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

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) was asked to deliver a scientific opinion on the safety and efficacy of a preparation of algae interspaced bentonite when used as aflatoxin B_1 binder for all animal species. The additive is composed of bentonite and algae belonging to *Ulva* spp. The additive is considered safe for weaned piglets, dairy cows and chickens for fattening at the maximum recommended dose of 125 mg/kg complete feed (a wide margin of safety is established in weaned piglets and dairy cows); this conclusion is extrapolated to all animal species. The additive is not genotoxic. As bentonite is essentially not absorbed from the gut lumen and algae from *Ulva* spp. are not expected to be of concern for human consumption, the FEEDAP Panel considers that the use of the additive in animal nutrition is safe for consumers. The additive is not an irritant to the skin or the eyes and it is considered to have low inhalation toxicity. However, the additive has a high dusting potential and contains a high proportion of fine particles. A high level of inhalation exposure to an inert dust may be hazardous. In the absence of data, the Panel could not conclude on dermal sensitisation. As the components of the additive are of natural origin (soil and marine environment), it is not expected that the use of the additive in animal nutrition would adversely affect the environment. The FEEDAP Panel could not conclude on the efficacy of the additive for all animal species.

Keywords: technological additives, mycotoxin binder, bentonite, algae, *Ulva* spp., all animal species

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Summary

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) was asked to deliver a scientific opinion on the safety and efficacy of a preparation of algae interspaced bentonite when used as aflatoxin B_1 (AfB_1) binder for all animal species (category: technological additives; functional group: substances for reduction of the contamination of feed by mycotoxins).

The additive is composed of bentonite feed grade and algae belonging to *Ulva* spp. harvested in the western coasts of France.

The additive is considered safe for weaned piglets, dairy cows and chickens for fattening at the maximum recommended dose of 125 mg/kg complete feed. This conclusion is extrapolated to all animal species. A wide margin of safety is established in weaned piglets and dairy cows as no adverse effects are seen when given 80 times the maximum recommended dose.

The additive is not genotoxic. As bentonite is essentially not absorbed from the gut lumen and algae from *Ulva* spp. are not expected to be of concern for human consumption, the FEEDAP Panel considers that the use of the additive in animal nutrition is safe for consumers.

The additive is not an irritant to the skin or the eyes and it is considered to have low inhalation toxicity. However, the additive has a high dusting potential and contains a high proportion of fine particles. A high level of inhalation exposure to an inert dust may be hazardous. In the absence of data, the Panel could not conclude on dermal sensitisation.

The components of the additive (bentonite and algae from *Ulva* spp.) are widely distributed in the environment being natural components of the soil and marine environment. Therefore, it is not expected that the use of the additive in animal nutrition would adversely affect the environment.

The results of the *in vitro* study indicate that the additive can adsorb AfB_1 at different concentrations and pH values. However, no adequate studies were available to confirm these effects *in vivo*. Therefore, the FEEDAP Panel cannot conclude on the efficacy of the additive for all animal species.

The FEEDAP Panel made recommendations regarding the specifications and conditions of use of the additive.

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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 Olmix SA[2] for authorisation of the product preparation of algae interspaced 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 21 April 2015.

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 preparation of algae interspaced bentonite, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

Bentonite is authorised as a feed additive, category: technological additives, functional groups: binder, anticaking agent (1m558i), substance for control of radionuclide contamination for all animal species and substance for reduction of the contamination of feed by mycotoxins (aflatoxin B_1 (AfB_1)) for ruminants, poultry and pigs (1m558).[3]

Bentonite is also authorised as a food additive.[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, 2013).

Algae are included in the Catalogue of feed materials.[5]

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 the product preparation of algae interspaced 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 and the applicable EFSA guidance documents.

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 to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the preparation of algae interspaced bentonite in animal feed. The Executive Summary of the EURL report can be found in Annex A.[7]

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Olmix SA. ZA du Haut Bois, 56580, Brehan, France.
[3] Commission 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 No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of theEuropean Parliament and of the Council by establishing a Union list of food additives, OJ L 295, 12.11.2011, p. 15.
[5] Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials, OJ L 29, 30.1.2013, p. 1
[6] FEED dossier reference: FAD-2014-0047.
[7] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrepfad_2014_0047_algae_interspaced_bentonite.pdf

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the preparation of algae interspaced bentonite is in line with the principles laid down in Regulation (EC) No 429/2008[8] 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 the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012c), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012d) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012e).

3. Assessment

The application is for the authorisation of the preparation of algae interspaced bentonite when used as AfB_1 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. Manufacturing process

The additive can be manufactured following a dry or a wet process.[9]

In the dry process, algae belonging to *Ulva* spp. are washed with tap water and crushed. Bentonite and algae are mixed, then dried (70–90°C), ground and sieved. The powder contains about 7% algae.

In the wet process, lactic acid is added as a preservative to the mixture of algae and bentonite. The algal cells are pressed, the solid fraction removed and the liquid part is mixed with bentonite. The resulting paste preparation is stored in cisterns for not more than 30 days and dried.

From the description provided, it would appear that the dry process results in a product containing whole algal cells, while the wet process results in a product containing an algal extract. The FEEDAP Panel notes that two different but related products are obtained.

3.1.2. Characterisation of the additive

The additive contains by specification 60–96% of smectite, 3–15% interspaced organic dry matter (DM) derived from the algae and 4–60 mg uronic acids/g DM.[9] The product is a pale green to beige/greenish free-flowing powder with a bulk density of 631 kg/m^3.[10]

Five batches of the additive were analysed. Total organic carbon analysed by flash dynamic combustion indicated an average algae content in the additive of 7.0% (range 4.6–10.4%).[11] Interspaced organic matter was 6.4% (range 5.0–8.0%) based on thermal gravimetric analysis (TGA) and differential thermal analysis (DTA).[12] Uronic acids were 9.1 mg/g DM (range 6.9–11.9 mg/g DM),[13] and iodine was 9.2 mg/kg (range 4.8–15 mg/kg).[14]

The mineral fraction of the same batches was analysed by X-ray powder diffraction (XRD) resulting in 85.2% smectite (range 78.8–89.9%), 6.8% quartz (range 5.3–8.5%), 2.9% feldspar (range 2.4–3.2%), 1.9% plagioclase (range 1.3–2.2%), 1.5% dolomite (0.4–3.9%), 0.6% calcite (range 0.5–5.0%) and 0.2% siderite (range 0.1–0.3%).[11] The chemical composition of the mineral fraction as determined by X-ray fluorescence was: SiO_2 43.1% (range 39.7–46.1%); MgO 12.9% (range 12.1–13.5%); Al_2O_3 10.0% (range 9.1–10.6%); Na_2O 9.1% (range 8.5–9.9%); Fe_2O_3 2.9% (range 2.5–3.4%); CaO 2.6% (range 1.3–6.5%); K_2O 1.9% (range 1.6–2.1%) and SO_3 1.2% (range 0.5–1.6%).[15]

[8] 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.
[9] Technical dossier/Section II/Sect. II Identity.
[10] Technical dossier/Section II/Annex_II_1_11 and 17.
[11] Technical dossier/Section II/Annex_II_1_1.
[12] Technical dossier/Section II/Annex_II_1_7.
[13] Technical dossier/Section II/Annex_II_1_5.
[14] Technical dossier/Section II/Annex_II_1_6.
[15] Technical dossier/Section II/Annex_II_1_2.

Loss on ignition (950°C) was 15.1% (range 12.9–16.9%) and $CaCO_3$ content (four batches) was 8.9% (range 8.7–9.2%).[16]

Three additional batches of the additive were analysed for moisture, heavy metals and arsenic, dioxins, dioxin-like polychlorinated biphenyls (PCBs) and non-dioxin-like PCBs.[17] Moisture ranged from 7.0 to 7.1%. Values for lead (6.0–6.1 mg/kg), cadmium (< 0.19 mg/kg), mercury (0.02 mg/kg), fluoride (< 76 mg/kg), arsenic (4.0–4.2 mg/kg), dioxins (0.08–0.4 ng WHO-PCDD/F-TEQ/kg), dioxin-like PCBs (0.1–0.4 ng WHO-PCDD/F-PCB-TEQ/kg) and non-dioxin-like PCBs (2.80 μg/kg) do not raise any safety concerns.[18] The same batches were analysed for asbestos by transmission electron microscopy, which was absent in all samples.[19] Three other batches were analysed for levels of aerobic mesophilic bacteria (< 30,000 colony-forming unit (CFU)/g) and sulfite-reducing anaerobes (< 10 CFU/g). Results showed compliance with the action limits set.[20] Levels of *Salmonella*, yeasts and enterobacteria were analysed but data were not submitted.

Each commercial batch of bentonite is analysed for heavy metals and arsenic before entering the production process. Additionally, fluorine, dioxins and dioxins-like PCBs are analysed once per year per origin.[9]

Each batch of algae is analysed for lead, cadmium, arsenic, mercury and fluorine levels. Once per year per origin, algae are also checked for dioxins, dioxins-like PCBs, non-dioxin-like PCBs, inorganic arsenic and nitrites content and subjected to genetic identification by polymerase chain reaction (PCR) to confirm identity (*Ulva* spp.).[21]

Binding capacity analysed in one batch according to the method specified in Commission Regulation 1060/2013[3] was 96.7%.[22]

Particle size distribution was measured in four batches of the additive by laser diffraction.[23] Results showed that 95.5% of the particles (range 94.8–96.5%) had a diameter below 100 μm, 83.9% of the particles (range 83.2–85.2%) had a diameter below 50 μm and 43.9% of the particles (range 43.1–45.4%) had a diameter below 10 μm. The mean dusting potential (Stauber–Heubach) of the same batches was 12.8 g/m^3.[24]

The content of crystalline silica in the dust filters used in the dusting potential analysis was analysed in the four batches.[25] Results showed that quartz was present at 61.6 mg/g dust and cristobalite at 173.1 mg/g dust.

3.1.2.1. Interference with the analysis of aflatoxins

The potential interference of the additive with the analysis of aflatoxins in feed was examined. The recovery of AfB_1 in ruminant feed spiked with AfB_1 solution to obtain the maximum authorised level was similar in unsupplemented (control) and supplemented (50 mg additive/kg) feeds (88.2 and 88.9%, respectively). Therefore, no interaction of the additive on the analytical determination of AfB_1 was observed.

3.1.3. Stability and homogeneity

The shelf life of the additive has been studied by comparing the binding capacity (AfB_1 adsorption) of two batches of the additive, one produced in 2011 and the second in 2013. Both batches were tested in late 2013. This approach does not provide any information on the shelf life of the same batch of the additive over time and therefore, it cannot be considered as appropriate.

The stability in a premixture was tested by adding the additive at 5% to a premixture (MMi.S) already containing 39% bentonite and 12% of algae. The stability of the additive has been studied by comparing the binding capacity (AfB_1 adsorption) of two batches of the premixture, one produced in 2015 and the second in 2016. Both batches were tested in mid-2016. Given the composition of the premixture (which contains bentonite and algae in addition to the additive) and the study design, these data cannot be further considered to assess stability.

[16] Technical dossier/Section II/Annex_II_2_13.
[17] Technical dossier/Section II/Annex_II_2_14.
[18] Technical dossier/Section II/Annex_II_1_8 and 9.
[19] Technical dossier/Section II/Annex_II_1_11.
[20] Technical dossier/Section II/Annex_II_1_10.
[21] Technical dossier/Section II/Annex II_2_6.
[22] Technical dossier/Section II/Annex_II_4_1.
[23] Technical dossier/Section II/Annex_II_1_13 and 14.
[24] Technical dossier/Section II/Annex_II_1_15.
[25] Technical dossier/Section II/Annex_II_1_16.

The effects of extrusion were tested in one batch of the additive under two different conditions of temperature and pressure (130°C/30 bars and 150°C/20 bars). No differences in the parameters analysed (apparent density, moisture, dry matter, organic matter, calcium and nitrogen content) were found before and after extrusion under the two above described conditions; X-ray diffraction indicated that the layered structure of the product was preserved.[26] However, the binding capacity of the additive after extrusion was not tested.

The capacity to homogeneously distribute in complete feed was measured in one batch of pig, poultry and ruminant mash feeds supplemented with the additive at a concentration of 0.05 g/kg.[27] After mixing, subsamples of pig (10), poultry (10) and ruminant (9) feeds were analysed for aluminium content. The analyses of aluminium showed coefficients of variation of 7.8, 9.8 and 11.6% in pig, poultry and ruminant feeds, respectively.

3.1.4. Conditions of use

The preparation of algae interspaced bentonite is intended to be used in premixtures and feedingstuffs for all animal species and categories at a recommended dose of 10–125 mg/kg complete feed. No withdrawal period is foreseen.

3.2. Safety

3.2.1. Safety for the target species

In order to support the safety for all animal species, the applicant has provided three tolerance studies in piglets, dairy cows and chickens for fattening.

3.2.1.1. Safety for weaned piglets

A total of 96 Pietrain × (Duroc × Landrace) male piglets (age at start 26 days) were randomly allotted to four dietary treatments each containing six replicates of four animals.[28] The diet (10 MJ net energy/kg, 18% crude protein (CP) and 1.2% digestible lysine), mainly based on barley, soybean and milk whey, was supplemented with the additive at 0 (control), 125 (1×, maximum recommended dose), 1,250 (10×) and 10,000 (80×) mg/kg complete feed. Feed in pellet form was offered *ad libitum* for 42 days. The concentration of the additive was confirmed by analysis of aluminium as the marker. The experimental design was a randomised complete block with six blocks and four treatments.

Body weight and feed consumption were recorded at 2-week intervals (14, 28 and 42 days of trial), and average daily gain (ADG), daily feed intake (FI) and feed to gain ratio (FG) were calculated for each period and for the overall experiment. Blood samples were obtained from two piglets per pen on day 42 for general haematology[29] and blood chemistry.[30] Data were analysed with ANOVA and Duncan's multiple range test was used to evaluate differences among treatment means. The pen was considered the experimental unit.

Mortality was very low (only one piglet from the recommended dose group died during the study). No significant differences were observed among groups for FI (808, 748, 789 and 750 g/day for the additive added at 0, 125, 1,250 and 10,000 mg/kg, respectively), ADG (550, 527, 535 and 519 g/day for the additive added at 0, 125, 1,250 and 10,000 mg/kg, respectively) and FG (1.5, 1.4, 1.5, 1.4 for the additive added at 0, 125, 1,250 and 10,000 mg/kg, respectively).

Glutamate dehydrogenase increased with dose reaching significance with the highest dose. Although the change was statistically significant, the magnitude was low (2.6 vs 3.6 U/L) and not considered indicative of cellular damage. None of the haematological data and the other blood analytes gave cause for concern.

[26] Technical dossier/Section II/Annex_II_4_2.
[27] Technical dossier/Section II/Annex_II_4_4.
[28] Technical dossier/Section III/Annex_III_1_1.
[29] Haemoglobin, red blood count, packed cell volume, mean cell volume, mean cell haemoglobin, platelets, white blood cell count, white blood cell differentials (neutrophils, lymphocytes, monocytes and eosinophils).
[30] Alanine transaminase, alkaline phosphatase, aspartate aminotransferase, creatine phosphokinase, gamma-glutamyl transpeptidase, glutamate dehydrogenase, glutathione peroxidase, lactate dehydrogenase, albumin, globulin, total protein, glucose, urea and phosphate.

3.2.1.2. Safety for dairy cows

A total of 48 Holstein primiparous and multiparous dairy cows, housed in free stalls and milked in a herringbone parlour, were assigned to four treatments (12 animals/treatment).[31] A total mixed ration (7.7 MJ net energy of lactation/kg, and 157 g CP, 326 g neutral detergent fibre and 436 g non-fibre carbohydrates per kg DM), mainly based on sorghum silage (20.0 kg/day), concentrate (9.1 kg/day) and corn grain (3.6 kg/day), was supplemented with the additive at 0 (control, 125 (1×), 1,250 (10×) and 10,000 (80×) mg/kg complete feed (concentration of the additive was not confirmed by analysis). Diets were fed for 57 days.

Body weight and milk production were individually recorded on a daily basis. FI was measured by group and milk composition (protein, fat and lactose) was individually determined at every milking using Fourier transform infrared spectroscopy (FTIR). Blood samples were obtained from seven cows per treatment on day 57 and analysed for general haematology[29] and blood chemistry.[32]

A mixed model was used to analyse all data (except feed intake) with the fixed effects of treatment, parity, week of study, and their two-way interactions, plus the random effect of animal. The days in milking entered the model as a covariate and the week of study as a repeated measure.

No health problems occurred during the study. Body weight (654, 651, 681 and 664 kg for the additive added at 0, 125, 1,250 and 10,000 mg/kg, respectively), milk composition (fat: 3.46, 3.63, 3.49 and 3.62%; protein: 3.16, 3.25, 3.22, 3.18% and lactose: 4.67, 4.72, 4.70 and 4.72% for the additive added at 0, 125, 1,250 and 10,000 mg/kg, respectively) and milk production (40.6, 41.6, 39.1 and 39.3 kg/day for the additive added at 0, 125, 1,250 and 10,000 mg/kg, respectively) were not affected by dietary treatments. The interactions 'treatment × week of study' and 'treatment × parity' were not statistically significant. Significant differences were observed for some haematological and blood biochemical parameters but none appeared treatment-related and the magnitude of change did not raise concern.

3.2.1.3. Safety for chickens for fattening

A total of 816 one-day-old male Ross 308 chickens were randomly allocated to four groups (6 replicates of 34 birds per treatment). The basal diet was mainly based on corn and soybean meal supplemented with the additive at 0 (control), 125 (1×), 1,250 (10×) and 10,000 (80×) mg/kg complete feed.[33] The concentration of the additive was confirmed by analysis of aluminium as the marker. The feeding program consisted of two phases: a starter period from 1 to 21 days of trial (12.3 MJ metabolisable energy (ME)/kg; 21.2% CP, 1.17% available lysine) and a growing period from 22 to 35 days of trial (12.8 MJ ME/kg; 20.0% CP; 1.0% available lysine). Both diets were supplemented at 0.05% with a commercial product containing monensin sodium. Feed was *ad libitum* offered as crumbs in the starter phase and as pellets during the growing period.

Animals were weighed on arrival and on days 21 and 35. Feed consumption was recorded by pen on these same days. Average body weight, ADG, FG and average daily feed intake were calculated. Mortality, including the most probable cause of mortality, was recorded. Blood samples were obtained from one chicken per pen on day 35 for general haematology[34] and blood chemistry.[35] Data were analysed by ANOVA. Duncan's multiple range test was used to evaluate differences among treatment means. The pen was considered the experimental unit.

Overall mortality was low (0.5–3%) and not treatment-related. No significant effects of the supplementation with the additive were seen on growth traits of chickens (final weight: 2,370, 2,440, 2,280 and 2,304 g; ADG: 66.5, 68.5, 64.0 and 64.6 g/day; FG: 1.47, 1.48, 1.49 and 1.48 g/g for the additive added at 0, 125, 1,250 and 10,000 mg/kg, respectively). As concerns blood biochemical parameters, albumin and haematocrit were significantly decreased in the highest level group (80×) compared with the control group (albumin 10.3 vs 12.2 g/L; haematocrit 28.4 vs 32.0%), indicating a potential adverse effect at the highest supplementation level. No treatment-related effects were observed for the remaining biochemical and haematological observations.

[31] Technical dossier/Supplementary information November 2015/Annex_1_C_Final Report.
[32] Amylase, gamma-glutamyl transpeptidase, alkaline phosphatase, alanine transaminase, lactate dehydrogenase, creatine kinase, calcium, phosphorous, magnesium, potassium, sodium, chlorine, cholesterol, lactate, albumin, total serum protein, urea, creatinine and non-esterified fatty acids.
[33] Technical dossier/Supplementary information November 2015/Annex_2_C.
[34] Red blood cells, haemoglobin, mean cell haemoglobin, packed cell volume, mean cell volume, white blood cell count, white blood cell differentials (segmented neutrophils, banded neutrophils, lymphocytes, monocytes and eosinophils).
[35] Alkaline phosphatase, gamma-glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine phosphokinase, glucose, uric acid, total protein and albumin.

3.2.1.4. Interaction with other constituents of the diet

The tolerance trial in chickens for fattening described above was combined with a digestibility trial carried out during the starter phase. The trial compared the control group and the maximum recommended dose group (125 mg/kg). The starter diet contained 0.5% TiO_2 as a marker. Between 15 and 18 days, fresh excreta were collected from each pen and analysed. Apparent digestibility measurements did not indicate significant differences in total nitrogen, monensin, zinc, pyridoxine, tocopherol acetate, α-tocopherol and total tocopherols. The digestibility of riboflavin was significantly improved (36.1 vs 29.4%; $p < 0.05$) by the addition of the additive.

The Panel notes that, in previous opinions, it has been shown that bentonite (montmorillonite) can bind coccidiostats and other medicinal substances (EFSA FEEDAP Panel, 2011a,b). This is reflected in the authorisation of bentonite[3] by 'The simultaneous oral use with macrolides shall be avoided'; in poultry: 'The simultaneous use with robenidine shall be avoided' and 'the simultaneous use with coccidiostats other than robenidine is contraindicated with level of bentonite above 5,000 mg/kg of complete feed'. Data have been provided showing that the recovery of monensin sodium in excreta was not affected by the additive up to 125 mg/kg complete feed. Therefore, the Panel considers that the use of the additive at the maximum recommended dose is compatible with monensin.

3.2.1.5. Conclusions on safety for the target species

The additive is considered safe at the maximum recommended dose of 125 mg additive/kg complete feed for the three target species examined (weaned piglets, chickens for fattening and dairy cows). This conclusion is extrapolated to all animal species.

Studies made with weaned piglets and dairy cows showed a wide margin of safety as no adverse effects were seen in groups given 80 times the maximum recommended dose. The margin of safety appeared narrower in chickens for fattening, as some potential adverse effects on blood parameters were seen at the highest dose (80×).

3.2.2. Safety for the consumer

The FEEDAP Panel considers it unlikely that bentonite, in common with other clays, will be degraded during its passage through the gastrointestinal tract of target animals or absorbed to any measurable extent and that harmful amounts of residues of any chemical component would occur in edible tissues/products as a consequence of the use of the additive. Clays are essentially not absorbed, and carry-over to tissues/products is therefore not relevant.

As concerns the safety of the algae fraction for humans, the applicant provided the results of a literature search (i.e. www.Algaebase.org, FAO) on the use of *Ulva* spp. as a food source. According to FAO (1976), it can be concluded that, although with differences due to local habits, algae belonging to *Ulva* spp. (commonly referred as sea lettuce), are historically consumed worldwide. The human consumption of *Ulva* spp. is specifically authorised in France (Fleurence et al., 2012).

The iodine content in the additive (9.2 mg/kg, range 4.8–15 mg/kg) does not rise any concern at the maximum recommended dose of 125 mg additive/kg complete feed.

3.2.2.1. Toxicological studies

An acute toxicity study of the additive in feed in rats following OECD Guideline 423[36] and using a single dose of 2,000 mg/kg body weight (bw) given by gavage showed no adverse effects.

The additive was tested for mutagenicity in a bacterial reverse mutation test following OECD Guideline 471.[37] It used concentrations of the additive of up to 5,000 μg/plate in two independent experiments, using strains TA1535, TA1537, TA98, TA100 and TA102 of *Salmonella* Typhimurium in the presence and absence of S9 from the liver of rats treated with Aroclor 1254. None of the strains showed any evidence of mutagenesis in either the presence or absence of metabolic activation. Positive control chemicals gave the expected results for each strain.

The additive was tested in an *in vitro* micronucleus test in mouse lymphocytes following OECD Guideline 487.[37] Based on the results of a preliminary cytotoxicity test, different concentrations of the additive (highest concentration 250 μg/mL) were tested in the absence (3 and 24 h treatments) and in the presence (3 h treatment, two experiments) of a metabolic activation (S9 mix from the liver of rats treated with Aroclor 1254). Cytotoxicity was evaluated by determining the population doubling (PD) of

[36] Technical dossier/Section III/Annex_III_2_1.
[37] Technical dossier/Section III/Annex_III_2_2.

cells. Quality of the cells on the slides was also taken into account. In the absence of S9 mix, no toxicity was induced at any tested dose following the 3 h treatment and a slight toxicity was induced at the highest tested dose level of 62.5 μg/mL following the 24 h treatment (33% decrease in the PD). No significant increase in the frequency of micronucleated cells was noted after both treatments. In the presence of S9 mix, slight toxicity was induced at the lowest tested dose of 7.81 μg/mL (29% decrease in the PD) after the first 3 h treatment and no toxicity was induced at any tested dose levels after the second 3 h treatment. No significant increase in the frequency of micronucleated cells was noted after both 3 h treatments. The additive did not induce any chromosome damage, or damage to the cell division apparatus, in cultured mammalian somatic cells, either in the presence or absence of metabolic activation.

The results of the studies show the absence of mutagenic or genotoxic activity of the additive.

3.2.2.2. Conclusions on safety for the consumer

The additive is not genotoxic. As bentonite is essentially not absorbed from the gut lumen and algae from *Ulva* spp. are not expected to be of concern for human consumption, the FEEDAP Panel considers that the use of the additive in animal nutrition is safe for consumers of food products from animals fed diets containing the additive.

3.2.3. Safety for the user

3.2.3.1. Effects on the respiratory system

The additive has a high dusting potential (12.8 g/m^3) and contains a high proportion of fine particles (83.9% ≤ 50 μm diameter; 43.9% ≤ 10 μm). Therefore, there is a potential for all parts of the respiratory tract of users to be exposed by inhalation of dust generated as a result of handling of the additive.

The additive contains crystalline silica (6.8% quartz, range 5.3–8.5%). Inhalation of silica is known to be hazardous and is associated with increased risk of lung cancer and the industrial disease, silicosis. The EU Scientific Committee on Occupational Exposure Limits concluded that silicosis is the main effect of respirable crystalline silica (SCOEL, 2003).

An acute inhalation toxicity study was performed with the additive using groups of five male and five female Sprague–Dawley rats exposed to 5.13 mg additive/L air for 4 h followed by a recovery period of 14 days (OECD 403).[38] The desired concentration of the additive in the chamber was determined in a pretest trial to establish suitable generation procedures. Animals were observed at 7 and 14 days and no adverse effects on signs of toxicity, body weight or gross pathology were found in any of the animals exposed. Therefore, the additive is considered to be of low acute toxicity by the inhalation route.

3.2.3.2. Effects on eyes and skin

An acute dermal irritation/corrosion study (OECD 404) was performed using three female New Zealand White rabbits.[39] Neither irritant nor corrosive effects were observed after a contact time of 4 h. No mortality and signs of toxicity were recorded and no significant changes of body weight were noted during the study period. The results indicate that the additive is not a skin irritant.

An acute eye irritation study (OECD 405) was performed using three female New Zealand White rabbits.[40] No mortality occurred and no signs of clinical toxicity were observed. Moderate chemosis and redness of the conjunctiva were observed 1 h after the instillation and were present in two animals after 2 days. No iris or corneal lesions were observed during the study period. The results of the test indicate that the additive is not an eye irritant.

No studies of skin sensitisation potential were available.

3.2.3.3. Conclusions on safety for the user

The additive is not an irritant to the skin or the eyes.

The additive is considered to have low inhalation toxicity. However, the additive has a high dusting potential and contains a high proportion of fine particles. A high level of inhalation exposure to an inert dust may be hazardous.

In the absence of data, the Panel cannot conclude on dermal sensitisation.

[38] Technical dossier/Section III/Annex_III_3_1_A.
[39] Technical dossier/Section III/Annex_III_3_3.
[40] Technical dossier/Section III/Annex_III_3_2.

3.2.4. Safety for the environment

The components of the additive (bentonite and algae from *Ulva* spp.) are widely distributed in the environment being natural components of the soil and marine environment. Therefore, it is not expected that the use of the additive in animal nutrition would adversely affect the environment.

3.3. Efficacy

The application is for use of the preparation of algae interspaced bentonite as AfB_1 binder for all animal species. As the substances used for the reduction in 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).

3.3.1. *In vitro* study

The binding capacity of the additive towards AfB_1 has been studied *in vitro*.[41] Adsorption isotherms were established for three pH values (3, 7 and 8) corresponding to the range found in the gastrointestinal tract of animals. The inclusion level of the additive was 0.33 mg/mL of AfB_1 solution. Twelve experimental solutions corresponding to AfB_1 concentrations from 3 to 48 μg/mL water were prepared from a 600 μg AfB_1/mL stock solution. Negative (buffer solution without mycotoxin) and positive controls (same volume of mycotoxin working solution) were prepared. The suspension was shaken at 37°C for 90 min to reach the adsorption equilibrium. After incubation, samples were centrifuged and AfB_1 concentrations determined in the supernatants by high-performance liquid chromatography (HPLC). Adsorption isotherms obtained from the experimental data were fitted to multiple isotherm equations (i.e. Langmuir, Freundlich and Sips and Hill equations).

The results indicate that the efficacy of the additive in adsorbing AfB_1 at different toxin concentrations varied from 70% to 99% at pH 3 and from 68% to 93% at pH 7 and 8. The experimental values for maximum adsorption were approximately 100 mg AfB_1/g additive independent of pH.

3.3.2. *In vivo* study

The applicant submitted only one efficacy study on dairy cows.[42] To evaluate the efficacy of the additive, a premix called MMi.S was used at a dose of 2.5 g/kg DM. The composition of MMi.S includes 0.4% of the additive which corresponds to the minimum recommended dose of 10 mg additive/kg complete feed. However, as shown in the certificate of analysis,[42] MMi.S included in addition to the 0.4% of the additive, 39% bentonite, 12% of algae, 6% molasses, and 42.6% carrier, which increased more than 100 times the dose of the active substances of the additive (858 mg of bentonite and 264 mg of algae per kg complete feed would be provided with 2.5 g MMi.S/kg DM). Consequently, the effects seen in the study could not be attributed to the additive only. Because of the presence of other active substances in addition to the additive as described, this study was not further considered.

3.3.3. Conclusions on efficacy for the target species

The results of the *in vitro* study indicate that the additive can adsorb AfB_1 at different concentrations and pH values. However, no acceptable studies were available to confirm these effects *in vivo*. Therefore, the FEEDAP Panel cannot draw conclusions on the efficacy of the additive for all animal species.

4. Conclusions

The additive is considered safe for weaned piglets, chickens for fattening and dairy cows at the maximum recommended dose of 125 mg/kg complete feed. This conclusion is extrapolated to all animal species.

[41] Technical dossier/Section IV/Annex_IV_1_18_A_Final Report.
[42] Technical dossier/Supplementary information November 2015/Annex_3_C Final report.

The additive is not genotoxic. As bentonite is essentially not absorbed from the gut lumen and algae from *Ulva* spp. are not expected to be of concern for human consumption, the FEEDAP Panel considers that the use of the additive in animal nutrition is safe for consumers.

The additive is not an irritant to the skin or the eyes and is considered to have low inhalation toxicity. However, the additive has a high dusting potential and contains a high proportion of fine particles. A high level of inhalation exposure to an inert dust may be hazardous. In the absence of data, the Panel could not conclude on dermal sensitisation.

The components of the additive (bentonite and algae from *Ulva* spp.) are widely distributed in the environment being natural components of the soil and marine environment. Therefore, it is not expected that the use of the additive in animal nutrition would adversely affect the environment.

The results of the *in vitro* study indicate that the additive can adsorb AfB_1 at different concentrations and pH values. However, no adequate studies were available to confirm these effects *in vivo*. Therefore, the FEEDAP Panel cannot conclude on the efficacy of the additive for all animal species.

5. Recommendations

The specification for the additive, as proposed by the applicant, should be modified to reflect the analytical data. The minimum content of smectite should be increased to 79% and that of uronic acids should be set to 7 mg/g DM.

The provisions applied to bentonite concerning the simultaneous use with macrolide antibiotics should be applied to the additive.

Documentation provided to EFSA

1. Preparation of algae interspaced bentonite for all animal species. January 2015. Submitted by Olmix S.A.S.
2. Preparation of algae interspaced bentonite for all animal species. Supplementary information. November 2015. Submitted by Olmix S.A.S.
3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for the preparation of algae interspaced bentonite.
4. Comments from Member States.

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Abbreviations

ADG	average daily gain
AfB_1	aflatoxin B_1
ANOVA	analysis of variance
BC	binding capacity
bw	body weight
CFU	colony-forming unit
CP	crude protein
DM	dry matter
DTA	differential thermal analysis
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FI	feed intake
FG	feed to gain ratio
FTIR	Fourier transform infrared
HPLC	high-performance liquid cromatography
ME	metabolisable energy
OECD	Organisation for Economic Cooperation and Development
PAIB	preparation of algae interspaced bentonite
PCBs	polychlorinated biphenyls
PCDD	polychlorinated dibenzo-*p*-dioxin
PCR	polymerase chain reaction
PD	population doubling
SCOEL	Scientific Committee on Occupational Exposure Limits
TEQ	toxic equivalent
TGA	thermal gravimetric analysis
WHO	World Health Organization
XRD	X-ray diffraction

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for feed additives on the method(s) of analysis for the preparation of algae interspaced bentonite

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 (BCAfB1) 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.