SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of bentonite (dioctahedral montmorillonite) as feed additive for all species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

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ABSTRACT

Bentonite (dioctahedral montmorillonite) is an additive proposed to be authorised as a substance for the reduction of the contamination of feed by mycotoxins for all animal species at a recommended use level ranging from 0.05 and 0.3 % in complete feed. The FEEDAP Panel considers 0.5 % bentonite as safe for all target animal species. Since (i) bentonite is authorised as food additive without any restriction, (ii) natural montmorillonite is not genotoxic, and (iii) a 28-week toxicity study in rats showed dietary levels up to 2 % calcium montmorillonite without toxicity, the FEEDAP Panel considers that there is no safety concern for consumers. Bentonite is not irritant to skin and mildly irritant to eyes. Bentonite airborne dust is associated with elevated susceptibility to pulmonary infections. Bentonite is ubiquitous in the environment and is not expected to adversely affect the environment. *In vitro* systems demonstrated the ability of bentonite to adsorb aflatoxins in aqueous media at different pH values and to a lower degree in gastric juice. Since *in vitro* systems do not completely mimic the complex situations during digestion, an efficacy assessment requires a minimum of *in vivo* data. Two *in vivo* studies in dairy cows (< 5 µg AfB_1/kg feed) demonstrated a significant reduction in AfB_1 excretion via milk by 0.03–1 % bentonite. The FEEDAP Panel concluded that the bentonite under application has the potential to be efficacious as aflatoxin binder in feed of dairy cows, and extended this conclusion to all ruminants. The Panel was not in a position to assess the efficacy of the bentonite under application as aflatoxin binder for any other animal species than ruminants due to the absence of data.

KEY WORDS

Technological additive, mycotoxin binder, bentonite, montmorillonite, aflatoxin, mycotoxins, safety, efficacy

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SUMMARY

Following a request from of the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver an opinion on the safety for the target animals, consumer, user and the environment and the efficacy of the product bentonite (dioctahedral montmorillonite). Bentonite is currently authorised as binder, anticaking and anticoagulant agent. The applicant is now seeking authorisation for bentonite as a substance for the reduction of the contamination of feed by mycotoxins, at a recommended use level ranging from 0.05 to 0.3 % in complete feed.

Although no typical tolerance studies with bentonites were found in the literature, the varying bentonite levels (0.2–10 %) used in a multitude of studies (with poultry, ruminants and trout) allow performing an assessment of safety for the target animals. Levels of 1–2 % were tolerated if only zootechnical parameters were considered. However, already 0.5 % bentonite reduced the availability of the essential trace element manganese. Bentonites also interact with coccidiostats and other medicinal substances but apparently not with vitamins. The FEEDAP Panel considers, as a conservative estimate, 0.5 % bentonite to be safe for all target animal species.

Since (i) bentonite is authorised as food additive without any restriction, (ii) natural montmorillonite is not genotoxic, and (iii) a 28-week toxicity study in rats showed dietary levels up to 2 % calcium montmorillonite without toxicity, the FEEDAP Panel considers that there is no concern in terms of safety for the consumer of food products from animals fed diets containing the bentonite for which authorisation is sought.

Bentonite is not irritant to skin and mildly irritant to eyes. No inhalation toxicity study has been performed despite the high dusting potential shown by the product under application. Bentonite airborne dust is associated with elevated susceptibility to pulmonary infections. Intratracheal administration of bentonite to rats revealed a high cytotoxic potential.

Bentonite is ubiquitous in the environment occurring as a natural soil component. Therefore, it is not expected that its use as a feed additive would adversely affect the environment.

All further conclusions refer to a bentonite with a minimum binding capacity of 100 mg aflatoxin per g bentonite as determined by a specific isothermal method used by the applicant.

A series of different *in vitro* systems was provided to demonstrate the ability of bentonite to adsorb aflatoxin in aqueous media at different pH values and to a lower degree in gastric juice. Since *in vitro* systems as described cannot completely mimic the complex situations during digestion, a reliable efficacy assessment without a minimum of *in vivo* data is not possible. Suitable *in vivo* data shall be generated under conditions reflecting maximum permitted mycotoxin contamination as established by EU legislation. Nearly all *in vivo* data in the literature do not fulfil this prerequisite.

Two experiments (one with an unspecified bentonite) allow the conclusion that bentonite is effective in reducing milk aflatoxin when added to rations of dairy cows contaminated below 0.005 mg AfB_1/kg. The experiment performed with the bentonite under application confirms the lowest dose (0.05 % of the complete diet) proposed by the applicant. This bentonite is considered to have the potential to be efficacious as an aflatoxin binder in feed of dairy cows. This conclusion is extended to all ruminants. The FEEDAP Panel is not in a position to assess the efficacy of the bentonite under application as an aflatoxin binder for any other animal species than ruminants due to the absence of data.

The FEEDAP Panel recommends to introduce the aflatoxin binding capacity in the specification of bentonite.

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BACKGROUND

Regulation (EC) No 1831/2003[4] 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 the company Biomin GmbH[5] for authorisation of the product bentonite, dioctahedral montmorillonite, to be used as a feed additive for all animal species (category: technological additive; functional group: substances for the reduction of the contamination of feed by mycotoxins) under the conditions mentioned in Table 1.

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.[6] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 21 May 2010.

The additive bentonite is authorised as technological additive (E558) as binder, anticaking agent and coagulant.[7]

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 animal(s), consumer, user and the environment and the efficacy of the product bentonite (dioctahedral montmorillonite), when used under the conditions described in Table 1.

[4] OJ L 268, 18.10.2003, p. 29.
[5] Biomin GmbH, Industriestraße 21, A-3130 Herzogenburg, Austria.
[6] EFSA Dossier reference: FAD-2010-0018.
[7] OJ C 50, 25.2.2004, p. 1.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Bentonite (Dioctahedral Montmorillonite)
Registration number/EC No/No (if appropriate)	
Category(ies) of additive	Technological feed additive (1)
Functional group(s) of additive	Substances for the reduction of the contamination of feed by mycotoxins (m)

Description

Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Bentonite: > 70 % dioctahedral montmorillonite	not applicable	Complies with EU law on undesireable substances including heavy metals, PCBs and PCDDs/PCDFs	Quanification of non-nutrient marker by a validated and ring-test trialed method

Trade name (if appropriate)	Mycofix® Secure
Name of the holder of authorisation (if appropriate)	Biomin GmbH

Conditions of use

Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg or Units of activity or CFU/kg of complete feedingstuffs (select what applicable)		
all animal species and categories	slaughter age	0.5 g/kg	3 g/kg	nil

Other provisions and additional requirements for the labelling

Specific conditions or restrictions for use (if appropriate)	Store in cool and dry place. Mixing with additives from the 'antibiotics', 'growth promoters', 'coccidiostats and other medical substances' groups is prohibited, except in the case of: monensin-sodium, narasin, lasalocid-sodium, flavophospholipol, salinomycin sodium and robenidine.
Specific conditions or restrictions for handling (if appropriate)	Breathing protection and safety glasses are recommended
Post-market monitoring (if appropriate)	Post-market monitoring will be carried out by Biomin GmbH in compliance with EU Regulations concerning Feed Hygiene and Feed and Food Controls, namely HACCP and Traceability, routine post-market sampling and analysis, and formal monitoring of customer feedback through product or service complaints.
Specific conditions for use in complementary feedingstuffs (if appropriate)	none

Maximum Residue Limit (MRL) (if appropriate)

Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
-	-	-	-

ASSESSMENT

1. Introduction

Bentonite (E558), a clay component, is authorised as binder, anticaking agent and coagulant under the category technological additives. The current application concerns bentonite (dioctahedral montmorillonite) as aflatoxin B_1 (AfB_1) binder (functional group (m): substances for the reduction of the contamination of feed by mycotoxins) with a bonding capacity of at least 100 mg AfB_1/g. The applicant requests the use of bentonite in all animal species and categories without time limitation and recommends a dose range of 0.05 % to 0.3 % for use in complete feed.

Bentonites are also permitted as food additives according to Directive 95/2/EC.[8]

2. Characterisation

2.1. Characterisation of the product

Mycofix® Secure consists of bentonite. The major component of the active substance is dioctahedral montmorillonite, a particular form of smectite.[9] Typical contents of the bentonite under application are > 70 % smectite, < 10 % opal and feldspar, < 4 % quartz and calcite. Five different batches were analysed by X-ray diffraction and differential thermal analysis for their content of dioctahedral montmorillonite (70–75 %) further characterised by its pH (8.6 to 9.5) and cation exchange capacity (63 to 67 cmol/kg).[10] The remainder of the product consists largely of water (~ 8 %). The SiO_2 modification cristobalite was found in traces in one batch.

A non-nutrient microtracer (Microtracer FS, > 97 % stainless steel grit) is included in the product to identify the product and for analysis in feed.

2.1.1. Purity

The product is regularly checked for heavy metals, PCBs and dioxins. The analysis of three batches[11] showed that levels for Pb (25–27 mg/kg), As (2–4 mg/kg), Cd (< 0.1 mg/kg), Hg (< 0.05 mg/kg), dioxin-like PCBs (< 0.01 ng WHO-TEQ/kg) and for dioxins (0.07–0.09 ng PCDDs/PCDFs WHO-TEQ/kg dry matter) were below the limits set in Directive 2002/32/EC.[12]

2.1.2. Physical state

The product is a fine greyish powder with a bulk density of 892 g/L and with a pH of 9.2 based on the determination of three batches.[13]

Particle size analysis based on a laser-diffraction analysis of three batches showed that more than 60 % of the particles have diameters below 50 µm, and 13 % below 10 µm.[14] The dusting potential according to the Stauber-Heubach method, based on three batches, is 6.3 g/m^3.[15]

2.1.3. Production process

The additive is obtained by mining.

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[8] OJ L 61, 18. 3. 1995, p. 1.
[9] Technical Dossier Section II Annex II:02.
[10] Technical Dossier Section II Annex II:03.
[11] Technical Dossier Section II Annexes II:7,8 and 9.
[12] OJ L 140, 30.5.2002, p. 10.
[13] Technical Dossier Section II Annexes II:17 and 20.
[14] Supplementary Information Annex_II_SIN_01.
[15] Technical Dossier Section II Annexes II:18.

2.2. Stability and homogeneity

Stability in premixtures and feed was measured by *in vitro* binding capacity of AfB_1. The FEEDAP Panel considers the approach acceptable to show that the additive maintains its properties. As would be expected for a mineral product, the binding activity was maintained for at least six months in premixtures and for three months in feedingstuffs.

The capacity to homogeneously distribute was measured by determining a microtracer which is added to the bentonite. Microtracer analysis in 12 subsamples each showed an adequate accuracy (recovery between 91 and 105 %) in a premixture (50 % bentonite), a complementary and four complete feedingstuffs (0.05–0.5 % bentonite) with coefficients of variation (CVs) of 7–10 %. However, the data confirms only that the microtracer was homogenously distributed. No proof that the bentonite followed the microtracer in its distribution in feed has been provided.[16]

2.3. Conditions of use

The additive is intended for all animal species and categories at a proposed dose ranging from 0.05 to 0.3 % in final feed. No maximum content is recommended.

2.4. Analytical methods

2.4.1. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in the Appendix.

2.4.2. Interference with the analysis of aflatoxin B1

Three samples of the additive were used for the evaluation of the potential interference of the product on the analytical determination of AfB_1 in feed. A ruminant complementary feed was spiked with AfB_1 at an intended concentration of 4.5 µg/kg and then the binder was added to three subsamples of the feed. Although the results showed that the addition of the product did not reduce the AfB_1 content in feed (control sample: 3.22 µg/kg; samples with bentonite: 3.95, 3.63 and 4.78 µg/kg),[17] the low control value makes the results of this experiment difficult to interpret.

3. Safety

3.1. Safety for the target species

No tolerance studies have been provided by the applicant. Upon request of the FEEDAP Panel, the applicant performed a structured database search using SciFinder, Pubmed, Scopus and diverse sources outside scientific databases to provide information on target animal safety, interactions with other feed components and on efficacy. Published research on bentonite was available since the early 1960s. The most relevant findings are summarised in the corresponding chapters.

3.1.1. Feeding different bentonite levels to food producing animals (literature review)

Poultry

Calcium-bentonite improved performance of chickens for fattening up to 2 % inclusion (Day et al., 1970), but tended to depress performance at high levels (5–10 %). Broiler performance with 1.5 or 3 % bentonite in the diet was studied by Katouli et al. (2010). It was found that 3 % bentonite improved weight gain, feed intake and growth rate. Pasha et al. (2008) tested the influence of bentonite at 0.5 and 1 % and acetic acid in the diet of broilers and found that weight gain, feed consumption, protein efficiency ratio and protein digestibility increased significantly. Inclusion of 0.5 % bentonite + zeolite

[16] Technical Dossier/Section II/Annex II:35.
[17] Supplementary Information Annex_II_SIN.03.

did not affect hematologic parameters, but increased organ weights (Prvulović et al., 2008). Tauquir et al. (2001) included bentonite in chickens' diets at 2.5, 3.5 and 4.5 %: Bentonite had no effect on weight gain and feed efficiency and improved feed consumption at lower levels. Addition of 2 % bentonite improved weight gain, feed efficiency and dressing percentage with no abnormalities in internal organs (Ali et al., 1996). No adverse effect of bentonite (0.5 %) on nutrient-deficient broilers was observed, on the contrary feed intake and gain was increased (Southern et al., 1994). Inclusion of 5 % bentonite in the diet did not have adverse effects on productive performance of broilers (Lon-Wo and Gonzalez, 1991). A use level of 2.5 % bentonite did not affect live weight and feed efficiency in turkeys (Salmon, 1985).

A negative influence of bentonite in laying hens on mortality, egg production and feed efficiency at inclusion levels of 1.5 % and below was observed by Ali et al. (1994). Bentonite also decreased egg yolk colour and tended to lower egg production (at 1–4 % inclusion), but to increase shell strength (Vogt, 1992). No effect on performance was noted at 0.25 and 0.5 % inclusion by Ambula et al. (2003). Olver et al. (1989) did not observe significant dietary effects with bentonite at 2–8 % inclusion. Bentonite at 1 and 1.5 % inclusion rate significantly improved egg production (Bhatti and Sahota, 1998; Tauqir et al., 2000). Egg yield and weight were not influenced by bentonite levels from 1.5 to 3.5 % in diets while feed efficiency was slightly higher with the highest inclusion rate (Inal et al., 2000). Bentonite increased body weight and egg size and decreased water content in hens' droppings (Quisenberry, 1966).

Ruminants

No effect on digestibility at supplementation of cow's diet with 1 % bentonite was found by Aguilera-Soto (2009). Ivan et al. (2001) found that a palm kernel cake diet supplemented with 2 % bentonite had no noticeable effects on rumen fermentation. Similarly, bentonite had no effect on milk yield and quality, ruminal pH and fatty acid concentration but it decreased ruminal ammonia nitrogen and digestibility of crude protein (Gulsen et al., 2000). Bentonite at 2 % inclusion did not prevent acidosis (Ha and Emerick, 1984). Bentonite had no effect on performance, rumen fermentation and volatile fatty acids but lowered digestibility when added at 0.6 and 1.2 % (Fisher and Mackay, 1983). Galyean and Chabot (1981) found no significant effects whatsoever with regard to digestibility at 3 % inclusion rate.

Bentonite at 2 % inclusion did not have any effect on the growth of calves (Mohini et al., 1999). Berthiaume et al. (2007) used 2 % dietary bentonite in steers and achieved an increase of average daily gain. Addition of up to 4 % bentonite to the diet of calves stimulated growth in a study by Losada et al. (1976). Bentonite supplemented to dairy cows at 2 or 3 % of the diet buffered the rumen fluid when feeding acidogenically, which lead to an improvement of milk yield, a decrease of downer-cow syndrome as well as improved Ca, Mg and P levels in blood (Slanina, 1974). Bentonite increased milk fat, milk production and ruminal acetate levels in a study of Rindsig et al. (1969), when added to fat-depressing rations of cows at 5 or 10 %.

Dietary bentonite at 2 % inclusion did not affect performance parameters or meat quality (other than meat redness and a couple of fatty acids) of lambs in a trial by Jerónimo et al. (2010). Khadem et al. (2007) tested the performance of lambs under addition of 2 or 4 % bentonite with positive effects with regard to feed intake and feed conversion rate as well as blood parameters. An improved feed intake caused by 5 % dietary bentonite in export sheep was noted by Round (2000). An increase in gain and gain-to-feed ratio as well as ruminal volatile fatty acids was observed in lambs with 0.75 % bentonite, but no effect on wool growth (Walz et al., 1998). No adverse effects of feeding bentonite at 2.5 % or 3 % to sheep were found by Fenn and Leng (1989) and Murray et al. (1990), respectively.

Trout

Eya et al. (2008) assessed the effects of the natural zeolites, bentonite and mordenite on the performance and body composition of rainbow trout. During the feeding trial, quadruplicate groups of 15 rainbow trout (average initial weight ± SD, 104.2 ± 0.7) were grown in freshwater (salinity: 0;

temperature: 14–16 °C) over 90 days. Fish were hand-fed, two times a day with diets containing 40 % crude protein supplemented with 0 (control), 2.5, 5.0 and 10 % bentonite or mordenite. Alpha cellulose replaced bentonite or mordenite in the control diet in order to keep the diet isonitrogenous and isoenergetic. There was a statistically significant decrease ($P < 0.05$) in percent weight gain, specific growth rate and feed efficiency for fish fed dietary bentonite at 5 and 10 % and dietary mordenite at 2.5 % compared to those fish on the control diet.

Pigs

No publications which could be used to derive safety of bentonite for pigs were found. A research note (Tavernera et al., 1984) indicates 2 and 4 % bentonite does not influence the performance of growing pigs, but reduces dry matter digestibility.

3.1.2. Interactions with other constituents of the diet

Several studies documented an adsorptive effect for heavy metals like cadmium, lead or caesium in pigs. Montmorillonite was found to be efficient in binding cadmium added to the basal diet at 0.5 % (Xu et al., 2004; Yu et al., 2009), showing less deposition in tissue and increased excretion. Concerning lead it was found that montmorillonite at an incorporation rate of 0.5 % reduced toxic effects in the liver (Yu et al., 2006) and decreased lead levels of tissues while improving performance parameters (Yu et al., 2005, 2008).

Grudnik et al. (2005) found that 3 % bentonite in the diet reduced thallium concentration in whole eggs and thallium accumulation in muscle of laying hens. In another study (Cabañero et al., 2005), it was shown that bentonite decreased inorganic mercury bioabsorption (60–100 %) and organic mercury bioaccumulation (29–67 %) in chickens. Cadmium and chromium deposition was also decreased by inclusion of bentonite in the diet (Srebocan et al., 1988). Bentonite (5 %) was found to decrease Caesium (^{137}Cs) levels in meat and did not adversely affect performance of the chickens (Andersson et al., 1990). Desheng et al. (2005) monitored in a three-week study the mineral contents in bone of chickens for fattening. The levels in bone of Ca, P, Cu, Fe and Zn were not affected by 0.5 % montmorillonite, but F, Mn and Pb were decreased. The authors concluded that an additional Mn supply to the diet becomes necessary when containing montmorillonite.

Bentonite (0.5 %) decreased the anticoccidial effects of low levels of monensin (55 mg/kg) and salinomycin (22 mg/kg) but not when the coccidiostats were used at the recommended levels (Gray et al., 1998). Bentonite (2 %) rendered tilmicosin ineffective (Shryock et al., 1994). Robenidine (and ipronidazol and buquinolate) were found to be incompatible with bentonite and ruled not to be used in combination by the FDA (Federal register, 1969, 1971, 1972). The Canadian Bureau of Veterinary Drugs (1992) reported a case of insufficient efficacy of tylosin when fed concurrently with bentonite to bovines.

3.1.3. *In vitro* tests on vitamin binding by bentonite

The *in vitro* tests to demonstrate the absence of vitamin binding of bentonite submitted by the applicant were carried out in a test system comparable to that used for conventional AfB_1 adsorption tests (see Section 4.1: *In vitro* studies). The vitamin concentrations in each buffer (citrate at pH 3.0; phosphate at pH 6.5) were 4 mg biotin/L and 10 mg pantothenic acid/L. Four batches of bentonite (0.2 %) were tested.

Contrary to the control substance (active charcoal), the bentonite (four batches) did not adsorb pantothenic acid (< 1 %) and biotin (< 3 %).

3.1.4. *In vivo* study on retinol binding

In a study of Pimpukdee et al. (2004), 0.5 % Ca-montmorillonite did not depress liver retinol storage indicating that vitamin A absorption was unaffected.

3.1.5. Conclusions on the safety of bentonite for the target animals

Although no typical tolerance studies with bentonites were found in the literature, the varying bentonite levels used in a multitude of studies allow performing an assessment of safety for the target animal.

A range of 1–2 % bentonite in complete feed for laying hens and poultry for fattening seems safe, hens being more susceptible than growing birds when only performance parameters are considered. However, already 0.5 % bentonite reduced manganese availability in chickens for fattening. Bentonite also reduced the availability of coccidiostats and other medicinal substances.

Ruminants seem to tolerate higher levels of bentonite. However, findings in the literature are controversial: 3 % bentonite is found without influence on digestibility, while in another study already 0.6 % depressed digestibility.

In trout, bentonite was tolerated at a 2.5 % level (the lowest concentration studied), but another clay (mordenite) not.

In vitro data with pantothenic acid and biotin may support the conclusion that the bentonite under application does not bind to water-soluble vitamins. Vitamin A availability in poultry is not affected by 0.5 % Ca-montmorillonite either.

Taken all together and considering the apparent lack of consistent data available to set a higher safe dose, the FEEDAP Panel considers 0.5 % as a bentonite concentration safe for all target animal species. This conservative conclusion is not in contrast to individual findings with a limited number of endpoints indicating higher dietary levels as safe for a certain animal species.

3.2. Safety for the consumer

No studies have been provided by the applicant to support the safety of bentonite for the consumer.

Since bentonite is authorised as food additive without any restriction, no studies concerning the safety for the consumer would be required for the present application of bentonite as feed additive, as far as the purity criteria of this product would satisfy those described in the Annex of Commission Directive 2008/84/EC[18] for bentonite (E558).

The major component dioctahedral montmorillonite (minimum 70 % instead of 80 % for bentonite E558) and other accompanying minerals have been identified unambiguously using the most relevant analytical techniques, including those listed in the Directive mentioned above. The content of arsenic and lead in the product were higher than the values retained in the Directive (2 mg/kg and 20 mg/kg, respectively). Thus, the bentonite under application does not fully fulfil the requirements of a food additive.

A systematic database search indicates recent studies showing that natural montmorillonite is not genotoxic (Sharma et al., 2010; Li et al., 2010; Prival et al., 1991). A long-term (28 weeks) toxicity study in rats (Afriyie-Gyawu et al., 2005) indicates that levels of calcium montmorillonite as high as 2.0 % (w/w) do not result in overt toxicity, considering zootechnical parameters, gross and histological pathology, haematological parameters or clinical chemistry.

The FEEDAP Panel considers that there is no concern in terms of safety for the consumer of food products from animals fed diets containing the bentonite seeking authorisation.

[18] OJ L 253, 20.9.2008, p. 1.

3.3. Safety for the user

Three studies on the dermal irritancy of the same bentonites, performed according to OECD Guideline 404, concluded to the absence of irritation or corrosion.[19]

Three studies on the eye irritancy potential of three different bentonites were performed according to OECD Guideline 405.[20] The elemental composition of the bentonites was close to that of the bentonite seeking authorisation, two exhibiting a similar pH (close to 9) and one a lower pH (3.8). Iridial inflammation and moderate conjunctival irritation with similar scores were observed in the three studies, which disappeared within 72 hours, leading to the classification as a mild irritant.

No inhalation toxicity study has been performed, despite the high dusting potential of the product and the particle size distribution in which 60 % was below 50 µm and 13 % below 10 µm. It has been shown that bentonite airborne dust is associated with elevated susceptibility to pulmonary infections (Hatch et al., 1985). The intratracheal administration of bentonite to rats revealed a high cytotoxic potential; however, acute inflammatory response was short-lived and the cell population returned to normal within few weeks (Sykes et al., 1983; Vallyathan, 1994).

3.4. Safety for the environment

Bentonite is ubiquitous in the environment occurring as a natural soil component. Therefore, it is not expected that its use as a feed additive would adversely affect the environment.

4. Efficacy

4.1. Efficacy of bentonites as aflatoxin binders (literature review from database search)

4.1.1. Pigs

Sodium-bentonite at 1 % inclusion level in piglets' diets containing 40 % corn with 922 µg AfB_1/kg partially restored performance and liver function of the control group (Schell et al., 1993a). The same authors (Schell et al., 1993b) found in another study with 0.5 % different clays (palygorskite, sepiolite, a treated Ca-bentonite (chemically and physically modified), hydrated sodium calcium aluminosilicate) and a diet with 800 µg AfB_1/kg, that considerable differences between the clays exist. The modified Ca-bentonite restored pig performance and serum biochemistry to control levels. In the same publication, Ca-bentonite at incorporation rates of 0.25 % to 2 % in the diet was effective against aflatoxin (800 µg/kg diet): it increased the average daily gain and average daily feed intake linearly and prevented some negative effects (seen in clinical serum biochemistry) of aflatoxin.

An aluminosilicate was fed to pigs at 0.5 % up to 2 % in the diet and prevented hepatic lesions caused by aflatoxin (3 mg/kg diet) as well as changes of serum parameters (Harvey et al., 1989).

The adverse effect of aflatoxins (200 µg Af/kg feed) in pigs was ameliorated by bentonite at 0.4 and 0.5 %, including restoration of performance loss, feed efficiency and abnormal blood profiles (Thieu et al., 2008).

Lindemann et al. (1993) examined the effect of hydrated sodium bentonite at inclusion levels of 0.5 % in the diet of weanling swine with aflatoxin levels of 420 and 840 µg AfB_1/kg. The bentonites improved average daily gain and restored clinical biochemistry profile, which had been affected by aflatoxin. In a second study, the authors compared the hydrated sodium bentonite with two other sodium bentonites (montmorillonite) at a 0.5 % inclusion level and the aflatoxin content was 800 µg/kg diet. All bentonites improved the performance and restored the clinical biochemistry profile. In a third study, with graded levels of bentonite (0.25, 0.5 and 0.75 %) and a diet with 800 µg AfB_1/kg, there was no benefit of bentonite doses higher than 0.5 %.

[19] Technical dossier/Section III/Annex III:07.
[20] Technical dossier/Section III/Annex III:06.

Montmorillonite applied at 0.3 % in feed did not protect from aflatoxin (110 µg/kg diet) in pigs, but a montmorillonite nanocomposite did (Shi et al., 2007). The nanocomposite at the same dose was able to restore decreased performance, organ damage and alterations in serum caused by aflatoxin (Shi et al., 2005).

4.1.2. Poultry

Miazzo et al. (2005) incorporated 0.3 % sodium bentonite in diets containing 2500 µg AfB_1/kg fed to chickens for fattening from day 23 to day 52. Aflatoxin B_1 significantly diminished body weight gain. Sodium bentonite significantly diminished the inhibitory effects of dietary AfB_1. Feeding AfB_1 alone caused significant increases in the relative weights of most observed organs. Addition of sodium bentonite to the diet containing AfB_1 reduced the relative weights of liver, kidney and spleen. Sodium bentonite decreased the incidence and severity of the histopathological hepatic changes associated with aflatoxicosis.

Pimpukdee et al. (2004) fed diets containing 5000 µg Af/kg and supplemented with 0, 0.125, 0.25 and 0.5 % Ca-montmorillonite to chickens for fattening for three weeks. Bentonite (0.25 and 0.5 %) restored average daily gain and hepatic vitamin A, both reduced by the aflatoxin.

The combined data of a six-week experiment with chickens for fattening fed pelleted diets (Bailey et al., 2006) showed that birds fed 0.5 % montmorillonite received significant but not complete protection against the effects of the aflatoxin (4000 µg Af/kg) for most parameters measured (average daily gain, feed to gain ratio, clinical blood serum biochemistry, relative weights of liver, kidney and spleen, tissue histology). Santurio et al. (1999) fed diets containing three levels of sodium bentonite (0, 0.25 and 0.5 %) at 0 and 3000 µg aflatoxin/kg to groups of 4 × 22 chickens for fattening for 42 days and measured the zootechnical parameters, clinical blood serum biochemistry and organ weights. Aflatoxin-induced changes were partially compensated by both levels of sodium bentonite. However, sodium 0.5 % bentonite appeared to reduce serum phosphorus. Adverse effects related to aflatoxicosis were also reduced by 0.5 and/or 1 % inclusion of bentonite in several studies: Kermanshahi et al., 2009 (500 and 1000 µg Af/kg), Pasha et al., 2007 (100 µg Af/kg), Keçeci et al., 1998 (2500 µg/kg, 83 % AfB_1), Desheng et al., 2005 (200 µg AfB_1/kg) and Phillips et al., 1988 (7500 µg Af/kg).

In a five-week study on chickens for fattening, Manafi et al. (2009) could show a reduction of the adverse effects of AfB_1 (500 µg/kg; significant reduction of body weight, feed consumption and antibody titres against Newcastle disease (ND) and Infectious Bursal Disease (IBD); increases in the relative liver weight) by 1 % bentonite in the diet. The adverse effects of aflatoxin (254 µg Af = 200 µg AfB_1/kg diet) on antibody production against IBD were reduced by 0.5 % bentonite in the diet of chickens (254 µg Af = 200 µg AfB_1/kg diet; Ghahri et al., 2009), on antibody production against ND by up to 0.6 % bentonite in the diet (2500 µg AF = 81 % AfB_1/kg diet; Ibrahim et al., 2000).

Eraslan et al. (2005) observed that aflatoxin decreased calcium and phosphate levels in blood of chickens, which was less marked when bentonite at 0.25 or 0.5 % was added to the diet. Utilisation of nutrients, depressed in case of aflatoxicosis, was improved by bentonite at inclusion rates of 0.15 and 0.3 % in the diet (Chaturvedi and Singh, 2004).

Zootechnical parameters of chickens for fattening (day 30 to day 52) depressed by the aflatoxin (5000 µg/kg) were moderately restored by 0.3 % of sodium bentonite. However, since Af-induced liver steatosis did not return to normal, the authors concluded that the sodium bentonite was ineffective. In a study by Shi et al. (2009), 0.3 % montmorillonite did not have protective effects against aflatoxin-induced (110 µg AfB_1/kg) depression of weight gain and feed to gain ratio and increase in the weights of liver, kidney, spleen and pancreas; however, a nanocomposite of montmorillonite at the same inclusion rate was effective.

4.1.3. Ruminants

Diaz et al. (2004) studied the effect of 1.2 % of different sodium bentonites and a calcium bentonite on the AfM_1 excretion in milk of dairy cows fed a diet with 55 µg AfB_1/kg (100 µg total Af/kg). The three sodium bentonites and the calcium bentonite significantly ($P < 0.01$) reduced AfM_1 contamination of milk (sodium bentonite 1: 61 %; sodium bentonite 2 : 65 %; sodium bentonite 3 : 50 %, and; calcium bentonite: 31 %). Similar effects were noted by Blüthgen and Schwertfeger (2000).

The data of the first trial of Veldman (1992) indicated a significant decrease ($P < 0.05$) in mean carry-over of aflatoxin of one third among cows with and without 1 % bentonite in the compound feed (10 µg AfB_1/kg). The results of the second trial showed that 1 % bentonite reduced the aflatoxin carry-over from 3 to 2.2 % at a lower level of aflatoxin (2.8 µg AfB_1/kg feed).

4.1.4. Conclusions

In the wide majority of experiments, bentonites, however not all, showed a positive effect on the reduction of symptoms of aflatoxicosis in pigs, poultry and ruminants. In most cases, and depending on the level of aflatoxin contamination, the bentonites could not fully restore the negative impact induced by aflatoxin. With the exception of one study in cows, all experiments were performed with dietary aflatoxin levels considerably above European legal limits. The design of those experiments does therefore not correspond to European conditions. As far as performance endpoints are considered, those parameters are inappropriate since at levels of AfB_1 contamination below the European legal limits no effects on performance are to be expected.

In the only acceptable study in dairy cows, the carry-over of aflatoxin from feed (2.8 µg AfB_1/kg feed) to milk could be reduced by about 26 % with 1 % bentonite in the ration.

4.2. Studies with the bentonite under application, provided by the applicant

4.2.1. *In vitro* binding studies

The applicant submitted a series of different *in vitro* studies to demonstrate the effect of the bentonite under application as a substance capable to adsorb aflatoxin (and some other mycotoxins) in aqueous solutions – originally developed as a screening system for identifying the most suitable compound to adsorb mycotoxins. All data reported result of triplicate determinations.

The opinion follows in the denomination of the *in vitro* tests the terms used by the applicant.

4.2.1.1. Conventional AfB_1 adsorption tests

These tests were conducted with buffer solutions (at different pH; e.g. 3.0 and 7.0) containing low concentrations of AfB_1 (e.g. 10–50 µg/L). A defined aliquot of AfB_1 containing buffer was spiked with the test substance (e.g. 0.05 and/or 0.3 % (w/v)) and incubated for a certain time (e.g. 1h at 37 °C) under permanent shaking. After centrifugation of the samples, the supernatants were analysed for AfB_1, which is considered to be representative to the non-adsorbed fraction.

All batches of the additive (three in test 1, five in test 2) adsorbed under all variable test conditions 100 % of the added toxin.

4.2.1.2. AfB_1 adsorption tests under intensified conditions

The main modifications of this test were related to an increase of the AfB_1 (4 mg/L) and a reduction of the test substance concentration (0.02 %) in buffer solutions. Seven batches were investigated.

Except for one batch, all other investigated products showed an AfB_1 adsorption between 92 and 95 %.

4.2.1.3. Chemisorption studies

This test is a further modification of the conventional test at high concentration of both the AfB_1 (4 mg/L) and the bentonite (0.5 %). After removal of the supernatant, the product is washed twice with acetate buffer and extracted three times with methanol, the supernatants of each washing and extraction step are analysed for AfB_1. The results are expressed as chemisorption index (amount of retained AfB_1 in relation to total AfB_1 in the sample). A theoretical maximum value of 1 indicates complete binding of AfB_1 by the test substance.

The three batches under test showed chemisorption indices of 0.94 to 0.96. Adsorption of AfB_1 under test conditions are considered by the applicant as primarily related to chemisorption (involving ionic or covalent binding) and not to physical binding.

4.2.1.4. AfB_1 adsorption tests in gastric juice

Preliminary studies have shown that the toxin adsorption in the more complex matrix (gastric juice) is lower than with aqueous solutions (buffer). The adsorption tests were carried out in similar conditions (AfB_1 being 4 mg/L and the test substance 0.02 %) with gastric juice from pigs. Six different batches were tested.

The results varied between 45 and 61 % AfB_1 adsorption. Three more recent batches (2009) showed an average of about 60 %, lower values are related to older batches.

4.2.1.5. Isothermal adsorption analyses

Adsorption isotherms with bentonite were carried out in order to determine two characteristics of a mycotoxin binder: the maximum toxin-binding capacity (Q_{max}) and its affinity to the toxin. These adsorption constants allow evaluation and comparison of the effectiveness of mycotoxin adsorbents.

The use of isotherms is considered as one of the most efficient mathematical approaches to describe surface adsorption, in which the amount of compound adsorbed per unit of weight of adsorbent is plotted against the concentration of the compound in the external phase, under equilibrium conditions. The Langmuir and Freundlich isotherms (multiple isotherm equations) have been used for the modelling of the adsorption of compounds in aqueous solutions to the surfaces of solids.

The first study[21] was performed to develop/optimise a standard protocol to assess the efficacy of some mycotoxin adsorbing materials based on their ability in binding zearalenone (ZEA) or AfB_1 under controlled *in vitro* conditions. Sixteen products were compared. Products of the applicant (the bentonite under application and a 70:30 premixture with inactivated yeast component) were tested for aflatoxin adsorption only.

For each product, three adsorption isotherms, each consisting of six concentrations (e.g. 0.5, 1, 2, 4, 10 and 20 µg AfB_1/mL), were obtained at pH 3 (citrate buffer), 5 (acetate buffer) and 7 (phosphate buffer). The inclusion level of feed additives was 1 mg/mL (0.1 % w/v) in all cases.

For the bentonite (and the premixture with inactivated yeast), toxin adsorption higher than 96 % was recorded at any toxin level and pH condition tested. Both products showed high efficacy in terms of highest affinity and capacity for AfB_1 near to the reference standard activated charcoal.

The second study[22] (four batches) was performed to identify the maximum adsorption capacity of bentonite (Q_{max}). Based on the principles of the laboratory study described above, the concentration of the binder in test solution was reduced (from 0.1 % to 0.002 %) as well as the number of pH values (two (3 and 7) instead of three (3, 5 and 7)). By this procedure, a Q_{max} at pH 7 of > 115 mg AfB_1/g bentonite was found for four batches.

4.2.1.6. Adsorption tests with mycotoxins other than AfB_1

The adsorbing properties of bentonite (one batch) for ergot alkaloids (ergine, ergotamine and ergovaline) were investigated at low product concentration (0.02 %) and high toxin concentration (2 mg ergine, ergotamine or ergovaline/L). High adsorption rates (89–95 %) were detected for all three ergot alkaloids (ergine < ergotamine < ergovaline) tested at pH 3 and pH 6.5. Almost no differences

[21] Technical dossier/Section IV/Annex IV:59.
[22] Technical dossier/Section IV/Annexes IV:64, 66 and 68.

were found between single- (i.e. one toxin individually present) and multi-toxin studies (i.e. all three toxins simultaneously present).

One batch was additionally investigated (product concentration 0.2 %) for its potential to adsorb ochratoxin A (200 µg/L), fumonisin B1 (500 µg/L), zearalenone (1000 µg/L), deoxynivalenol (1000 µg/L) and AfB_1 (200 µg/L). While good AfB_1-binding of the bentonite batch was confirmed in this study (100 % at pH 3, 95 % at pH 6.5), the product showed only partial pH-dependent adsorption activity against ochratoxin A (76 % at pH 3, 9 % at pH 6.5), fumonisin B1 (73 % at pH 3, 5 % at pH 6.5) and zearalenone (42 % at pH 3, 68 % at pH 6.5). Practically no binding activity was detected for deoxynivalenol (1 % at pH 3, 11 % at pH 6.5).

4.2.2. *In vivo* studies

4.2.2.1. Chickens for fattening

A total of 200 one-day-old birds was distributed to ten treatments (four replicates of five chickens) and fed diets containing 0 and 2 mg AfB_1/kg, the aflatoxin contaminated diets without or with 0.5 % bentonites/zeolites of different origin for 21 days.[23] The parameters measured were feed intake, body weight, blood proteins, and liver and kidney weight. Liver samples (six per treatment) were collected for histopathological examination. No measurement of Af excretion and/or deposition in tissues was made.

The AfB_1 concentration studied in the experiment was 200 times higher than the maximum amount permitted by Directive 2002/32/EC. In addition, the level of the mycotoxin binders was higher (0.5 %) than the maximum recommended by the applicant (0.3 %).

4.2.2.2. Dairy cows

A total of 18 dairy cows (Italian Friesian breed; parity 1.9; 130 days in milk; average milk production 33.6 kg/day) were distributed following a 3 × 3 Latin square with seven days per period (without washout) to a control without an adsorbent, and to groups treated with 0, 20 and 50 g/day of a premixture (Mycofix®Plus).[24] Aflatoxin was provided by a daily amount of 1 kg contaminated corn meal (91.7 µg AfB_1/kg) into which the daily dose of Mycofix®Plus was incorporated. The total mixed ratio without corn meal contained 0.24 µg AfB_1/kg DM.

Mycofix®Plus consists of 38 % bentonite, 30 % inactivated yeast, 25 % diatomaceous earth, 5 % seaweed meal and 2 % of a plant component. An *in vitro* study in which bentonite from Mycofix®Plus was substituted with sand, showed that 80 % of the binding capacity was related to the bentonite.[25]

Based on feed intake figures of 23.3, 23.5 and 23.7 kg dry matter for the groups with 0, 20 and 50 g Mycofix®Plus per day, the following key data could be calculated:

- AfB_1 content of the daily ration: 4.1 µg/kg DM (below the legal limit of 5 µg/kg)
- Bentonite in the daily ration: 0.32 and 0.80 g/kg DM (= 0.03 and 0.08 %)

These concentrations are close to the lowest recommended dose (range: 0.05–0.3 %).

The AfM_1 concentration in the milk of control cows was 0.120 µg/kg. Mycofix®Plus at 20 and 50 g per head and day reduced significantly ($P < 0.008$) the AfM_1 content to 0.083 and 0.072 µg/kg milk, respectively. However, even the reduced AfM_1 content is above the maximum permitted level in milk (0.05 µg/L; Commission Regulation (EC) No 1881/2006).[26]

[23] Technical dossier/Section IV/Annex IV:88.
[24] Technical dossier/Section IV/Annex IV:97.
[25] Technical dossier/Section IV/Annex IV:100.
[26] OJ L 364, 20.12.2006, p. 5, ammended by Commission Regulation (EU) No 165/2010, OJ L 50, 27.2.2010, p. 8.

The carry-over rate was 3.85 in the control cows, 2.66 and 2.27 in the groups fed 20 and 50 g Mycofix®Plus/cow and day, respectively. The reduction of the carry-over rate (by 31 and 41 %) was in the same order of magnitude as observed by Veldmann (1992) with 2.8 µg AfB_1/kg feed and 1 % bentonite.

4.2.3. Conclusions

There is clear evidence of the capacity of the bentonite to bind AfB_1 provided by the *in vitro* studies, but this is indicative only and does not guarantee that the same effects would be observed *in vivo*. The binding capacity of the bentonite in gastric juice was considerably lower than under the more artificial aqueous buffer solutions. The isothermal method, although impressive in its mathematical precision, shares this concern. The method is considered predictive for bentonite as candidate for the functional group (m) of additives (mycotoxin binder) and important particularly when considering the diversity of naturally occurring clay minerals in structure and functionality. The isothermal method is therefore suitable to provide a physical parameter as an element of bentonite specification, but not conclusive for the *in vivo* effects of bentonite.

The results of the *in vitro* binding capacity are specific to the form of bentonite selected by the applicant based on the criteria to ensure a minimum AfB_1 binding capacity.

The *in vitro* data with other mycotoxins show that the bentonite effective in binding aflatoxins is (i) effective in binding selected ergot mycotoxins, (ii) less effective in binding other mycotoxins (ochratoxin, fumonisin B1 and zearalenone) and (iii) ineffective in binding deoxynivalenol.

The study on dairy cows demonstrates the efficacy of a 38 % bentonite-containing premixture (Mycofix®Plus) by significantly reducing milk AfM_1 resulting from a complete feed contamination within the limits of European legislation. However, the bentonite concentration tested and proven to be efficacious was low (0.03 to 0.08 %) compared (i) to the recommended use range (0.05–0.3 %) and (ii) to levels used with almost equal effects on AfM_1 which were about 1 %. An *in vitro* study showed that 80 % of the binding capacity of Mycofix®Plus was related to the bentonite.

Considering the multitude of different *in vitro* studies provided by the applicant and two studies with bentonites on dairy cows with an aflatoxin exposure in the frame of European legislation, the FEEDAP Panel considers that the bentonite under application has the potential to be efficacious as an aflatoxin binder in dairy cows. This conclusion can be extended to all ruminants.

For other animal species and categories, no *in vivo* studies with aflatoxin levels at or below the permitted concentration (Directive 2002/32/EC) and in the range of the proposed bentonite inclusion in the ration were submitted. The FEEDAP Panel is therefore not in a position to assess the efficacy of the bentonite under application as an aflatoxin binder in any other species than ruminants.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Although no typical tolerance studies with bentonites were found in the literature, the varying bentonite levels used in a multitude of studies allow performing an assessment of safety for the target animals.

A range of 1–2 % bentonite in complete feed for laying hens and poultry for fattening seems safe, hens being more susceptible than growing birds when performance parameters are considered only. However, already 0.5 % bentonite reduced manganese availability in chickens for fattening. Bentonite also reduces the availability of coccidiostats and other medicinal substances. Ruminants seem to tolerate higher levels of bentonite. However, findings in the literature are controversial. In trout, bentonite was tolerated at a 2.5 % level. Intestinal vitamin availability is apparently not affected by bentonite as shown by *in vitro* data with pantothenic acid and biotin and a poultry study on hepatic retinol storage.

Taken all together and considering the apparent lack in consistent data, the FEEDAP Panel considers as a conservative estimate 0.5 % bentonite to be safe for all target animal species.

Since (i) bentonite is authorised as food additive without any restriction, (ii) natural montmorillonite is not genotoxic, and (iii) a 28-week toxicity study in rats showed dietary levels up to 2 % calcium montmorillonite without toxicity, the FEEDAP Panel considers that there is no concern in terms of safety for the consumer of food products from animals fed diets containing the bentonite for which authorisation is sought.

Bentonite is not irritant to skin and mildly irritant to eyes. No inhalation toxicity study has been performed despite the high dusting potential shown by the product under application. Bentonite airborne dust is associated with elevated susceptibility to pulmonary infections. Intratracheal administration of bentonite to rats revealed a high cytotoxic potential.

Bentonite is ubiquitous in the environment occurring as a natural soil component. Therefore, it is not expected that its use as a feed additive would adversely affect the environment.

All further conclusions refer to a bentonite with a minimum binding capacity of 100 mg aflatoxin per g bentonite as determined by a specific isothermal adsorption method used by the applicant.

In vitro studies also showed that bentonite has a limited or no capacity to bind mycotoxins other than aflatoxins subject of EU legislation.

Considering the series of different *in vitro* studies provided by the applicant and two studies with bentonites on dairy cows with an aflatoxin exposure in the frame of European legislation, the FEEDAP Panel considers that the bentonite under application has the potential to be efficacious as an aflatoxin binder in dairy cows. This conclusion can be extended to all ruminants.

For other animal species and categories, no *in vivo* studies with aflatoxin levels at or below the permitted concentration (Directive 2002/32/EC) and in the range of the proposed bentonite inclusion in the ration were submitted. The FEEDAP Panel is therefore not in a position to assess the efficacy of the bentonite under application as an aflatoxin binder in any other species than ruminants.

RECOMMENDATIONS

Since materials which fall within the definition of bentonites can show a wide range of binding capacities, any authorisation should include a specification which defines a minimum binding capacity e.g. for AfB_1. The FEEDAP Panel proposes that such specification should approximate to a minimum binding capacity equivalent to 100 mg AfB_1/g bentonite measured according to the method described in this application.[27] However, such a specification would require an official analytical basis. The method described in Directive 2001/59/EC[28] (see also OECD Guideline 106[29]) could be used as a model.

Considering the potential authorisation of bentonite within a functional group of the technological category, the authorisation would refer to a generic substance. Not all bentonites would fulfil the criteria set and effects demonstrated for the bentonite under application. In the view of the FEEDAP Panel, such an official method should be available before authorisation of bentonite as a mycotoxin binder is made.

[27] Technical dossier/Section II/Annex II:31.
[28] OJ L 225 , 21.08.2001, p. 1.
[29] OECD Guidelines for the Testing of Chemicals / Section 1: Physical-Chemical properties. Test No. 106: Adsorption - Desorption Using a Batch Equilibrium Method.

The maximum inclusion level of bentonite should be set at 5 g/kg complete feedingstuffs, considering target animal safety. If so, the simultaneous use of coccidiostats would not need to be prohibited. If the current maximum level (20 g/kg compete feed) is retained, other provisions should contain: 'simultaneous use with coccidiostats is not allowed' (without exceptions).

The interactions between bentonite and other medicinal substances (macrolide antibiotics) in poultry and bovines would support a warning statement in the labelling of bentonite, e.g. 'The simultaneous oral use with certain medicinal substances (e.g. macrolides) should be avoided'.

Given the particle size distribution and high dusting potential of the additive, it is recommended that appropriate protection measures are taken to reduce exposure of workers/users via inhalation.

The use of microtracers should be restricted to more important uses, where potential safety concerns for the consumer related to the feed dose exist.

Documentation provided to EFSA

1. Bentonite (Dioctahedral montmorillonite). March 2010. Submitted by Biomin GmbH.
2. Bentonite (Dioctahedral montmorillonite). Supplementary information September 2010. Submitted by Biomin GmbH.
3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for bentonite.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Bentonite for all animal species/categories

Mycofix® Secure is a product for which authorisation as *feed additive* is sought under the category "Technological feed additive", functional group 1(m) "substances for the reduction of the contamination of feed by mycotoxins" according to Annex I of Regulation (EC) No 1831/2003. The active substance of *Mycofix® Secure* is *bentonite (dioctahedral montmorillonite).* In the current application submitted according to Article 4(1) of Regulation (EC) No 1831/2003, the authorisation is sought for all animal species and categories. The proposed inclusion level of bentonite in complete feedingstuffs is 0.5 g/kg for the minimum content and 3 g/kg for the maximum content.

For the determination of the mineralogical and geological parameters of the *bentonite* in the *feed additive*, the Applicant proposed several chemical and mineralogical methods, commonly used in geological studies. The X-ray diffraction (XRD) analysis indicates that the product contains a minimum of 70 % *dioctahedral montmorillonite*.

The CRL recommends for official control the X-ray diffraction (XRD) method proposed by the Applicant for the determination of *bentonite* in the *feed additive.*

The direct determination of the *bentonite* content added to *premixtures* or *feedingstuffs* is not achievable by analysis. The Applicant proposed instead an indirect method for the determination of the *bentonite* in *premixtures* and *feedingstuffs*, based on the addition of a non-nutrient marker in the *feed additive*. The method consists in counting coloured stainless steel particles extracted from the samples. This method was originally used to monitor the homogeneity of the product. The transferability of the method was further investigated through a collaborative trial organised by the Applicant. The following satisfactory performance characteristics were re-calculated by the CRL based on the experimental data provided: - a relative standard deviation for *repeatability* and *reproducibility* ranging from 10.8 to 12.2 %; and - a *recovery* rate ranging from 87 to 103 %.

Based on the above mentioned performance characteristics, the CRL conditionally considers the indirect method (using non-nutrient marker particles) proposed by the Applicant as suitable for the determination of the *bentonite* in *premixtures* and *feedingstuffs* for official control only, when (1) the content of the specific marker is established - expressed as number of particle per gram of *feed additive* – and (2) the specific marker proposed in this dossier is exclusively used for this feed additive.

In addition, EFSA requested the CRL to evaluate the analytical method applied by the Applicant to monitor the stability of the active substance in the product, *premixtures* and *feedingstuffs*. In this dossier the Applicant used the capability of *bentonite* to adsorb aflatoxin B1 (AfB1) from buffer solutions as indicator for its stability and measured this parameter after having applied different storage conditions. For the analysis the Applicant used liquid chromatography coupled to a diode array detector (LC-DAD). Based on the obtained sensitivity of the LC-DAD method and due to the high AfB1 concentration in the butter solution, the CRL considers the LC-DAD method suitable for the purpose of the stability study.