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JRC F.5/CvH/ZE/AS/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**

Sepiolite and diatomaceous earth
(FAD-2019-0011; CRL/180074)



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Dossier related to: **FAD-2019-0011 - CRL/180074**

Name of Product: ***Sepiolite and diatomaceous earth***

Active Agent (s): **Sepiolite**
Diatomaceous Earth

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
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Date: **20/01/2020**

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Date: **20/01/2020**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for a preparation of *sepiolite and diatomaceous earth* 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 terrestrial animal species.

According to the Applicant, the *feed additive* is a preparation consisting of 90 % (w/w) of *sepiolite* and 10 % (w/w) of *diatomaceous earth*. The product also contains a minimum (expressed as mass fraction) of 54 % of SiO₂, 15 % of MgO and 7 % of Al₂O₃. The *feed additive* is intended to be included into *feedingstuffs* through *premixtures*. The Applicant suggested an inclusion level of the *feed additive* ranging from 0.5 to 5 g/kg complete *feedingstuffs*.

For the characterisation of the *feed additive* the Applicant proposed the EN ISO 12677 method based on X-ray fluorescence (XRF) spectrometry to determine the elemental composition of the *feed additive*. Based on the experimental evidence presented, the EURL recommends for official control the EN ISO 12677 method based on XRF spectrometry for the elemental characterisation of the *feed additive*.

For the determination of the capacity of the *feed additive* to adsorb aflatoxin B₁ (AfB₁) the Applicant proposed a single-laboratory validated and further verified method based on an adsorption experiment at specific conditions, where the product as such is added to a buffer solution containing aflatoxin B₁. The content of this analyte in the solution is subsequently determined by high performance liquid chromatography with fluorescence detection (HPLC-FLD). Based on the experimental evidence presented, the EURL recommends this method for official control.

Moreover, the EURL recommends that a target value of the adsorption capacity of the *feed additive* including the conditions of the adsorption experiment is established to allow a proper implementation of this method. It is important to underline that the adsorption capacity of the product obtained by using the current method shall not be compared with the binding capacity for bentonite (1m558) as specified in the Commission Implementing Regulation (EU) No 1060/2013, because the conditions of the two adsorption experiments are different.

Even though the Applicant provided the EURL with the criteria for the mineralogical composition and the relevant experimental data, no corresponding method was submitted by the Applicant. Nevertheless, the EURL identified the EN 13925 method based on X-ray

diffraction (XRD) which can be recommended for official control for the mineralogical characterisation of the *feed additive*.

As the unambiguous determination of *sepiolite* and *diatomaceous earth* or the *feed additive* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control for the determination of *sepiolite* and *diatomaceous earth* 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Sepiolite and diatomaceous earth, *sepiolite*, *diatomaceous earth*, technological feed additives, substances for the reduction of the contamination of feed by mycotoxins, all terrestrial animal species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a feed additive) for the preparation of *sepiolite and diatomaceous earth* as a 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 [1,2]. The authorisation is sought to use the *feed additive* for all terrestrial animal species [1,2,3].

According to the Applicant, the *feed additive* is an off-white powder preparation consisting of 90 % (w/w) of *sepiolite* and 10 % (w/w) of *diatomaceous earth* [2,3]. The product also contains a minimum (expressed as mass fraction) of 54 % of SiO₂, 15 % of MgO and 7 % of Al₂O₃ [3]. The *feed additive* is intended to be included into *feedingstuffs* through *premixtures*. The Applicant suggested an inclusion level of the *feed additive* ranging from 0.5 to 5 g/kg complete *feedingstuffs* [3].

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 *sepiolite and diatomaceous earth* 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)

As the unambiguous determination of *sepiolite* and *diatomaceous earth* or the *feed additive* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control for the determination of *sepiolite* and *diatomaceous earth* in *premixtures* and *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 characterisation of the *feed additive* the Applicant proposed the EN ISO 12677 method based on X-ray fluorescence (XRF) spectrometry [4] to determine the elemental composition of the *feed additive* and the single-laboratory validated and further verified method based on an adsorption experiment followed by high performance liquid chromatography with fluorescence detection (HPLC-FLD) for the determination of the capacity of the *feed additive* to adsorb aflatoxin B₁ (Afb₁) [5].

When applying the EN ISO 12677 method, the powdered sample is fused with a suitable flux to destroy its mineralogical and particulate composition. The resultant melt is cast into the shape of a glass bead which is then introduced into an XRF spectrometer. The intensities of the fluorescent X-rays of the elements in the bead are measured and the chemical composition of the sample is determined by using calibration graphs or equations applying corrections for inter-elemental effects [4].

Table 1: Elemental composition of *sepiolite*, *diatomaceous earth* and the *feed additive*, expressed as an average content \pm standard deviation

Content, % (mass fraction)	Sepiolite	Diatomaceous earth	Feed additive	Feed additive
	Experimental			Expected / Calculated
SiO ₂	56.5 \pm 0.4	71.2 \pm 0.7	58.4 \pm 0.3	58.0
MgO	18.3 \pm 0.2	1.4 \pm 0.01	16.3 \pm 0.1	16.6
Al ₂ O ₃	7.3 \pm 0.1	9.5 \pm 0.3	7.6 \pm 0.1	7.5
CaO	2.5 \pm 0.04	1.3 \pm 0.1	2.4 \pm 0.01	2.4
Fe ₂ O ₃	1.9 \pm 0.02	6.2 \pm 0.3	2.4 \pm 0.02	2.3
K ₂ O	1.9 \pm 0.02	1.3 \pm 0.02	1.9 \pm 0.02	1.9
Na ₂ O	0.5 \pm 0.01	0.5 \pm 0.1	0.5 \pm 0.01	0.5
TiO ₂	0.3 \pm 0.01	1.1 \pm 0.03	0.4 \pm 0.01	0.4
Reference	[6]			

The Applicant analysed five batches of *sepiolite*, *diatomaceous earth* and the *feed additive* and the elemental composition derived [6] is presented in Table 1. The experimental values obtained for the *feed additive* samples are in good agreement with the expected/calculated ones, derived from the average values of the preparation containing 90 % (w/w) of *sepiolite* and 10 % (w/w) of *diatomaceous earth*.

Based on the experimental evidence presented the EURL recommends for official control the EN ISO 12677 method based on X-Ray Fluorescence (XRF) spectrometry for the elemental characterisation of the *feed additive*.

For the determination of the capacity of the *feed additive* as such to adsorb Aflatoxin B₁ (AfB₁) the Applicant proposed the following protocol [5]: 1 mg of the *feed additive* was added to 1 ml of a buffer solution (0.1 mM phosphate buffer at pH 7.0) containing AfB₁ at 1 μ g/ml and the suspension was shaken at 37 °C for 90 min. Then it was centrifuged at 14000 rpm for 20 min and the supernatant was filtered for further analysis by high performance liquid chromatography (HPLC) coupled to fluorescence detection (FLD) (excitation wavelength - 365 nm; emission wavelength - 435 nm). The same experimental protocol is applied to the blank sample not containing the *feed additive*.

The adsorption capacity of the *feed additive* is calculated in percentage as a ratio of the difference of AfB₁ content after adsorption experiments in the blank sample (B) and the sample containing the *feed additive* (A) to the content of AfB₁ in the blank sample (B) according to the formula [5]:

$$\text{Adsorption Capacity (\%)} = \frac{B - A}{B} \times 100$$

For the single-laboratory validation [5] and the verification [7,8] of the above mentioned method the Applicant reported the following results: a relative standard deviation for *repeatability* (RSD_r) and *intermediate precision* (RSD_{ip}) of 0.1 % for the adsorption capacity of the *feed additive* ranging from 99.5 to 99.7 % (w/w). Furthermore, the Applicant reported a limit of quantification (LOQ) of 1 ng AfB₁ / ml solution.

Based on the experimental evidence presented the EURL recommends for official control the above mentioned single-laboratory validated and further verified method for the determination of the capacity of the *feed additive* to adsorb Aflatoxin B₁ (AfB₁).

In addition, the EURL recommends that a target value of the adsorption capacity of the *feed additive* as such, along with the conditions of the adsorption experiment, is established. It is important to underline that the adsorption capacity of the product obtained by using the current method shall not be compared with the binding capacity for bentonite (1m558) as specified in the Commission Implementing Regulation (EU) No 1060/2013, because the conditions of the two adsorption experiments are different.

Even though the Applicant provided the EURL with the criteria for the mineralogical composition [2,3] and the relevant experimental data [9] no corresponding method was submitted by the Applicant. Nevertheless, the EURL identified the EN 13925 method based on X-ray diffraction (XRD) [10] which can be recommended for official control for the mineralogical characterisation of the *feed additive*.

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 the current authorisation the EURL recommends for official control:

- the EN ISO 12677 method based on X-Ray Fluorescence (XRF) spectrometry for the elemental characterisation of the *feed additive*.
- the EN 13925 method based on X-ray diffraction (XRD) spectrometry for the mineralogical characterisation of the *feed additive*.
- the single-laboratory validated and further verified method based on the adsorption experiment followed by high performance liquid chromatography with fluorescence detection (HPLC-FLD) for the determination of the capacity of the *feed additive* to adsorb aflatoxin B₁ (AfB₁).

In addition, the EURL recommends that a target value of the adsorption capacity of the *feed additive* as such along with the conditions of the adsorption experiment is established. Furthermore, the adsorption capacity of the product obtained by using the current method shall not be compared with the binding capacity for bentonite (1m558) as specified in the Commission Implementing Regulation (EU) No 1060/2013, because the conditions of the two adsorption experiments are different.

As the unambiguous determination of *sepiolite* and *diatomaceous earth* or the *feed additive* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control for the determination of *sepiolite* and *diatomaceous earth* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the characterisation of the *feed additive*:

- X-ray fluorescence (XRF) spectrometry (EN ISO 12677) and
- X-ray diffraction (XRD) spectrometry (EN 13925)

For the determination of the adsorption capacity of the *feed additive*:

- Adsorption test carried out in a buffer solution at pH 7.0 with a concentration of 1 µg/ml for AfB₁ and 0.1 % (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 *sepiolite and diatomaceous earth* 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. 1831-0019-2019 & Annex I – submission number 1540474739942-2316
- [2] *Application, proposal for Register entry – Annex A
- [3] *Technical dossier, Section II: Identify, characterisation and conditions of use of the additive; methods of analysis
- [4] EN ISO 12677:2011 – *Chemical analysis of refractory products by X-ray fluorescence (XRF) – fused cast-bead method*
- [5] *Technical dossier – Annex_II_6_3
- [6] *Technical dossier – Annex_II_1_3_1_3
- [7] *Supplementary information – 2_Annex II_6_4_Study_Report_APO0009

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- [8] *Supplementary information – 4_Annex II_6_5_Verification_Form_APO0009
- [9] *Technical dossier – Annex_II_2_1_1_0
- [10] EN 13925-1,2:2003; EN 13925-3:2005 – *Non-destructive testing. X-ray diffraction from polycrystalline and amorphous materials. General principles, procedures, instruments*
- *Refers to Dossier no: FAD-2019-0011

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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- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 — Labore Landwirtschaft, Nossen (DE)

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