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Safety and efficacy of a natural mixture of illite, montmorillonite and kaolinite (Argile Verte du Velay) as a feed additive for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

The additive, a natural mixture of illite, montmorillonite and kaolinite with a minor amount of calcite and sanidine, is intended to be used as a technological additive (functional groups: binders and anticaking agents) in feedingstuffs for all animal species. The additive is safe in complete feed for cattle for fattening at a maximum concentration of 50,000 mg/kg and at a maximum concentration of 20,000 mg/kg for piglets and pigs for fattening. No conclusions can be drawn for all the other animal species/categories. The additive is not genotoxic. As the additive is essentially not absorbed from the gut lumen, the Panel on Additives and Products or Substances used in Animal Feed considers that use of the additive in animal nutrition is safe for consumers of food products from animals fed diets containing the additive. The additive is not an irritant to the eyes and the skin and it is of low toxicity by the inhalation route. No systemic toxicity is expected following dermal exposure. Due to its nickel content, the additive should be considered a potential dermal and respiratory sensitiser. The components of the additive are natural constituents of soil. Consequently, the use of the additive in animal nutrition will not pose a risk to the environment. The additive is effective as an anticaking agent and a binder at an inclusion rate of 50,000 mg/kg complete feed.

Keywords: illite, montmorillonite, kaoline, safety, efficacy, anticaking agent, binder

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Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

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Summary

Following a request from the European Commission, the 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 the natural mixture of illite, montmorillonite and kaolinite (MIMK).

The additive is intended for use as a technological additive (functional groups: binders and anticaking agents) in feedingstuffs for all animal species at a recommend use level of 20,000 to 50,000 mg/kg complete feed.

The additive is safe in complete feed for cattle for fattening at a maximum concentration of 50,000 mg/kg and at a maximum concentration of 20,000 mg/kg for piglets and pigs for fattening. No conclusions can be drawn for all the other animal species/categories.

The additive is not genotoxic. As the additive is essentially not absorbed from the gut lumen, the FEEDAP Panel considers that use of MIMK in animal nutrition is safe for consumers of food products from animals fed diets containing the additive.

The additive is not an irritant to the eyes and the skin and it is of low toxicity by the inhalation route. No systemic toxicity is expected following dermal exposure. Due to its nickel content, the additive should be considered a potential dermal and respiratory sensitiser.

The components of the additive (illite, montmorillonite, kaolinite, calcite and sanidine) are natural constituents of soil. Consequently, the use of the additive in animal nutrition will not pose a risk to the environment.

The additive is effective as an anticaking agent and a binder at an inclusion rate of 50,000 mg/kg complete feed.

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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 (EC) received a request from Argile Du Velay - Arvel[2] for authorisation of the product natural mixture of illite, montmorillonite and kaolinite (Argile Verte du Velay), when used as a feed additive for all animal species (category: technological additives; functional group: binders, anticaking agents).

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 15 May 2013.

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 product natural mixture of illite, montmorillonite and kaolinite (Argile Verte du Velay), when used under the proposed conditions of use (see Section 3.1.6).

1.2. Additional information

The additive Argile Verte du Velay is a natural mixture of illite, montmorillonite and kaolinite. This product has not been previously authorised in the Community as feed additive. Montmorillonite is authorised for use as feed additive in feedingstuffs for all animal species with a maximum content of 20,000 mg/kg feed. Kaolinitic clay is authorised for use as feed additive in feedingstuffs for all animal species with no maximum content. The active substances montmorillonite and kaolinite are authorised for use in food with no maximum content.

EFSA has delivered several opinions on the safety and efficacy of bentonite–montmorillonite as a feed additive (EFSA FEEDAP Panel, 2011a, b, 2012a, 2013), one opinion on a mixture of montmorillonite – illite mixed layer clay (EFSA FEEDAP Panel, 2014) and one opinion on the safety of kaolinite as a food additive (EFSA CEF Panel, 2013).

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[3] in support of the authorisation request for the use of natural mixture of illite, montmorillonite and kaolinite as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003 and the applicable EFSA guidance documents.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies and peer-reviewed scientific papers to deliver the present output.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Argile Du Velay – Arvel. Zone d'activités de Nolhac, 43350, Saint-Paulien, France.
[3] FEED dossier reference: FAD-2012-0025.

EFSA has verified the European Union Reference Laboratory (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 Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of natural mixture of illite, montmorillonite and kaolinite is consistent with the principles laid down in Regulation (EC) No 429/2008[5] 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), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

The product under assessment is a natural mixture mainly composed of illite, montmorillonite and kaolinite (MIMK). Illite is not currently authorised as a feed or food additive in the European Union (EU). Kaolinite (without maximum content) and montmorillonite (as bentonite–montmorillonite, maximum content of 20,000 mg/kg) are currently authorised as technological feed additives (binders, anticaking agents and coagulants). Kaolinite and montmorillonite are also authorised for use as food additives under Regulation (EC) No 1333/2008,[6] with a specification established by Regulation (EU) No 231/2012.[7]

The applicant is seeking an authorisation for the use of the product as a technological additive (functional groups: (g) binders and (i) anticaking agents) in feedingstuffs for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the additive

The product is obtained by mining from a quarry located in France. Extraction is followed by crushing, drying and packaging. It is beige/green finely powdered clay with a bulk density of about 515 kg/m^3.[8]

The product is specified to contain at least 40% of illite, as the main component. The other components are montmorillonite and kaolinite and minor amounts of calcite and sanidine (potassium feldspar). The characteristics of the main constituents are listed in Table 1.

Table 1: Main characteristics of illite, montmorillonite and kaolinite as provided by the applicant.

	Illite	Montmorillonite	Kaolinite
Phyllosilicates family	Phyllosilicate sheets of six-membered rings with 2:1 layers (dioctahedral micas)	Phyllosilicate sheets of six-membered rings with 2:1 layers (dioctahedral smectites)	Phyllosilicate sheets of six-membered rings with 1:1 layers
CAS number	106958-53-6	67479-91-8	1318-74-7
ICDD number	26-0911	02-0009	29-1488
Chemical formula	$K(Al,Fe)_2AlSi_3O_{10}(OH)_2.H_2O$	$Na_x[(Al_{2-x}Mg_x)Si_4O_{10})\ (OH)_2]$	$Al_2(OH)_4(SiO_5)$
Molecular weight (g/mol)	389	-	258.16

CAS: Chemical Abstracts Service; ICDD: International Center for Diffraction Data

[4] The full report is available on the EURL website:https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2012-0025-Velay-Clay.doc .pdf

[5] 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.

[6] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16

[7] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

[8] Technical dossier/Section II/Annex 2-1-12

X-ray diffraction (XRD) is commonly used to provide a mineralogical analysis of clays, which can also be characterised by their elemental composition, usually determined/expressed as the corresponding oxides. Both mineralogical and chemical approaches have been used to characterise the additive. The mineralogical composition (XRD of 10 batches) is summarised in Table 2. The concentration of crystalline silica (quartz), analysed in three batches of the product, was in the range of 0.43–1.36%. The elemental composition (six batches) is given in Table 3.

Table 2: Mineralogical composition of 10 batches of the product (X-ray diffraction).

	Illite (%)	Montmorillonite (%)	Kaolinite (%)	Calcite (%)	Sanidine (%)
Mean	53.3	16.2	16.5	7.8	6.2
Minimum	41.5	10.3	8.4	4.8	2.3
Maximum	59.7	24.0	20.2	14.5	12.2

Table 3: Elemental composition of six batches of the product (expressed as oxide)

	SiO_2 (%)	Al_2O_3 (%)	Fe_2O_3 (%)	CaO (%)	K_2O (%)	MgO (%)
Mean	41.0	24.4	14.9	8.5	7.9	3.2
Minimum	35.9	21.1	10.6	6.7	6.7	2.6
Maximum	44.4	26.6	18.8	14.6	9.4	3.8

The product is further characterised by (average of three batches) loss of ignition (900°C): 14.5%;[9] pH (10% solution): 7.9;[10] moisture (4 h at 105°C): 3.7%;[11] ash (450°C): 94.2%;[12] carbonates: 10.9%.[13]

3.1.2. Purity

The concentrations of lead (17–23 mg/kg), cadmium (0.11–0.29 mg/kg), mercury (0.005-0.01 mg/kg) and arsenic (13–26 mg/kg) analysed in six batches of the product[14] do not raise safety concern. Nickel concentration was 30 mg/kg.

Dioxins in four batches were ≤ 0.076 ng WHO PCDD/F-TEQ/kg, sum of dioxins and dioxins-like polychlorinated biphenyls (PCBs) ≤ 0.10 ng WHO-PCDD/F-PCB-TEQ/kg and of non-dioxin-like PCBs ≤ 0.36 µg/kg.[15] These concentrations are below the limits set in the Directive 2002/32/EC.[16]

3.1.3. Physical state of the product

Particle size distribution, analysed by laser diffraction in three batches of the additive,[17] showed that about 93% of particles (v/v) were of diameter ≤ 100 µm, 72% ≤ 50 µm and 11.5% ≤ 10 µm. The mean diameter of the particles of the additive was about 30 µm.

The additive showed a dusting potential (analysed by Stauber–Heubach method in three batches)[18] of about 2.5 g/m^3.

[9] Technical dossier/Section II/Annex 2-2-3
[10] Technical dossier/Section II/Annex 2-2-4
[11] Technical dossier/Section II/Annex 2-2-3
[12] Technical dossier/Section II/Annex 2-2-3
[13] Technical dossier/Section II/Annex 2-2-5
[14] Technical dossier/Section II/Annex 2-1-7
[15] Technical dossier/Section II/Annex 2-1-8
[16] Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels and action thresholds for dioxins and polychlorinated biphenyls. OJ L 140, 30.5.2002, p.10.
[17] Technical dossier/Section II/Annex 2-1-10
[18] Technical dossier/Section II/Annex 2-1-11

3.1.4. Stability and homogeneity

Stability studies are not required for mineral-based products, which are assumed to be stable.

To analyse the homogenous distribution, a poultry, a pig and a ruminant feed were each supplemented with the additive at a concentration of 3 g/kg feed.[19] A microtracer (FSS-red lake, about 250,000 particles/g) was also added to the feeds (0.01 g/kg). After mixing, ten subsamples of each feed were analysed for the microtracer and aluminium content (taken as a marker for the product). The concentration of MIMK showed coefficients of variation of 5.1, 7.1 and 4.3% in the poultry, pig and ruminant feeds respectively.

3.1.5. Physico-chemical interactions in feed

An *in vitro* trial was performed[20] to study the effect MIMK on the analytical determination of different diet components. MIMK was added at concentrations of 5% to mash and pelleted feeds for poultry, piglets and cattle for fattening. The feeds (control and treated with MIMK) were then analysed for concentrations of basic nutrients of the diets,[21] macro and trace elements,[22] vitamins and amino acids[23] and of coccidiostats.[24] The results showed that the analytical recovery of the tested constituents was not affected by the additive. No physico-chemical incompatibilities with feed materials, other additives or medicinal substances in feed are therefore expected.

3.1.6. Conditions of use

The additive is intended to be used in premixtures and feedingstuffs for all animal species and categories, with no minimum or maximum content. The applicant suggested use levels in premixtures and feedingstuffs of 20,000–50,000 mg/kg.

3.2. Safety

3.2.1. Safety for the target species

Safety for weaned piglets

A total of 96 male piglets (Pietrain × (Duroc × Landrace)) of about 26 days of age was fed pelleted diets supplemented with 0, 20,000 or 100,000 (2 × highest recommended use level) mg MIMK/kg for 42 days.[25] Group size was eight replicates with four piglets each (initial body weight: 8.3 kg). The diets consisting mainly of barley, biscuit meal and soybean meal were isonitrogenous (about 18% crude protein (CP)) and isocaloric (about 10 MJ net energy (NE)/kg, by an increase in full fat extruded soybeans with increasing content of the additive). The concentrations of the additive were analytically confirmed (by analysis of aluminium as the marker). Body weight and feed intake were recorded fortnightly. Feed-to-gain ratio was calculated for the different periods. At the end of the experiment, a blood sample was taken from two piglets per pen for haematology[26] and clinical chemistry.[27] The experiment was statistically considered as a randomised complete block design with the pen as experimental unit. The effects of the additive were evaluated by a set of linear contrasts. Group differences were analysed by Duncan's multiple range test.

Only one animal died during the study (day 5, use level group) due to pneumonia. The cumulative performance data did not show statistically significant differences among groups. However, at nearly

[19] Technical dossier/Section II/Annex 2-4-5
[20] Technical dossier/Section II/Annex 2-4-6
[21] Moisture, ash, crude cellulose, crude protein, crude fat, starch, total sugar, metabolisable energy
[22] Calcium, sodium, phosphorus, iron, zinc, copper, manganese, cobalt, iodine, selenium
[23] Vitamin A, vitamin E, vitamin D3, lysine, methionine, methionine hydroxy analogue
[24] Monensin sodium, narasin, salinomycin sodium, decoquinate, diclazuril, halofuginone, lasalocid A sodium, maduramycin ammonium alpha, nicarbazin, robenidine hydrochloride, semduramicin sodium.
[25] Supplementary Information February 2014/Annex_III_1_1_A; Annex_III_1_1_B and Annex_III_1_1_C.
[26] Haemoglobin, red blood cell count, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, platelets, white blood cell count, white blood cell differentials (segmented neutrophils, banded neutrophils, lymphocytes, monocytes, eosinophils).
[27] Alanine transaminase, alkaline phosphatase, aspartate aminotransferase, creatine phosphokinase, gamma-glutamyl transpeptidase, glutamate dehydrogenase, glutathione peroxidase, lactate dehydrogenase, albumins, globulins, total protein, glucose, urea, phosphate.

equal feed intake (average 750 g/day), daily weight gain (485 g) and feed-to-gain ratio (1.55) of the high-dose group appeared somewhat inferior to the corresponding figures of the control (520 g, 1.46) and the use level group (511 g, 1.46). Although the diets were isocaloric by calculation, the results do not support this assumption. It cannot be excluded that dietary fat of the high-dose diet was less effectively utilised because of its high fat content (11.3%) than that of the control and the use level diets with lower fat concentration (6.2 and 7.3%, respectively).

There were no significant differences among treatments for most of the endpoints of clinical chemistry except ß-globulins and alanine transaminase for the use level only, and for phosphate at the high dose compared to the use level and the control group. Serum-phosphate was significantly ($P \leq 0.001$) reduced in the high level group (7.54 mg/100 mL) compared to the control and the use level groups (8.96 and 9.02 mg/100 mL, respectively). This is considered as an adverse effect indicating disturbed intestinal absorption of dietary phosphorus. Haematological parameters did not show significant differences, except for a non-treatment-related decrease in banded neutrophils at both doses and a decrease in mean corpuscular volume (MCV) and an increase in monocytes at the high dose. The results indicate that 20,000 mg MIMK/kg complete feed is safe for piglets. A margin of safety could not be determined.

Safety for chickens for fattening

A total of 960 one-day-old male chickens (Ross 308) was fed pelleted diets supplemented with 0, 20,000, 50,000 (1 × highest recommended use level) or 100,000 (2 ×) mg MIMK/kg for 35 days.[28] Group size was 6 replicates with 40 birds each. The diets (starter, from day 0 to day 21; grower, from day 22 to day 35) consisting mainly of maize and soybean meal, were isonitrogenous (starter: about 21.5% CP; grower: about 19.5% CP) and isocaloric (starter: about 12.3 MJ metabolisable energy (ME)/kg; grower: about 12.8 MJ ME/kg, by an increase in full fat extruded soybeans with increasing content of the additive). The concentration of the additive was analytically confirmed (by analysis of aluminium as the marker). The diets contained 100 mg monensin sodium/kg; the starter diet included also 0.5% titanium oxide, used as an inert marker for the digestibility measurements. Body weight and feed intake were recorded at days 21 and 35. Feed-to-gain ratio was calculated for the different periods. At the end of the experiment, a blood sample was taken from one chicken per pen for haematology[29] and clinical chemistry.[30] On days 18 to 21, excreta samples were collected for the determination of nitrogen and uric acid, vitamin E (tocopherol acetate, alpha tocopherol and total tocopherols), riboflavin, pyridoxine, zinc and monensin, and the digestibility was calculated for the groups receiving 0, 20,000 and 50,000 mg MIMK/feed. The experiment was statistically considered as a randomised complete block design. The effects of the additive were evaluated by a set of linear contrasts with the pen as experimental unit. Group differences were analysed by Duncan's multiple range test.

Final body weight of the control group was 2602 g; that of the treated groups was not significantly different (mean: 2614 g). As feed consumption increased linearly ($P < 0.001$) with the addition of MIMK, feed-to-gain ratio of the treated groups was inferior to that of the control group (Control (1.43) significantly different from the groups with 20,000 and 50,000 mg MIMK/kg feed (1.47 and 1.48, respectively), and all three groups significantly different from the group with 100,000 MIMK/kg feed (1.53)).

Blood measurements showed an increase in alkaline phosphatase in the diets associated with the increased level of the additive, although no significant differences were observed among the different levels of the treated groups. Phosphate was not measured. Concerning the blood haematological values, results showed a slight decrease in mean corpuscular haemoglobin and a linear and quadratic effect in the percentage of monocytes, although the differences were small and not significant between the control group and the high-dose group. No safe level of MIMK for chickens for fattening could be identified as feed-to-gain ratio and plasma alkaline phosphatase were higher in all treated groups compared to control.

[28] Supplementary Information July 2015/Annex_ii_1_A; Annex_ii_1_B; Annex_ii_1_C; Annex_ii_1_D;

[29] Haemoglobin, red blood cell count, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, platelets, white blood cell count, white blood cell differentials (segmented neutrophils, banded neutrophils, lymphocytes, monocytes, eosinophils).

[30] Alanine transaminase, alkaline phosphatase, aspartate aminotransferase, creatine phosphokinase, gamma-glutamyltranspeptidase, lactate dehydrogenase, albumins, globulins, total protein, glucose, uric acid.

The excretion of nitrogen, tocopherol acetate, alpha tocopherol and total tocopherols, riboflavin, pyridoxine, zinc and monensin sodium was not significantly affected by the additive at concentrations of 20,000 and 50,000 mg/kg.

Safety for cattle for fattening

A total of 63 Holstein bulls (initial average age: 144 days; initial average body weight: 168 kg) was fed diets composed of straw and mash concentrates supplemented with 0, 20,000, 50,000 (1 × highest recommended use level) or 100,000 (2 ×) mg MIMK/kg for 42 days, both, the concentrate and straw offered ad libitum.[31] Group size was four replicates with four bulls each (except one with three bulls in the control group). The concentrates were isonitrogenous (about 15.7% CP) and isocaloric (about 8.4 MJ NE/kg), mainly by an increase in corn gluten feed and palm oil with increasing content of the additive. It is noted that the incorporation 100,000 mg MIMK/kg in an isonitrogenous and isocaloric diet involved many changes in the diet composition that the comparability of this diet with the three others is questionable. The concentration of the additive was analytically confirmed (by analysis of aluminium as the marker). Individual body weight and pen feed intake (including straw consumption) were recorded at days 0, 14, 28 and 42. Feed-to-gain ratio was calculated for the different periods. At the end of the experiment, blood samples were obtained from seven animals per treatment and analysed for haematology[32] and clinical chemistry.[33] An analysis of variance was done with the body weight and haematology and clinical chemistry data; the model considered the treatment only. Feed intake, daily weight gain and feed-to-gain ratio data was analysed with a mixed model considering the data from the different periods. For all performance parameters, the statistical analysis considered the initial body weight as a covariate. The pen was the experimental unit.

Final body weight in the control group was 242 kg. The average daily weight gain for the groups with 20,000, 50,000 or 100,000 mg MIMK/kg feed was 1.76, 1.71, 1.67 and 1.60 kg, the total dry matter (DM) intake (mean 6.64 kg concentrate and 0.49 kg straw DM/day) to gain ratio was 4.07, 4.33, 4.28 and 4.44 respectively. No significant differences between the groups were identified. No differences were seen in the haematological parameters among the groups. Significant differences were seen for six endpoints of clinical biochemistry: gamma-glutamyl transferase (lower values in the treated groups compared to control), glutathione peroxidase (mid-dose group higher than the other groups), alkaline phosphatase (mid-dose group higher than low-dose and control groups), urea (higher in the high dose), albumin and total protein (lower in the low- and high-dose groups than in the control group). Serum phosphate was not significantly influenced by MIMK. The differences identified refer to some endpoints of clinical chemistry but not dose related.

The results show that diets containing 100,000 mg MIMK/kg feed can be formulated without causing adverse effects in cattle for fattening.

Nickel as a contaminant

As the additive contains nickel as a contaminant (30 mg/kg), the FEEDAP Panel assessed the impact of nickel on the safety for the target species. Supplementing complete feed with MIMK at the highest amount proposed (50,000 mg/kg feed), 1.5 mg nickel/kg would be added.

This level, together with the background concentration in animal feed (0.5–4 mg/kg DM feed; Nicholson et al., 1999; Van Paemel et al., 2010), would amount to 4 to 11% of the lowest maximum tolerable nickel level in animal species (50 mg Ni/kg DM feed for rodents and fish) set by NRC (NRC, 2005).

Interactions *in vivo*

The excretion of nitrogen, tocopherol acetate, alpha tocopherol and total tocopherols, riboflavin, pyridoxine and zinc was not significantly affected by the additive in concentration at 20,000 and 50,000 mg/kg in diets for chickens for fattening. It is therefore expected that the additive, up to a concentration of 50,000 mg/kg complete feed, will not interfere with the nutrient/micronutrients supply of animals.

[31] Supplementary Information July 2015/Annex_i_3_A-G

[32] Haemoglobin, red blood cell count, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell count, white blood cell differentials (neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets).

[33] Alanine transaminase, alkaline phosphatase, aspartate aminotransferase, creatine phosphokinase, gamma- glutamyl transpeptidase, glutamate dehydrogenase, glutathione peroxidase, lactate dehydrogenase, albumins, total protein, glucose, urea, phosphate.

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). This is reflected in the authorisation of bentonite 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'.
The additive contains montmorillonite and therefore, in principle, the same provision should apply to MIMK. However, data has been provided that shows that the recovery of monensin sodium in faeces was not affected by the additive up to 50,000 mg/kg complete feed. Therefore, the Panel considers that the use of MIMK is compatible with monensin sodium.

Conclusions on the safety for target species
The FEEDAP Panel concludes that 20,000 mg MIMK/kg complete feed is safe for piglets (weaned); and extends this conclusion to pigs for fattening. For cattle, for fattening a concentration of 50,000 mg MIMK/kg complete feed is considered safe with a margin of safety of two. No conclusion can be drawn on the safety for poultry or any other species/categories.

MIMK in chickens for fattening up to 50,000 mg/kg feed did not influence the absorption of nutrients/micronutrients and monensin sodium.

3.2.2. Safety for the consumer

The FEEDAP Panel considers it unlikely that illite, montmorillonite, kaolinite, calcite and sanidine, in common with other clays, will be degraded during their 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.

The applicant has provided some reports of toxicological studies performed with MIMK.

The additive was tested for mutagenicity in a bacterial reverse mutation test. The study was GLP compliant and conformed to OECD Guideline 471.[34] It used concentrations of the additive of up to 5,000 µg/plate in two independent experiments, using strains TA1537, TA1535, TA98, TA100 and TA102 of *Salmonella* Typhimurium in the presence and absence of S9 from the livers 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 tester strain.

The chromosome aberration test was GLP-certified and complied with OECD Guideline 473.[35] It used Chinese hamster ovary (CHO-K1) cells to test the clastogenicity at metaphase of the additive in three independent experiments at concentrations of up to 5,000 µg/mL in the presence or absence of S9 from the livers of rats treated with Aroclor 1254. None of the experiments gave results showing any treatment-related increase in the numbers of cells with chromosomal aberrations, polyploidy or endoreduplication. Positive control chemicals (mitomycin C and cyclophosphamide) produced the expected results.

The micronucleus test was good laboratory practice (GLP) compliant conformed to OECD Guideline 474.[36] It used groups of five male and five female Swiss albino mice in three groups: a negative control (given vegetable oil), a test group (given 2,000 mg/kg body weight (bw)/day of the additive) and a positive control (given 1 mg/kg bw/day of mitomycin C). All administrations were as two daily oral gavage doses. Toxicity to bone marrow (as indicated by the polychromatic to total erythrocyte ratio) was not seen in the group treated with the additive, therefore, there was no evidence of target exposure. There was no increase in the number of micronucleated polychromatic erythrocytes seen in the bone marrow of the additive-treated group. The positive control group responded as expected.

[34] Technical dossier/Section III/Annex 3-2-2
[35] Technical dossier/Section III/Annex 3-2-3
[36] Technical dossier/Section III/Annex 3-2-4

Conclusions on safety for the consumer

The additive is not genotoxic. As the additive is essentially not absorbed from the gut lumen, the FEEDAP Panel considers that use of MIMK in animal nutrition is safe for consumers of food products from animals fed diets containing the additive.

3.2.3. Safety for the user

Effects on the respiratory system

The additive has a quite high dusting potential (2.5 g/m^3) and contains a high proportion of fine particles (72% ≤ 50 µm diameter; 11.5% ≤ 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.

An acute inhalation toxicity study was performed with the additive using groups of five male and five female Wistar rats exposed to 0 or 3.9 mg additive/L air for 4 h followed by a recovery period of 14 days, using a protocol that conformed to OECD Guideline 403.[37] No adverse effects on signs of toxicity, body weight or gross pathology were found in any of the animals in either the control or treatment group. Therefore, the additive is considered to be of low toxicity by the inhalation route.

Effects on eyes and skin

An eye irritation study that conformed to OECD Guideline 405 was performed using three female New Zealand White rabbits.[38] The only reaction in treated eyes was a slight transient redness. All eyes appeared normal at 72-h postdosing. The results of this test indicate that the additive does not require labelling as an eye irritant.

An acute dermal toxicity study conforming to OECD Guideline 402 was performed on the additive using groups of five male and five female Wistar rats.[39] No mortality was observed. No adverse effects on signs of toxicity, body weight or gross pathology were found in any of the animals in either the control or the treatment group. Therefore, the additive is considered to be of low toxicity by the dermal route.

No studies of skin sensitisation potential were available.

The nickel content of the additive is up to 30 mg/kg additive; given its well-known sensitisation potential (EC, 2011), the additive should be considered a potential dermal and respiratory sensitiser (Nemery, 1990; Schnabel et al., 2010; Nordberg et al., 2015).

Conclusions on safety for the user

The additive is not an irritant to the eyes and it is of low toxicity by the inhalation route. No conclusions could be drawn on skin irritation. The additive should be considered a potential dermal and respiratory sensitiser.

3.2.4. Safety for the environment

The components of the additive (illite, montmorillonite, kaolinite, calcite and sanidine) are ubiquitous in the environment, being natural components of soil. Therefore, it is not expected that its use as a feed additive would adversely affect the environment.

3.3. Efficacy

3.3.1. Anticaking agent

The efficacy of the additive as an anticaking agent was tested in five subsamples each of a laying hen,[40] a pig[41] and a cattle[42] pelleted feed (pellet dimensions 3x4, 3x8 and 4x24 mm, respectively).

[37] Technical dossier/Section III/Annex 3-3-1
[38] Technical dossier/Section III/Annex 3-3-2
[39] Technical dossier/Section III/Annex 3-3-3
[40] Technical dossier/Section IV/Annex 4-2-A

After mixing the feeds with the additive at concentrations of 0, 5,000, 10,000, 20,000 and 50,000 mg/kg, samples of 500 g of each were loaded into a standard cone with an orifice of 25 mm (laying hens feed) or 43 mm (pig and cattle feed) and left to fall from a standard height of 120 mm (laying hens) and 80 mm (pig and cattle feed). Below the cone, the sample formed a small pile, forming an angle of response α, calculated as a quotient of the height (h) and the diameter (D) of the pile (tan α = h/0.5 D). The angle (α) gives the tendency of the material to be cohesive or free-flowing, with lower values (25–30°) for very flowing materials and higher values (> 66°) for cohesive materials (Carr, 1965). The speed of flow through the cone (S) gives an indication of the flowability; the data were statistically analysed using Student's t-test to compare the series. The outcome is expressed as a percentage improvement by the additive I (I = 1-$S_{test}/S_{control}$).

In the three feedingstuffs tested, the addition of 5,000 and 10,000 mg of the additive reduced the flowability of feed as indicated by higher values for the angle of response α and the speed S. The data of the inclusion of 20,000 mg MIMK were ambiguous for the pig feed, α showing lower values but S being still higher. No improvement of flowability was seen in the feedingstuffs for laying hens and cattle. The average α for the laying hens, pigs and cattle feed containing 50,000 mg MIMK, compared to the control, was 32.6° vs. 33.2°, 27.4° vs. 28.5 and 27.2° vs. 27.8 respectively. The improvement I was 1.3%, 1.6% and 1.2% respectively. Both endpoints of flowability α and S support for all types of feed tested the efficacy of the additive as anticaking agent at inclusion level of 50,000 mg/kg.

3.3.2. Binders

In a first trial,[43] commercial broiler and pig feedingstuffs were supplemented with 0, 10,000, 20,000 or 50,000 mg of the additive, mixed for three minutes, and then pelleted at 77°C (broiler feed) and 82°C (pig feed) to obtain 2 x 7 mm pellets for broiler feed and 3 x 10 mm pellets for pig feed. After cooling, the abrasion characteristics of 100–500 g samples of pellets (four repetitions) were evaluated with three different methods (Pfost, Quick Test and New Holmen (pig feed only)). The abrasion tests expose pellets to mechanical action and simulate stress (e.g. during transport, bin-filling). The hardness of the pellets (10 samples) was tested using the Kahl hardness tester. The data were statistically analysed using Student's t-test. At inclusion levels of 1 and 2%, no effect of the additive on either feed was observed. At the inclusion level of 5%, a statistically significant improvement of pellet durability shown by reduced abrasion (Pfost, -14.3% and Quick, -15.5%) and by increased pellet hardness (+29.2%) was observed for the poultry feed. A tendency in decreasing pellet abrasion was shown in pig feed supplemented with 50,000 mg MIMK (New Holmen (-15.5%)), whereas the hardness of the pellets was improved (+33.9%).

In a second trial,[44] two batches of a commercial turkey feed were supplemented with 0 or 20,000 mg of the additive, mixed and then pelleted at 70°C, to obtain pellets of 10 mm length. After cooling, pellet durability was tested using the Pfost and the Quick Test methods. The study design did not allow statistical evaluation of the results, which were therefore not considered further.

In a third trial,[45] a commercial concentrate for heifer (87% DM, 17% CP, 10% crude fibre) was supplemented with 0, 10,000, 20,000 or 50,000 mg of the additive and then pelleted to obtain 3.2 mm pellets. The hardness of the pellets (100 samples per treatment) was subsequently tested using the Kahl hardness tester. The data were statistically analysed using Student's t-test. At inclusion levels of 10,000, 20,000 and 50,000 mg/kg, a statistically significant ($P < 0.05$) improvement of pellet hardness was observed (+3.0%, +6.4% and +10.3%, respectively).

Conclusions on efficacy

MIMK was effective as pellet binder by increasing pellet hardness and reducing pellet abrasion in feeds for two animal species at 50,000 mg/kg and at levels of 10,000 mg/kg or greater in feed for a third animal species. Efficacy as anticaking agent was demonstrated at 50,000 mg/kg in feedingstuffs

[41] Technical dossier/Section IV/Annex 4-2-B
[42] Technical dossier/Section IV/Annex 4-2-C
[43] Technical dossier/Section IV/Annex 4-3
[44] Technical dossier/Section IV/Annex 4-4
[45] Supplementary Information July 2015/Annex_iii_1

for three animal species, lower concentrations (5,000 and 10,000 mg/kg) showed rather a cohesive than an anticaking action.

The additive is effective as a binder and an anticaking agent at a dietary inclusion rate of 50,000 mg/kg in feed for all animal species.

4. Conclusions

The additive is safe in complete feed for cattle for fattening at a maximum concentration of 50,000 mg/kg and at a maximum concentration of 20,000 mg/kg for piglets and pigs for fattening. No conclusions can be drawn for all the other animal species/categories.

The additive is not genotoxic. As the additive is essentially not absorbed from the gut lumen, the FEEDAP Panel considers that use of MIMK in animal nutrition is safe for consumers of food products from animals fed diets containing the additive.

The additive is not an irritant to the eyes and the skin, and it is of low toxicity by the inhalation route. No systemic toxicity is expected following dermal exposure. Due to its nickel content, the additive should be considered a potential dermal and respiratory sensitiser.

The components of the additive (illite, montmorillonite, kaolinite, calcite and sanidine) are natural constituents of soil. Consequently, the use of the additive in animal nutrition will not pose a risk to the environment.

The additive is effective as an anticaking agent and a binder at an inclusion rate of 50,000 mg/kg complete feed.

5. Remark

The FEEDAP Panel notes that the iron content of the product (average 10.4% and ranges between 7.6 to 13.2%) would limit its use in compound feedingstuffs for which a maximum content for iron is set by the EU legislation.[46]

[46] Commission Regulation (EC) No 1334/2003 of 25 July 2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements (OJ L 187, 26.7.2003, p. 11)

Documentation provided to EFSA

1. Natural mixture of illite, montmorillonite and kaolinite (Argile Verte du Velay) for all animal species. April 2011. Submitted by Argile Du Velay - Arvel

2. Natural mixture of illite, montmorillonite and kaolinite (Argile Verte du Velay) for all animal species. Supplementary information. February 2014. Submitted by Argile Du Velay – Arvel

3. Natural mixture of illite, montmorillonite and kaolinite (Argile Verte du Velay) for all animal species. Supplementary information. July 2014. Submitted by Argile Du Velay – Arvel

4. Natural mixture of illite, montmorillonite and kaolinite (Argile Verte du Velay) for all animal species. Supplementary information. July 2015. Submitted by Argile Du Velay – Arvel

5. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for natural mixture of illite, montmorillonite and kaolinite.

6. Comments from Member States.

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Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ADI	average daily intake
BW	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CP	Crude Protein
CV	Coefficient of variation
DM	Dry matter
EC	European Commission
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
GLP	Good laboratory practice
HACCP	hazard analysis and critical control points
ICDD	International Center for Diffraction Data
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
ME	Metabolisable energy
MIMK	natural mixture of illite, montmorillonite and kaolinite
NE	Net energy
OECD	Organisation for Economic Co-operation and Development
PCBs	Polychlorinated biphenyls
PCDD/F	Polychlorinated dibenzodioxins/dibenzofurans
TEQ	Toxic equivalent
WHO	World Health Organisation
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 natural mixture of illite, montmorillonite and kaolinite.

In the current application authorisation is sought under article 4(1) for Velay green clay, a *natural mixture of illite, montmorillonite* and *kaolinite* (MIMK), under the category/functional group 1(g) and 1(i) 'technological additives'/'binders' and 'anticaking agents', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the *feed additive* for all animal species. According to the Applicant, the *feed additive* contains a minimum of 40% *illite*. The *feed additive* is intended to be used in *premixtures* and *feedingstuffs*. The Applicant did not specify any maximum or minimum concentration of MIMK in *feedingstuffs* but recommends a dosage of 2–5% for all animal species.

For the determination of mineralogical composition of the *feed additive*, the Applicant submitted experimental data obtained using X-ray diffraction (XRD) method. Furthermore, the chemical composition of the *feed additive* was characterised by the Applicant using X-Ray fluorescence (XRF). Based on the experimental evidence provided, the EURL recommends for official control the two methods (XRD and XRF) for the characterisation of *Velay green clay.*

As the quantification of *MIMK* in *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot recommend any method for official control in these matrices. However, the addition of *Velay green clay* in *feedingstuffs* could be monitored indirectly by determining the increased iron and aluminium content, if identical *feedingstuffs* without the product is available for comparison.

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.