glycolate (free glycolate), the sample (0.5 g) is moistened with glacial acetic acid and water and stirred until it is completely dissolved. Then, acetone and sodium sulfate are added to the latter solution, the mixture is stirred until complete precipitation of carboxymethyl cellulose and filtered. The aliquot of the filtrate is mixed with blank solution prepared with water, glacial acetic acid and acetone, and heated in a water bath until acetone is evaporated. The remaining mixture is cooled down to room temperature, naphthalenediol is added and the mixture is heated for 20 min. Then, an absorbance of the latter sample solution is measured at 540 nm wavelength. The determination of sodium glycolate (free glycolate) is performed using calibration with an external standard, namely solutions of glycolic acid treated in the same way as the samples [8].

For the determination of sodium, atomic absorption spectrometry or flame photometry is used [9].

For the determination of sodium chloride, the sample (5 g) is heated until charcoal formation and subsequently burned in electrical oven at 500 ^{o}C for 15 min. After cooling down, the formed ash is extracted with water several times, the extracts are filtered and the filtrates are acidified with nitric acid. The sodium chloride content is determined by the method of Volhard using silver nitrate and ammonium thiocyanate [8].

The determination of the sodium carboxymethyl cellulose content is performed by subtracting from 100 the relative contents of sodium chloride and sodium glycolate (free glycolate) determined separately by the procedures described above [8].

The EURL recommends for the identification/characterisation of the *feed additives* the above mentioned methods described in the FAO JECFA *'microcrystalline cellulose'* and *'sodium carboxymethyl cellulose'* monographs and the *'volume 4'* of FAO JECFA combined compendium for food additives specifications.

As the accurate quantification of *microcrystalline cellulose* and *sodium carboxymethyl cellulose* added to *premixtures* or *feedingstuffs* is not achievable experimentally the EURL cannot evaluate nor recommend any method for official control to quantify *microcrystalline cellulose* and *sodium carboxymethyl cellulose* in *premixtures* or *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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for the identification/characterisation of the *feed additives* the above mentioned methods described in the FAO JECFA *'microcrystalline cellulose'* and *'sodium carboxymethyl cellulose'* monographs and the *'volume 4'* of FAO JECFA combined compendium for food additives specifications.

As the accurate quantification of *microcrystalline cellulose* and *sodium carboxymethyl cellulose* added to *premixtures* or *feedingstuffs* is not achievable experimentally the EURL cannot evaluate nor recommend any method for official control to quantify *microcrystalline cellulose* and *sodium carboxymethyl cellulose* in *premixtures* or *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the identification/characterisation of *microcrystalline cellulose* in the *feed additive*:

- The FAO JECFA *'microcrystalline cellulose'* monograph and the *'volume 4'* of FAO JECFA combined compendium for food additives specifications

For the identification/characterisation of *sodium carboxymethyl cellulose* in the *feed additive*:

- The FAO JECFA *'sodium carboxymethyl cellulose'* monograph and the *'volume 4'* of FAO JECFA combined compendium for food additives specifications

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *microcrystalline cellulose* and *sodium carboxymethyl cellulose* 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. 1831-0016-2019 & Annex I – submission number 1288166956555-1178

[2] *Application, proposal for Register entry – Annex A

[3] +Application, proposal for Register entry – Annex A

[4] *Technical dossier, Section II: Identify, characterisation and conditions of use of the additive; methods of analysis

[5] +Technical dossier, Section II: Identify, characterisation and conditions of use of the additive; methods of analysis

[6] Commission Regulation (EU) No 231/2012 of 9 March 2012, laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council

[7] FAO JECFA Combined Compendium of Food Additive Specifications, *'microcrystalline cellulose'*, Monograph No. 7 (2009) http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/monograph7/additive-280-m7.pdf (last visited on 28/05/2019)

[8] FAO JECFA Combined Compendium of Food Additive Specifications, *'sodium carboxymethyl cellulose'*, Monograph No. 11 (2011) http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/monograph11/additive-396-m11.pdf (last visited on 28/05/2019)

[9] FAO JECFA Combined Compendium for Food Additive Specifications - Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications, Vol. 4 http://www.fao.org/docrep/pdf/009/a0691e/a0691e.pdf (last visited on 28/05/2019)

*Refers to Dossier no: FAD-2016-0062

+Refers to Dossier no: FAD-2016-0064

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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- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 — Labore Landwirtschaft, Nossen (DE)