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Safety and efficacy of bentonite as a feed additive for all animal species

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Abstract

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) received a request from the European Commission to assess the safety and efficacy of bentonite when used as a technological feed additive (substances for reduction of the contamination of feed by mycotoxins) for all animal species. The applicant, EUBA aisbl (European Bentonite Association) representing six companies, submitted to EFSA a technical dossier to support the application. The applicant proposes to use bentonite at the maximum level of 20,000 mg/kg complete feed. The additive apparently interferes with the analysis of aflatoxin B1 in feed. The safety of the additive was already evaluated by the Panel in an opinion delivered in 2012. Bentonites are safe for all animal species, the consumers and the environment when used at a maximum level of 20,000 mg/kg complete feed. The results of a new genotoxicity study reinforced the previous conclusion that smectites are non-genotoxic. Bentonites are not skin irritants but might be mildly irritant to the eye; based on a new study submitted, the additive is not a skin sensitiser. Owing to its silica content, the additive is a hazard by inhalation for the users. The *in vitro* study showed that the di- and tri-octahedral smectites tested can adsorb aflatoxin B1 at different concentrations and at pH 5; however, no adequate *in vivo* studies were available. Therefore, the Panel cannot draw conclusions on the additive's efficacy. The Panel further considers the safety and efficacy conclusions to apply equally to the di- and tri-octahedral smectites under assessment. The FEEDAP Panel posted some recommendations regarding the maximum content of other minerals in the additive and the incompatibilities of the additive with other medicinal substances. The Panel also drew a remark concerning the denomination of the additive and the current regulatory definition of *Bentonite*.

Keywords: technological additives, mycotoxin binder, bentonite, smectites, safety, efficacy

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Amendment: An editorial correction was carried out that does not materially affect the contents or outcome of this scientific output. The sentence in Section 2.1 referring to the Bentonite authorisation as food additive, and the footnote linked to it, have been amended. To avoid confusion, the older version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from EUBA aisbl (European Bentonite Association)[2] for authorisation of the product bentonite, when used as a feed additive for all animal species (category: technological additives; functional group: substances for reduction of the contamination of feed by mycotoxins).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 24 October 2016.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the additive bentonite, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

Bentonite is authorised as a feed additive[3] under the category technological additives and the functional groups:

- 'binder', 'substance for control of radionuclide contamination' and 'anticaking agent' (1m558i), for all animal species and
- 'substance for reduction of the contamination of feed by mycotoxins (aflatoxin B1 (AfB1))' for ruminants, poultry and pigs (1m558).

The authorisation of Bentonite as food additive expired on the 31st of May 2013.[4]

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has delivered several scientific opinions on the safety and/or efficacy of bentonites when used as feed additives (EFSA FEEDAP Panel, 2011a,b, 2012a[5], 2013, 2014, 2016.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[6] in support of the authorisation request for the use of bentonite as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[7] and the applicable EFSA guidance documents.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] EUBA aisbl, Rue des Deux Eglises 26 box 2 B-1000 Brussels, Belgium. There are six companies belonging to this consortium and which provided data to the current application. The companies have been referred to in the text as (A1) to (A6).
[3] Commission Implementing Regulation (EU) No 1060/2013 of 29 October 2013 concerning the authorisation of bentonite as a feed additive for all animal species, OJ L 289, 31.10.2013, p. 33.
[4] Commission Regulation (EU) No 380/2012 of 3 May 2012 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the conditions of use and the use levels for aluminium-containing food additives. OJ L 119, p. 14.
[5] The FEEDAP Panel notes that the dossier supporting the application behind the EFSA FEEDAP Panel 2012a opinion was submitted by the same applicant as the current submission.
[6] FEED dossier reference: FAD-2016-0051.
[7] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as peer-reviewed scientific papers or other scientific reports.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of bentonite in animal feed. The Executive Summary of the EURL report can be found in Annex A.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Bentonite is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on technological additives (EFSA FEEDAP Panel, 2012b), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011c), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

The applicant is representing the consortium of the European Bentonite Association (EUBA aisbl – composed by six companies and founded as the official body representing the European bentonite producers) and is now seeking the authorisation of bentonite when used as AfB1 binder for all animal species (category: technological additives; functional group: substances for reduction of the contamination of feed by mycotoxins).

3.1. Characterisation

3.1.1. Characterisation of the additive

The applicant used in the dossier the term 'bentonite' as a synonym for both di-octhaedral and tri-octahedral smectites while the term bentonite generally describes any highly colloidal and plastic clay material composed largely, but not exclusively, of montmorillonite (a species of di-octahedral smectite) without reference to a particular origin.

Smectites are phyllosilicates characterised by a sheet structure made of layers of polyhedra of silicon oxide with tetrahedral coordination between which there is an octahedral layer. The octahedral layers contain atoms of aluminium, iron (II or III) or magnesium in their interior. When the cation of the octahedral layer is trivalent, as for example in the case of aluminium, one of every three cation positions is unoccupied; the octahedral layer has the structure of gibbsite, $Al(OH)_3$, and the smectite is di-octahedral: this group includes montmorillonite, beidellite and nontronite. In tri-octahedral smectites, the cation in the octahedral layer is divalent and, as a result, all the cation positions are occupied; this gives rise to an octahedral layer with the geometry of brucite, $Mg(OH)_2$, and it is the case for the saponites and hectorites (Hurlbut and Klein, 1982).

The smectite content analysed by X-ray diffraction and batch-to-batch variation of the samples from all companies of the consortium are shown in Table 1.[9] It should be noted that some samples contained di-octahedral smectite (montmorillonite), whereas others contained tri-octahedral smectite (saponite). A full mineralogical analysis was provided by five companies, detailing the mineral composition in addition to smectite (Table 1).

[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2016-0051-bentonite.pdf
[9] Technical Dossier/Section II/Annex II_1_2 to II_1_7.

Table 1: Mineralogical analysis (including content of smectite and other minerals) of the batches provided by each company

Company	No. of batches (Cation occupancy)	Mineralogical analysis (%)[a]		
		Smectite	Other minerals	
A1	2 (di-octahedral)	85/97	Opal 1/0 Illite 0/7 Plagioclase feldspar 0/3	Quartz 1/2 Calcite 0/2
A2	3 (di-octahedral)	70.9–86.5	Quartz 4.3–14.7 Plagioclase 1.4–6.7 Calcite 0.4–1.3 Muscovite 0–11 Kaolinite 0–6.2	Dolomite 0–1.7 Opal-cristobalite 0–1.6 K-feldspar 0–1.1 Phlogopite 0–1.2
	2 (tri-octahedral)	85.5/94.0	Quartz 2.5/3 K-feldspar 1.4	Calcite 0.4/1.3 Plagioclase 0.6/0.8
A3	2 (di-octahedral)[b]	71.9/76.0	Not analysed	
A4	1 (di-octahedral)	74.7	Cristobalite 11.6 Calcite 3.4 Feldspar and illite 3.2	Clinoptilolite 3.1 Quartz 0.4 Dolomite 0.4
A5	3 (tri-octahedral)	92–94	Quartz 2–3 Feldspar 1 Plagioclases 1	Calcite 0–3 Illite 0–2
A6	1 (tri-octahedral)	88.0	Dolomite 5 Quartz 4	Feldspar 3
	1 (di-octahedral)	89.0	Feldspar 5 Quartz 3	Gypsum 3

(a): Individual values (when up to two figures) or range (more than two figures).
(b): As determined by the magnesium and aluminium oxides content of the chemical analysis.

According to Regulation 1060/2013 (concerning the authorisation of bentonite as a feed additive for all animal species), bentonite used as a mycotoxin binder should contain ≥ 70 % smectite (di-octahedral montmorillonite), < 10 % opal and feldspar and < 4% quartz and calcite. Although all samples contained more than 70% smectite, two batches from company A2, three batches from company A5 and one batch from company A6 containing tri-octahedral smectite do not meet the current legal specification for di-octhaedral montmorillonite minimum level. The FEEDAP Panel also notes that one sample of di-octahedral smectite from company A1, three samples of di-octahedral smectite provided by A2, two samples of tri-octaedral smectite from A5[10] and one sample of tri-octahedral smectite from A6[11] exceeded the limit for quartz and calcite set by Regulation 1060/2013 (< 4%) for bentonite when used as a mycotoxin binder. The di-octahedral smectite from the company A4 has a high content of cristobalite, a polymorph of quartz.

The main chemical elements which characterise the various clay samples are silicon dioxide (SiO_2), aluminium oxide (Al_2O_3) and magnesium oxide (MgO). They were determined using X-ray fluorescence analysis, inductively coupled plasma optical emission spectrometry or atomic absorption spectrometry.[12,13] The results of the analyses are summarised in Table 2.

Table 2: Chemical analysis (% values) of the main elements found in the clay samples

Company	No. of batches (Cation occupancy)	Mineral oxides (%)[a]				
		SiO_2	Al_2O_3	MgO	Na_2O	Fe_2O_3
A1	1 (di-octahedral)	59.0	17.8	2.2	2.7	5.7
A2	3 (di-octahedral)	57.0–63.0	18.4–19.3	2.4–4.9	0.4–2.1	1.2–1.5
	2 (tri-octahedral)	55.6/58.5	4.7/6.7	21.8/24.6	0.3/0.4	0.3/0.4

[10] The compliance of the other sample from company A5 remains with uncertainties as the opal content was not provided.
[11] The compliance of the other sample from company A6 remains with uncertainties as the opal and calcite contents were not provided.
[12] Technical Dossier/Section II/Annex II_6_3 and II_6_4.
[13] Technical Dossier/Section II/Annex II_1_8 to II_1_13.

Company	No. of batches (Cation occupancy)	Mineral oxides (%)[a]				
		SiO_2	Al_2O_3	MgO	Na_2O	Fe_2O_3
A3	3 (di-octahedral)	53.4–55.0	16.6–17.0	2.9–4.0	2.2–3.1	4.0–5.3
A4	5 (di-octahedral)	55.1–58.4	22.0–26.1	2.1–3.1	1.0–1.4	3.5–3.9
A5	3 (tri-octahedral)	53.8–54.5	7.6–9.4	18.3–20.4	2.2–2.4	2.9–3.3
A6	1 (tri-octahedral)	53.97	4.02	25.5	2.0	1.2
	1 (di-octahedral)	61.70	18.29	2.45	3.3	4.8

(a): Individual values (when up to two figures) or range (more than two figures).

From the comparison between di-octahedral (montmorillonite) and tri-octahedral smectites (saponites), it can be noted that the latter characteristically have lower Al_2O_3 and higher MgO contents. Values regarding the other compounds are comparable between the two smectite types.

At least three batches of the different products from all companies were each analysed for lead and cadmium (25 samples analysed).[14] In all cases, values were below the maximum levels specified in the Directive on Undesirable substances[15] of 30 mg Pb/kg and 2 mg Cd/kg for additives classified as *binders*. Arsenic was analysed in at least three batches from five companies (21 samples analysed). Mercury was analysed in at least three batches from four companies (18 samples analysed). Although arsenic and mercury are not specifically regulated for binders, Directive 2002/32/EC sets maximum contents for arsenic in mineral feed of 12 mg/kg and 20 mg/kg in magnesium oxide, which can be taken as indicative. With the exception of one sample (company A5, 21.2 mg/kg), the analysed values for arsenic (range 0.1–19.9 mg/kg) comply with this latter maximum limit. The arsenic content in feed resulting from the use of the additive produced by company A5 would be 0.53 mg As/kg feed; this concentration is below the maximum limit of arsenic (2 mg/kg) in complete feed set by Directive 2002/32/EC. Mercury contents were low and would result in feed concentrations below the value accepted for complete feedingstuffs (0.1 mg Hg/kg).

Dioxins were measured in at least three batches from each company (except company A4 and A6 which assayed only one and two batches, respectively) and ranged between 0.08 and 0.55 ng PCDD/F (polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofuran WHO toxic equivalent (TEQ))/kg. Dioxin and dioxin-like polychlorinated biphenyls (PCBs) were measured in at least three batches from four companies and levels were found to range between 0.12 and 0.44 ng PCDD/F-PCBs (WHO-TEQ)/kg.[16] All analysed levels were below the limits set for 'clays' in Directive 2002/32/EC.

3.1.1.1. Physical properties

The bulk density of the smectite samples shows a wide variation (600–1260 kg/m^3), as might be expected given the differences in mineral composition.

All samples examined were characterised by a low particle size,[17] as determined by laser diffraction, and, where measured, a correspondingly high dusting potential,[18] as measured by the Stauber–Heubach method (Table 3).

[14] Technical Dossier/Section II/Annex II_1_14 to II_1_19. (Analysis of lead, cadmium, arsenic and mercury).
[15] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10.
[16] Technical Dossier/Section II/Annex II_1_20 to II_1_27. The FEEDAP Panel notes that data from companies A1, A3 and partially those from A2 are the same as those provided in the previous submission of the same applicant (Dossier FAD-2010-0233).
[17] Technical Dossier/Section II/Annex II_1_28 to II_1_32. The FEEDAP Panel notes that data from companies A1, A2, A3 and A6 are the same as those provided in the previous submission of the same applicant (Dossier FAD-2010-0233).
[18] Technical Dossier/Section II/Annex II_1_33 to II_I_35. The FEEDAP Panel notes that data from companies A1, A2, A3 and A6 are the same as those provided in the previous submission of the same applicant (Dossier FAD-2010-0233).

Table 3: Particle size distribution and dusting potential of the smectite samples (n=number of batches)

Company	Particle size distribution	Dusting potential (g/m^3)
A1	< 10 μm: 17.6–18.6% < 50 μm: 63.8–67.7% < 100 μm: 88.3–92.8% (n = 3)	ND
A2	< 10 μm: 11.4–18.9% < 50 μm: 46.3–64.2% < 100 μm: 71.8–84.5% (n = 3)	11.4 (n = 1)
A3	< 10 μm: 12.4–25.3% < 50 μm: 67.0–78.6% < 100 μm: 91.2–95.8% (n = 3)	ND
A4	< 10 μm: 3.0–3.3% < 50 μm: 14.0–15.3% < 100 μm: 100% (n= 3)	1.6–2.0 (n = 3)
A6	< 45 μm: 67.0–68.8% (n = 3)	8.8–12.3 (n = 3)

ND: Not determined

3.1.1.2. Production process

The additives are obtained by mining from a variety of locations, including some within the European Union. Extraction is followed by crushing, drying, grinding and packaging for shipment. Some producers use a 'soda activation' process with calcium bentonites, using sodium carbonate to increase the swelling and adsorption behaviour and to raise the pH.

3.1.2. Stability and homogeneity

3.1.2.1. Shelf-life

Studies demonstrating stability are generally not required for mineral-based products which are assumed stable. However, three studies were provided by two of the companies. In the two studies from company A3, the smectite content (determined by the methylene blue method) showed no variation after three or four years' storage.[19] The third study from company A6 showed that the mineral composition measured by X-ray diffraction of three batches of bentonite remained constant after six years' storage.[20]

3.1.2.2. Homogeneity

A single sample of smectite (from company A6), which included cobalt (100 mg/kg feed) as a microtracer, was mixed with an unspecified feed.[21] A total of 15 samples were collected and assayed for the cobalt content. Using this method, the microtracer showed good distribution with a coefficient of variation of 4.2%.[22]

3.1.2.3. Interference with the analysis of mycotoxins in feed

The applicant provided a study in which a feed for dairy cows was spiked with 5 μg/kg aflatoxin B1 in the presence or absence of the samples of bentonite provided from all companies (supplemented feed with 20,000 mg/kg of bentonite, either di- or tri-octahedral). In the absence of smectites,

[19] Technical Dossier/Section II/Annexes II_4_1 and II_4_2. The FEEDAP Panel notes that these data are the same as those provided in the previous submission of the same applicant (Dossier FAD-2010-0233).
[20] Technical Dossier/Section II/Annex II_4_3. The FEEDAP Panel notes that these data are the same as those provided in the previous submission of the same applicant (Dossier FAD-2010-0233).
[21] Technical Dossier/Section II/Annex II_4_6. The FEEDAP Panel notes that these data are the same as those provided in the previous submission of the same applicant (Dossier FAD-2010-0233).
[22] Technical Dossier/Supplementary Information/August 2017.

aflatoxin was recovered at the expected concentration. However, in the presence of both di- and tri-octahedral smectites, recoveries of aflatoxin were reduced by approximately 40%.

3.1.3. Conditions of use

The applicant proposes the use of bentonite as an aflatoxin B1 binder in feed for all animal species at a maximum inclusion level 20,000 mg/kg complete feedingstuffs.

3.2. Safety

In the previous opinion based on a technical dossier on bentonite submitted by the same applicant of the current submission, the safety of the additive was assessed (EFSA FEEDAP Panel, 2012a).[23]

3.2.1. Safety for the target species

The FEEDAP Panel concluded that bentonite at 20,000 mg/kg complete feed was safe for all animal species (EFSA FEEDAP Panel, 2012a). The conclusion was based on tolerance studies with a tri-octahedral smectite[24] in lactating cows, piglets and chickens for fattening, and two literature studies[25] on laying hens and fish.

In the previous opinion, the Panel also noted that the data available suggested that addition of bentonites to diets was incompatible with the use of robenidine as a coccidiostat, and that levels of bentonite higher than 0.5% are also expected to reduce the effectiveness of other coccidiostats (EFSA FEEDAP Panel, 2012a). Moreover, the FEEDAP Panel drew a recommendation concerning the simultaneous use of bentonites with other medicinal substances.

No new data or studies have been provided in the current application.

3.2.2. Safety for the consumers

The FEEDAP Panel concluded in its previous opinion that there was no concern for the safety of consumers of food products derived from animals fed diets containing bentonite" (EFSA FEEDAP Panel, 2012a).

In the current submission, the applicant has provided a new genotoxicity study.

Bentonite (di-octahedral smectite) was examined for the potential to induce gene mutations using mouse lymphoma L5178Y cells (OECD guideline 476) both with and without metabolic activation with S9 from livers of phenobarbital/β-naphthoflavone treated rats.[26] The dose tested under both conditions ranged from 312.5 to 5,000 μg/mL. No increases in mutant frequency were seen at any dose with or without activation thus it is concluded that bentonite is not mutagenic *in vitro* under the conditions used.

3.2.3. Safety for the users

The FEEDAP Panel concluded in its previous opinion that bentonites were not skin irritants but might be mildly irritant to the eye. Skin sensitisation was not considered, but the Panel noted that bentonites are widely used in cosmetics. The Panel also considered it prudent to assume that all bentonite dusts posed a hazard to those handling the additive (EFSA FEEDAP Panel, 2012a).

In the current submission, the applicant has provided two studies relating to users safety, which address the data gaps identified previously.

An acute inhalation toxicity (nose only) study was performed using six Wistar rats (three males and three females) exposed to 0 or 5.3 mg bentonite (di-octahedral smectite)/L air for 4 h followed by an observation period of 14 days (OECD guideline 436).[27] One death occurred at 170 min after exposure. Common abnormalities noted during the study included increased respiratory rate, hunched posture, pilo-erection and wet fur. Surviving animals recovered to appear normal on day 4 post-exposure. With the exception of one female, animals had reasonable body weight gain during the observation period. No macroscopic abnormalities were detected at necropsy among animals that survived until the end of

[23] This previous submission regarded Bentonite as a technological additive and functional group 'binders, anticaking agents and substances for control of radionuclide contamination'.
[24] The applicant clarified that the test item used in the tolerance studies was a tri-octahedral smectite.
[25] The identity of the smectite (i.e. di-octahedral or tri-octahedral smectite) was unknown.
[26] Technical Dossier/Section II/Annex III_2_4.
[27] Technical Dossier/Section II/Annex III_3_1.

the 14-day observation period. Pale lungs were noted at necropsy in the animal that died during the course of the study.

A local lymph node assay (LLNA) was performed using female CBA/Ca mice (OECD guideline 429).[28] The concentration of bentonite (di-octahedral smectite) at which no toxic signs were observed (25%) was selected in a preliminary screening test and used as the highest dose investigated. Three groups (five mice/group) were treated with 50 μL (25 μL per ear) of the test item as a suspension in propylene glycol at concentrations of 25%, 10% or 5% w/w. No proliferation response was elicited by the treatment. No clinical signs, mortality or changes in body weight were observed. The test item under assessment is not considered a skin sensitiser.

The FEEDAP Panel also notes that the additive contains crystalline silica (range 1–14.7%). Inhalation of silica is known to be hazardous and is associated with increased risk of lung cancer and the industrial disease, silicosis. The EC Scientific Committee on Occupational Exposure Limits (SCOEL) concluded that silicosis is the main effect of respirable crystalline silica (European Commission, 2003) and proposed an occupational exposure limit of 0.05 mg/m^3 of respirable silica dust.

3.2.4. Safety for the environment

The FEEDAP Panel concluded in its previous opinion that bentonites are ubiquitous in the environment, being natural components of soil; therefore, it was not expected that its use as a feed additive would adversely affect the environment. No new data or studies have been provided in the current submission.

3.2.5. Conclusions on the safety of the additive

The FEEDAP Panel reiterates its previous conclusion that bentonites are safe for all animal species, the consumers and the environment when used at a maximum level of 20,000 mg/kg complete feed. The results of a newly submitted genotoxicity study reinforced the previous conclusion that smectites are non genotoxic.

The FEEDAP Panel maintains its previous conclusion that bentonites are not skin irritants but might be mildly irritant to the eye. Based on a new study submitted, the additive is not a skin sensitiser. Owing to its silica content, the additive is a hazard by inhalation for the users.

The Panel considers these conclusions to apply equally to the di- and tri-octahedral smectites under assessment.

3.3. Efficacy

As the substances used for the reduction of the contamination of feed by mycotoxins do not affect the characteristics of feed but produce their effects in the animal, efficacy can only be fully demonstrated by *in vivo* studies. *In vitro* studies are considered as a screening tool for the potential of substances to act as mycotoxin binders. However, *in vitro* studies alone cannot be used to demonstrate efficacy under practical conditions (EFSA FEEDAP Panel, 2012b).

***In vitro* study**

The binding capacity of the additive towards AfB_1 has been studied *in vitro*, according to the provisions set by Regulation (EU) No 1060/2013.[29] For each batch of smectite from the different producers (one sample each from companies A1 and A3, two samples from company A6, three samples each from companies A4 and A5, five samples from company A2), an adsorption isotherm was prepared with a concentration of 4 μg AfB_1/mL at pH 5. The inclusion level of the additive was 0.2 mg/mL (0.02% w/v) in all cases.

A control (a solution with mycotoxin without binder) and test solutions containing both mycotoxin and binder were prepared. The suspensions were shaken and incubated at 37°C for 60 min and then centrifuged for 15 min. AfB1 concentrations were determined in the supernatants by high performance liquid chromatography. The binding capacity was calculated from the difference in mycotoxin concentration in the control and the supernatant of the incubated binder sample. The results indicate that the efficacy of the additive in adsorbing AfB_1 varied from 90.0% to 95.3% at pH 5.

[28] Technical Dossier/Section II/Annex III_3_10.
[29] Technical Dossier/Section IV/Annex IV_1 to IV_7.

In vivo study

The applicant submitted only one study on dairy cows performed with the product from company A6.[30] The study reports a survey carried out in ten Italian dairy farms with a total of 742 animals. The cows ingested naturally contaminated feed ingredients; analysis of corn and complementary concentrates from three farms revealed an average content AfB1 content of 26.1 and 4.9 ng/kg, respectively. The authors determined the reduction in AfB1 in feed and in milk following the application of bentonite in feed.

The FEEDAP Panel notes that the study cannot be considered suitable to scientifically demonstrate the efficacy of the product because of several inadequacies in the experimental design: e.g. no control animals, different duration of the treatments.

No further studies *in vivo* were submitted.

Conclusions on efficacy for the target species

The results of the *in vitro* study show that the samples of the di- and tri-octahedral smectites tested can adsorb aflatoxin B1 at different concentrations and at pH 5. However, no adequate studies were available to confirm these effects *in vivo*. Therefore, the FEEDAP Panel cannot draw conclusions on the efficacy of the additive as AfB1 binder for all animal species.

4. Conclusions

The additive apparently interferes with the analysis of aflatoxin B1 in feed.

The FEEDAP Panel reiterates its previous conclusion that bentonites are safe for all animal species, the consumers and the environment when used at a maximum level of 20,000 mg/kg complete feed. The results of a newly submitted genotoxicity study reinforced the previous conclusion that smectites are non-genotoxic.

The FEEDAP Panel maintains its previous conclusion that bentonites are not skin irritants but might be mildly irritant to the eye. Based on a new study submitted, the additive is not a skin sensitiser. Owing to its silica content, the additive is a hazard by inhalation for the users.

In the absence of adequate *in vivo* studies, the FEEDAP Panel cannot draw conclusions on the efficacy of the additive for all animal species.

The Panel considers the safety and efficacy conclusions to apply equally to the di- and tri-octahedral smectites under assessment.

5. Recommendations

The FEEDAP Panel recommends to maintain the limits for opal and feldspar (< 10%) and for quartz and calcite (< 4%), as set in the Regulation (EU) No 1060/2013, should the additive be authorised.

The other provisions currently drawn in the Regulation (EU) No 1060/2013 concerning the simultaneous oral use of bentonites with macrolides and in poultry with robenidine should be also maintained.

6. Remark

In view of the FEEDAP Panel, the term 'Smectites' defines the additive better than the term 'Bentonite'; the latter often refers only to montmorillonite, a di-octahedral smectite. The FEEDAP Panel also notes that the present specification of *Bentonite* when used as a mycotoxin binder in Commission Implementing Regulation (EU) No 1060/2013, indicating a minimum content of di-octahedral montmorillonite of 70%, would exclude in practice all tri-octahedral smectites.

Documentation provided to EFSA

1) Bentonite for all animal species. September 2016. Submitted by EUBA aisbl.
2) Bentonite for all animal species. Supplementary information. December 2016. Submitted by EUBA aisbl.
3) Bentonite for all animal species. Supplementary information. August 2017. Submitted by EUBA aisbl.
4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Bentonite.
5) Comments from Member States.

[30] Technical Dossier/Section IV/Annex_IV_8.

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Abbreviations

AAS	atomic absorption spectrometry
EUBA	European Bentonite Association
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
ICP-AES	inductively coupled plasma atomic emission spectroscopy
LLNA	local lymph node assay
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofuran
TEQ	toxic equivalent
SCOEL	Scientific Committee on Occupational Exposure Limits
WHO	World Health Organization
XRD	X-ray diffraction
XRF	X-ray fluorescence

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for bentonite

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.