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DIRECTORATE GENERAL  
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**European Union Reference Laboratory for 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**

**Carrageenan**

*(FAD-2010-0205; CRL/100197)*

*(FAD-2010-0319; CRL/100292)*

*amended version*





**Evaluation Report on the Analytical Methods submitted  
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Additive according to Regulation (EC) No 1831/2003**

Dossier related to: **FAD-2010-0205 - CRL/100197**  
**FAD-2010-0319 - CRL/100292**

Name of Feed Additive: **Carrageenan (refined and semi-refined)**

Active Agent (s): **Carrageenan**

Rapporteur Laboratory: **European Union Reference Laboratory for  
Feed Additives (EURL-FA)  
Geel, Belgium**

Report prepared by: **Zigmas Ezerskis**

Report checked by: **Piotr Robouch (EURL-FA)**  
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Report approved by: **Christoph von Holst**  
Date: **18/08/2014**  
**17/09/2014**

*amended version*

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## EXECUTIVE SUMMARY

In the current applications authorisation is sought under article 10(2) for *Carrageenan* under the 'category' / 'functional groups' 1(c), 1(d), 1(e) and 1(f) 'technological additives' / 'emulsifiers'<sup>1</sup>, 'stabilisers'<sup>1,2</sup>, 'thickeners'<sup>1,2</sup>, 'gelling agents'<sup>1,2</sup> 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 'pets and other non-food producing animals'<sup>1</sup>, 'cats and dogs'<sup>2</sup>.

*Carrageenan* is colourless-to-tan powder produced from natural strains of red seaweeds *Rhodophyceae*. The *feed additive* mainly consists of carrageenan polysaccharides. According to the Applicants, two types of the products exist: refined *Carragennan*<sup>1</sup> and semi-refined *Carragennan*<sup>1,2</sup>, which mainly differ in the acid insoluble matter (AIM) content derived from algal cellulose. Refined *Carragennan* contains less than 2% of AIM, while the AIM content in semi-refined *Carragennan* ranges from 8 to 15%. Furthermore, the Applicants suggested the following additional specifications to characterise the *feed additive*: - more than 5 mPa·s for viscosity; - less than 12% for loss on drying; - 15 to 40% for sulphate and total ash contents; and - less than 1% for acid insoluble ash content. The Applicants stated that the specific purity criteria set in the Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012 for the food additives are applicable for the *feed additive*.

The *feed additive* is intended to be incorporated directly into *feedingstuffs*. No recommended minimum or maximum concentration levels were proposed by the Applicants. However, the typical inclusion levels range from 1 to 30 g *feed additive* /kg *feedingstuffs*.

For the characterisation of *Carrageenan* in the two forms of the *feed additive* (i.e. refined and semi-refined) the Applicants submitted two internationally recognised FAO JECFA monographs for food additives ('*Carrageenan*' and '*Processed Eucheuma Seaweed*'), recommended by Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012. Identification is based on: - solubility; - infrared spectroscopy; positive tests for - galactose & anhydrogalactose and - hydrocolloid & predominant type of copolymer. Additional characterisation is based on the following quantitative assays: - viscosity; - pH values; - loss on drying; - sulphate; - total ash; - acid insoluble ash; and - acid insoluble matter contents.

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<sup>1</sup> FAD-2010-0205; <sup>2</sup> FAD-2010-0319

Even though no performance characteristics are provided, the EURL recommends for official control the two FAO JECFA monographs mentioned above to characterise refined and semi-refined *Carrageenan* in the *feed additive* - as recommended by Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012.

The Applicants provided no experimental data or any analytical methods for the quantification of *Carrageenan* in *feedingstuffs*, as the accurate determination of *Carrageenan* in *feedingstuffs* is not achievable experimentally. Therefore the EURL cannot evaluate nor recommend any method for official control to quantify *Carrageenan* 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) is not considered necessary.

## KEYWORDS

*Carrageenan*, technological additives, emulsifiers, stabilisers, thickeners, gelling agents, pets and other non-food producing animals, cats and dogs

## 1. BACKGROUND

In the current applications authorisation is sought under article 10(2) (re-evaluation of the already authorised additives under provisions of Council Directive 70/524/EEC) for *Carrageenan* under the 'category' / 'functional groups' 1(c), 1(d), 1(e) and 1(f) 'technological additives' / 'emulsifiers'<sup>1</sup>, 'stabilisers'<sup>1,2</sup>, 'thickeners'<sup>1,2</sup>, 'gelling agents'<sup>1,2</sup> 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 'pets and other non-food producing animals'<sup>1</sup> [3], 'cats and dogs'<sup>2</sup> [4].

*Carrageenan* is colourless-to-tan powder produced from natural strains of red seaweeds *Rhodophyceae* [5,6]. The *feed additive* mainly consists of carrageenan polysaccharides: *kappa-carrageenan* (co-polymer of D-galactose-4-sulphate with 3,6-anhydro-D-galactose); *iota-carrageenan* (co-polymer of D-galactose-4-sulphate with 3,6-anhydro-D-galactose sulphated at carbon 2); and *lambda-carrageenan* (co-polymer of 1,3-linked D-galactose-2-sulphate with 1,4-linked D-galactose-2,6-disulphate) [5].

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<sup>1</sup> FAD-2010-0205; <sup>2</sup> FAD-2010-0319

According to the Applicants, two types of the products exist: refined *Carragennan* (FAD-2010-0205) and semi-refined *Carragennan* (FAD-2010-0205 and FAD-2010-0319), which mainly differ in the acid insoluble matter (AIM) content derived from algal cellulose. Refined *Carragennan* contains less than 2% of AIM, while AIM content in semi-refined *Carragennan* ranges from 8 to 15% [5,6].

The Applicants suggested the following technical specifications to characterise the *feed additive* [5,6]:

<i>Parameter</i>	<i>Value</i>
Viscosity, mPa.s	≥ 5 (1.5 % aq. solution, 75 °C)
Loss on drying, %	≤ 12 (105 °C, 4h)
Sulphate content, % (dry w/w)	15 to 40
Total ash, % (dry w/w)	15 to 40 (550 °C) (refined) 15 to 30 (550 °C) (semi-refined)
Acid insoluble ash, % (dry w/w)	≤ 1 (10% HCl)
Acid insoluble matter, % (dry w/w)	≤2 (1% H <sub>2</sub> SO <sub>4</sub> ) (refined) 8 to 15 (1% H <sub>2</sub> SO <sub>4</sub> ) (semi-refined)

Furthermore, the Applicants state that the specific purity criteria set in the Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012 for the food additives are applicable for the *feed additive* [5,6].

The *feed additive* is intended to be incorporated directly into *feedingstuffs*. No recommended minimum or maximum concentration levels were proposed by the Applicants [3,4]. However, the typical inclusion levels range from 1 to 30 g of *feed additive* /kg complete *feedingstuffs* [5,6].

## 2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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 authorisation of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Carrageenan* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

### 3. EVALUATION

#### ***Identification /Characterisation of the feed additive***

##### ***Qualitative and quantitative composition of impurities in the additive***

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, salmonella, mycotoxins and dioxins) are available from the respective European Union Reference Laboratories [7].

##### ***Description of the analytical methods for the determination of the active substance in feed additive and feedingstuffs***

For the characterisation of *Carrageenan* in the two forms of the *feed additive* (i.e. refined and semi-refined) the Applicants submitted two internationally recognised FAO JECFA monographs for food additives '*Carrageenan*' and '*Processed Eucheuma Seaweed*' [8,9], recommended by Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012. Identification is based on: - solubility; - infrared spectroscopy; positive tests for - galactose & anhydrogalactose and - hydrocolloid & predominant type of copolymer. Additional characterisation is based on the following quantitative assays: - viscosity; - pH values; - loss on drying; - sulphate; - total ash; - acid insoluble ash; and - acid insoluble matter contents. The experimental protocols for generic tests are provided in the FAO JECFA compendium of methods [10].

Note: The FAO JECFA monograph '*Processed Eucheuma Seaweed*' should be applied when characterising semi-refined *Carrageenan*.

Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned methods described in the FAO JECFA monographs - as recommended by Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012 to characterise refined and semi-refined *Carrageenan* in the *feed additive*.

The Applicants provided no experimental data or any analytical methods for the quantification of *Carrageenan* in *feedingstuffs*, as the accurate determination of *Carrageenan* in *feedingstuffs* is not achievable experimentally. Therefore the EURL cannot evaluate nor recommend any method for official control to quantify *Carrageenan* 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) is not considered necessary.

#### 4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the analytical methods recommended by Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012, and described in the FAO JECFA monographs to characterise *Carrageenan* in the two forms of the *feed additive*.

The Applicant provided no experimental data or any analytical methods for the quantification of *Carrageenan* in *feedingstuffs*, as the accurate determination of *Carrageenan* in *feedingstuffs* is not achievable experimentally. Therefore the EURL cannot evaluate nor recommend any method for official control to quantify *Carrageenan* in *feedingstuffs*.

##### ***Recommended text for the register entry (analytical method)***

For the determination of *Carrageenan* (refined) in the *feed additive*:

- FAO JECFA Monograph '*Carrageenan*' as referred in Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012

For the determination of *Carrageenan* (semi-refined) in the *feed additive*:

- FAO JECFA Monograph '*Processed Eucheuma Seaweed*' as referred in Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012

#### 5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Carrageenan* 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, Ref.: SANCO/G1:Forw.Appl.1831/0044-2013
- [2] +Application, Ref.: SANCO/G1:Forw.Appl.1831/0045-2013
- [3] \*Application, Proposal for Register Entry - Annex A
- [4] +Application, Proposal for Register Entry - Annex A
- [5] \*Technical dossier, Section II – Identity, characterisation and conditions of use of the additive; Methods of analysis
- [6] +Technical dossier, Section II – Identity, characterisation and conditions of use of the additive; Methods of analysis
- [7] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories



- [8] FAO JECFA Combined Compendium of Food Additive Specifications, 'Carrageenan', Monograph No. 4 (2007)  
<http://www.fao.org/ag/agn/jecfa-additives/specs/monograph4/additive-117-m4.pdf>  
(last visited on 01/07/2014)
- [9] FAO JECFA Combined Compendium of Food Additive Specifications, 'Processed *Eucheuma Seaweed*', Monograph No. 4 (2007)  
<http://www.fao.org/ag/agn/jecfa-additives/specs/monograph4/additive-353-m4.pdf>  
(last visited on 01/07/2014)
- [10] FAO JECFA Combined Compendium for Food Additive Specifications - *Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications*, Monographs No. 1, Vol. 4  
<http://www.fao.org/docrep/009/a0691e/a0691e00.htm>  
(last visited on 01/07/2014)

\*Refers to Dossier no: FAD-2010-0205

+Refers to Dossier no: FAD-2010-0319

## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, 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 (EC) No 885/2009.

## 8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (IT)
- Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)<sup>1</sup>
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Danish Veterinary and Food Administration, Ringsted (DK)<sup>2</sup>
- National Veterinary Research Institute (NVRI), Puławy (PL)

<sup>1</sup> Name and address according to Regulation (EC) No 885/2009: Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim

<sup>2</sup> Official name and address according to Regulation (EC) No 885/2009: Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby

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- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Nossen (DE)<sup>3</sup>
  - Univerza v Ljubljani, Veterinarska fakulteta, Nacionalni veterinarski inštitut, En ota za patologijo prehrane in higieno okolja, Ljubljana (SI)
  - Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)

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<sup>3</sup> Name and address according to Regulation (EC) No 885/2009: Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 – Landwirtschaftliches Untersuchungswesen, Leipzig