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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**

Bentonite
(FAD-2016-0051; CRL/160015)



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in connection with the Application for Authorisation of a
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Dossier related to: **FAD-2016-0051 - CRL/160015**

Name of Feed Additive: ***Bentonite***

Active Agent (s): **Bentonite-Smectite**

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

Report prepared by: **Zigmas Ezerskis**

Report checked by: **Piotr Robouch (EURL-FA)**
Date: **03/02/2017**

Report approved by: **Christoph von Holst**
Date: **06/02/2017**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Bentonite*, under the category/functional group 1(m) "technological additives" / "substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action" 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. The *feed additive* is already authorised by the Regulation (EU) No 1060/2013 under the category/functional group: "technological additives" / "binders", "anticaking agents" and "substances for control of radionuclide contamination (134/137 Cs)" (identification number of additive - 1m558i) with a minimum content of smectite of 50 %.

In the current dossier, the product is a beige to brown powder or granulates, containing of minimum of 70 % di- or tri-octahedral smectite, therefore higher than the corresponding minimum smectite content of 50 %, as specified in Regulation (EC) No 1060/2013. According to the Applicant, the *Bentonite* has a minimum Aflatoxin B1 binding capacity (BC_{AfB1}) of 90 %. The *feed additive* is intended to be incorporated in *premixtures*, complete or complementary *feedingstuffs*, with a maximum proposed level of 20 g/kg *feedingstuffs*.

For the characterisation of *Bentonite (feed additive)*, the Applicant submitted several analytical methods, such X-ray Diffraction (XRD) based on the EN 13925 method, X-Ray Fluorescence (XRF), Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) and Atomic Absorption Spectrometry (AAS). The EURL recommends for official control the above mentioned the X-ray diffraction (XRD) method for the characterisation of the *feed additive*.

Furthermore, the Applicant performed an adsorption test to assess the capacity of *Bentonite* to bind Aflatoxin B1 (AfB1) applying the method recommended in Commission Implementing Regulation (EU) No 1060/2013 and the EURL report related to dossier FAD-2010-0018. Based on the experimental evidences submitted, the EURL recommends for official control the method for determination of BC_{AfB1} of the *feed additive*.

The Applicant provided no experimental data or analytical method for the determination of *Bentonite* in *premixtures* and *feedingstuffs* as the unambiguous determination of the *feed additive added* to the matrices is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control for the determination of *Bentonite* in *premixtures* and *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

Bentonite, smectite, technological additives, substances for the reduction of the contamination of feed by mycotoxins, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new use of a feed additive already authorised) for *Bentonite*, under the category/functional group 1(m) "technological additives" / "substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action" 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 additive* for all animal species [1,2]. The *feed additive* is already authorised by the Regulation (EU) No 1060/2013 under the category/functional group: "technological additives" / "binders", "anticaking agents" and "substances for control of radionuclide contamination (134/137 Cs)" (identification number of additive - 1m558i) with a minimum content of smectite of 50 %.

In the current dossier, the product is a beige to brown powder or granulates, containing of minimum of 70 % di- or tri-octahedral smectite [2,3], therefore higher than the corresponding minimum smectite content of 50 %, as specified in Regulation (EC) No 1060/2013. According to the Applicant, the *Bentonite* has a minimum Aflatoxin B1 binding capacity (BC_{AfB1}) of 90 % [3].

The *feed additive* is intended to be incorporated in *premixtures*, complete or complementary *feedingstuffs*, with a maximum proposed level of 20 g/kg *feedingstuffs* [2].

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 *Bentonite* 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, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [4].

Description of the analytical methods for the determination of the active substance in feed additive, premixtures and feedingstuffs

For the mineralogical analysis of the *feed additive* the Applicant applied the single-laboratory validated X-Ray diffraction (XRD) method [3] based on the EN 13925 method [5]. In addition, for the quantitative mineral analysis the Applicant used the XRD method described by Omotoso *et al* [6]; and (ii) methylene-blue adsorption method based on titrimetry [7].

The characterisation of the *feed additive* is based on comparison of the bentonite XRD pattern to the reference patterns published by the International Centre for Diffraction Database; smectite and other associated minerals are further quantified by means of normalised full pattern reference intensity ratios. In addition, the Applicant suggested to quantify smectite content in the *feed additive* by methylene-blue adsorption method, where well-characterised bentonite standards are used for calibration. However, this method does not provide additional information about other minerals present in the bentonite.

The Applicant analysed *Bentonite* samples of different origin and reported smectite contents ranging from 72 to 94 % [3].

For the elemental analysis the Applicant submitted three methods based on: i) X-Ray Fluorescence (XRF) spectrometry described in the EN ISO 12677 standard [8]; ii) Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) [9]; and iii) Atomic Absorption Spectrometry (AAS) [10]. The following ranges of chemical composition were reported: SiO₂ from 53 to 63 %; Al₂O₃ from 4.0 to 26 %; and MgO from 2.4 to 25 % [3].

The use of all the above mentioned methods is not necessary in the frame of official control. The EURL recommends for official control an X-ray diffraction (XRD) based on the EN 13925 method for the characterisation of the *feed additive*.

Furthermore, the Applicant performed an adsorption test to assess the *Bentonite* capacity to bind Aflatoxin B1 (Afb1) using the single-laboratory validated and further verified method recommended in Commission Implementing Regulation (EU) No 1060/2013 and the EURL report related to dossier FAD-2010-0018 [11]. This experimental protocol was applied by the Applicant on several bentonite products in the frame of efficacy studies and BC_{Afb1} ranging from 90 to 96 % was reported [12-16].

Based on the experimental evidences submitted, the EURL recommends for official control the single-laboratory validated and further verified method for the determination of AfB1 binding capacity.

The Applicant is aware that the direct determination of the *Bentonite* content added to *premixtures* or *feedingstuffs* is not achievable experimentally [3] and did not provide any experimental method or data for the determination of *Bentonite* in *premixtures* and *feedingstuffs*. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *Bentonite* in *premixtures* and *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 i) X-ray diffraction (XRD) based on the EN 13925 method for the characterisation of the *feed additive*; and ii) the adsorption test for determination of the Aflatoxin B1 binding capacity (BC_{AfB1}).

The Applicant provided no experimental data or analytical method for the determination of *Bentonite* in *premixtures* and *feedingstuffs* as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control for the determination of *Bentonite* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the characterisation of the *feed additive*:

- X-ray diffraction (XRD)

For the determination of BC_{AfB1} of the *feed additive*:

- adsorption test carried out in a buffer solution at pH 5.0 with the concentration of 4 mg/l for AfB1 and 0.02 % (w/v) for the *feed additive*

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Bentonite* 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: Forw. Appl. 1831/0038-2016
- [2] *Application, Proposal for Register Entry – Annex A
- [3] *Technical dossier, Section II
- [4] 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
- [5] EN 13925-1,2:2003; EN 13925-3:2005 – Non-destructive testing. X-ray diffraction from polycrystalline and amorphous materials. General principles, procedures, instruments
- [6] *Technical dossier, Section II – Annex_II_6_1 (cf. O. Omotoso *et al.* Clays and Clay Minerals, vol. 54, no.6, 748-760, 2006)
- [7] *Technical dossier, Section II – Annex_II_6_2
- [8] EN ISO 12677:2011 – Chemical analysis of refractory products by X-ray fluorescence (XRF) – fused cast-bead method
- [9] *Technical dossier, Section II – Annex_II_6_3
- [10] *Technical dossier, Section II – Annex_II_6_4
- [11] #EURL Evaluation Report – JRC.D.5/SFB/CvH/PRO/mds/ARES(2013)179391
- [12] *Technical dossier, Section IV – Annex_IV_6_2
- [13] *Technical dossier, Section IV – Annex_IV_6_3
- [14] *Technical dossier, Section IV – Annex_IV_6_4
- [15] *Technical dossier, Section IV – Annex_IV_6_6
- [16] *Technical dossier, Section IV – Annex_IV_6_7

*Refers to Dossier no: FAD-2016-0051

#Refers to Dossier no: FAD-2010-0018

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

The following National Reference Laboratories contributed to this report:

- Fødevarestyrelsens Laboratorie Ringsted (kemisk og mikrobiologisk) (DK)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- RIKILT Wageningen UR, Wageningen (NL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
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- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
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