EUROPEAN COMMISSION
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JRC.D.5/SFB/CvH/ZE/Ares

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

Preparation of algae interspaced bentonite

(FAD-2014-0047; CRL/140023)

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-2014-0047 - CRL/140023**
Name of Product / Feed Additive:	**Preparation of algae interspaced bentonite**
Active Agent (s):	**-**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
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Report checked by: Date:	**Piotr Robouch (EURL-FA)** **18/08/2015**
Report approved by: Date:	**Christoph von Holst** **18/08/2015**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for the *preparation of algae interspaced bentonite* (*PAIB*) as *feed additive* under the category "technological feed additives", functional group 1(m) "substances for the reduction of the contamination of feed by mycotoxins" according to the classification system of Annex I of Regulation (EC) No 1831/2003. The authorisation is sought to use the *feed additive* for all animal species and categories.

PAIB is a free-flowing, pale green to beige powder consisting of smectite (60 to 96%); interspaced organic matter (3 to 15%); and uronic acids (4 to 60 mg /g *feed additive*, based on anhydrous weight). It is intended to be included directly into *feedingstuffs* or through *premixtures* with no minimum or maximum recommend concentrations. However, the Applicant suggested typical inclusion levels of *PAIB* in complete *feedingstuffs* ranging from 10 to 125 mg/kg.

For the characterisation of the mineralogical composition of *PAIB* the Applicant submitted the X-Ray powder diffraction, a well-established and widely used crystallographic method. For the quantification of the organic matter content in *PAIB* the Applicant submitted two complementary analytical methods based on differential thermal analysis (DTA) and thermal gravimetry analysis (TGA). For the quantification of the uronic acids content in *PAIB* the Applicant submitted a single-laboratory validated and further verified method based on spectrophotometry at 520 nm, similar to the AOAC 994.13 method.

For the determination of the Aflatoxin B1 (AfB1) binding capacity (BC_{AfB1}) the Applicant applied the method prescribed by the EURL in the frame of the FAD-2011-0002 dossier. The experimental results indicate that at least 19.4 mg AfB1 are adsorbed when 1g of *PAIB* is added to 1 L solution containing 20 mg AfB1.

Based on the experimental evidence presented the EURL recommends for official control all the above mentioned methods for the proper characterisation of the *PAIB*.

As stated by the Applicant the direct quantification of *algae interspaced bentonite* preparation added to *premixtures* or *feedingstuffs* is not achievable experimentally. No experimental data were provided. Therefore the EURL cannot evaluate nor recommend any method for official control to quantify *PAIB* 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

Preparation of algae interspaced bentonite, technological feed additives, substances for the reduction of the contamination of feed by mycotoxins, all animal species

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new feed additive) for the *preparation of algae interspaced bentonite* (*PAIB*) as *feed additive* under the category "technological feed additives", functional group 1(m) "substances for the reduction of the contamination of feed by mycotoxins" according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. The authorisation is sought to use the *feed additive* for all animal species and categories [1,2].

PAIB is a free-flowing, pale green to beige powder consisting of smectite (60 to 96%); interspaced organic matter (3 to 15%); and uronic acids (4 to 60 mg/g *feed additive*, based on anhydrous weight) [2,3].

PAIB is intended to be included directly into *feedingstuffs* or through *premixtures* with no minimum or maximum recommend concentrations [2]. However, the Applicant suggested typical inclusion levels of *preparation of algae interspaced bentonite* in complete *feedingstuffs* ranging from 10 to 125 mg/kg [3].

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 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 *preparation of algae interspaced 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 characterisation of the mineralogical composition of *PAIB* the Applicant submitted an analytical method based on X-Ray powder diffraction [5] a well-established and widely used crystallographic technique also described in the generic European Pharmacopoeia monograph [6]. *Smectite* and the other minerals were identified comparing the XRD patterns to the reference ones published in the Handbook of Mineralogy [7] or available at International Centre for Diffraction Data® (ICDD®) [8], while quantification was performed using normalised full pattern reference intensity ratio [5]. Five different batches of the product were analysed and the following mineral composition was reported [5]:

smectite	from 78.8 to 89.9 %;
quartz	from 5.3 to 8.5 %;
K-feldspar	from 2.4 to 3.1 %;
plagioclase	from 1.3 to 2.2 %
calcite	from 0.5 to 5 %; and
dolomite	from 0.3 to 3.9 %

For the quantification of the organic matter content in *PAIB* the Applicant submitted two complementary analytical methods based on differential thermal analysis (DTA) and thermal gravimetry analysis (TGA) [9]. The sample (20 to 30 mg) is deposited in an aluminium oxide pan and placed in a furnace close to a reference pan. The rise in temperature is programmed from 25 to 1000 ^{o}C at the rate of 10 ^{o}C/ min. DTA and TGA follow the heat flow variation and the weight decrease as a function of temperature [9]. Five batches of the product were analysed and an organic matter content ranging from 5 to 8% (based on anhydrous weight) was reported [9].

For the quantification of the uronic acids content in *PAIB* Applicant submitted single-laboratory validated and further verified method based on spectrophotometry, similar to the AOAC 994.13 method. The sample is hydrolysed with 2 M hydrochloric acid, filtered and derivatised with 3-phenylphenol in the presence of sulfamic acid and sodium tetraborate [10]. The uronic acids are then determined by spectrophotometry at 520 nm. The validation and verification studies were performed analysing a blank clay (bentonite) spiked with glucuronic (uronic) acid at the concentration of 50 mg/g [11,12]. The following performance characteristics were reported: - a relative standard deviation for *repeatability* (RSD_r) ranging from 1.8 to 3.2%; - a relative standard deviation for *intermiadiate precision* (RSD_{ip}) ranging from 2.8 to 5.1%; and - a *recovery rate* (R_{rec}) ranging from 92 to 108%. The Applicant also analysed five batches of *PAIB* containing 7.1 to 11.6 mg uronic acids per gram of product and reported a relative *precision* of 4.7% [13], in agreement with the validation data.

For the determination of the Aflatoxin B1 (AfB1) binding capacity (BC_{AfB1}) the Applicant applied the method prescribed by the EURL in the frame of the FAD-2011-0002 dossier [3,14,15]. The adsorption test is carried out in a buffer solution at pH 5.0 with the concentrations of *feed additive* and AfB1 of 200 mg /L and 4 mg /L of the solution, respectively. The solution is centrifuged and the supernatant is directly analysed for Aflatoxin B1 using high performance liquid chromatography with diode fluorescence detection (HPLC-FLD) [14]. The Applicant analysed two batches of *PAIB* in triplicate and reported a binding capacity (BC_{AfB1}) ranging from 96.7% to 97.5% with a relative standard deviation of 0.2% [14].

Based on the experimental evidence presented the EURL recommends for official control all the methods mentioned above for the proper characterisation of *PAIB*.

Furthermore, the Applicant provided the following information:

- An elemental compositon: SiO_2 (40 to 46 %); MgO (12 to 13.5 %); Al_2O_3 (9 to 10.6 %); and Na_2O (8.5 to 9.5 %) [16], applying the ISO 29581-2:2010 method based on X-Ray fluorescence spectrometry (XRF) [17];
- Iodine concentration ranging from 4.8 to 15.0 mg/kg [18], applying the ring-trial validated method EN 15111:2007 based on inductively coupled plasma mass spectrometry (ICP-MS) [19].

As stated by the Applicant the direct quantification of *PAIB* added to *premixtures* or *feedingstuffs* is not achievable experimentally [3]. No experimental data were provided. Therefore the EURL cannot evaluate nor recommend any method for official control to quantify *PAIB* 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 the following methods to characterise the *feed additive* (*preparation of algae interspaced bentonite*):

- X-ray diffraction (XRD) for the characterisation of the mineralogical composition; and
- Differential thermal analysis (DTA) together with thermal gravimetry analysis (TGA) for the quantification of the organic matter content; and
- The single-laboratory validated and further verified method based on spectrophotometry for the quantification of the uronic acids content; and
- Adsorption test carried out in a buffer solution at pH 5.0 with a concentration of 4 mg AfB1/L and 200 mg *feed additive*/L for determination of the Aflatoxin B1 (AfB1) binding capacity (BC_{AfB1}).

As stated by the Applicant the direct determination of the *preparation of algae interspaced bentonite* added to *premixtures* or *feedingstuffs* is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify the preparation in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the characterisation of the mineralogical composition of the *feed additive*:

- X-ray Diffraction (XRD)

For the quantification of the organic matter content in the *feed additive*:

- Differential thermal analysis (DTA) together with thermal gravimetry analysis (TGA)

For the quantification of the uronic acids content in the *feed additive*:

- Spectrophotometry

For the determination of BC_{AfB1} of the additive: adsorption test carried out in a buffer solution at pH 5,0 with a 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 preparation of *algae interspaced 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 SANCO/G1: Forw. Appl. 1831/0004-2015

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

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; Methods of analysis

[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] *Technical dossier, Section II – Annex_II_1_1

[6] European Pharmacopoeia Monograph 01/2008:20933

[7] John W. Anthony, Richard A. Bideaux, Kenneth W. Bladh, and Monte C. Nichols, Eds., Handbook of Mineralogy, Mineralogical Society of America, Chantilly, VA 20151-1110, USA, http://www.handbookofmineralogy.org/

[8] The International Centre for Diffraction Data (ICDD®), http://www.icdd.com/

[9] *Technical dossier, Section II – Annex_II_1_7

[10] *Technical dossier, Section II – Annex_II_6_3

[11] *Supplementary information – Annex of validation and verification data

[12] *Supplementary information – Verification report

[13] *Technical dossier, Section II – Annex_II_1_5

[14] *Technical dossier, Section II – Annex_II_4_1

[15] FAD-2011-0002 – JRC.D.5/SFB/CvH/GDA/mds/Ares (2013)3071856 https://ec.europa.eu/jrc/sites/default/files/corr_finrep_fad-2011-0002-addendum.pdf

[16] *Technical dossier, Section II – Annex_II_1_2

[17] ISO 29581-2:2010 Cement – Test methods – Part 2: Chemical analysis by X-ray fluorescence

[18] *Technical dossier, Section II – Annex_II_1_6

[19] EN 15111:2007 - *Foodstuffs – Determination of trace elements – Determination of iodine by ICP-MS (inductively coupled plasma mass spectrometry)*

*Refers to Dossier no: FAD-2014-0047

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 sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Fødevarestyrelsens Laboratorie Aarhus (kemisk) (DK)[1]
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)[2]
- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen, Jena (DE)
- 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)

Name and address according to Regulation (EC) No 885/2009:
[1] Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby.
[2] Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes.