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Safety and efficacy of a feed additive consisting of sepiolite for all animal species (Sepiol S.A and Tolsa, S.A)

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the re-evaluation of the authorisation of sepiolite as a feed additive for all animal species. The FEEDAP Panel considered that sepiolite is unlikely to be absorbed. Harmful amounts of residues of any chemical component in edible tissues/products, as a consequence of the use of sepiolite as a feed additive, are not expected. Sepiolite is not genotoxic and does not induce any toxicity effects following oral administration and, therefore, it was considered safe for the consumers. The additive was considered safe for dairy cows at the recommended use level with a safety factor of 2.5. The conclusion was extrapolated to other dairy ruminants but owing to the lack of sufficient data, no conclusions can be drawn on the safety of the additive for the other target species/categories. Based on the results of a chronic inhalation toxicity study, the additive is considered a respiratory irritant. Owing to the dusting potential of the additive and its silica content, handling the additive was considered a risk by inhalation for the users. It is not irritant or corrosive to skin or eyes. Due to the nickel content, it is considered a skin and respiratory sensitiser. The additive was considered safe for the environment. The FEEDAP Panel concluded that sepiolite was efficacious as a thickener-suspending agent, binder and anticaking agent in feed for all animal species under the proposed conditions of use.

Keywords: example: sepiolite, thickener-suspending agent, binder, anticaking agent, all animal species, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2019-00428

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Amendment: In the whole document, the term 'sepiolite' has been reported in capital letter when it refers to the additive itself and in lower case letter when it refers to the mineral contained in the additive. An editorial correction was carried out that does not materially affect the contents or outcome of this scientific output. To avoid confusion, the original version of the output has been removed from the EFSA Journal, but is available on request.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC. In particular, Article 10(2) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of seven years after the entry into force of this Regulation.

The European Commission received a request from Sepiol S.A. and Tolsa S.A.[2] for re-evaluation of the authorisation of Sepiolite, 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 10(2) (re-evaluation of an authorised 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 8 August 2019.

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 sepiolite, when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

Sepiolite is authorised as binder, anti-caking agent and coagulant for all animal species with a maximum content of 20,000 mg/kg feed.[3]

EFSA has adopted one opinion on the safety and efficacy of sepiolite for all animal species as a preparation in combination with bentonite (EFSA FEEDAP 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[4] in support of the re-evaluation of the authorisation request for the use of Sepiolite (E 562) as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the sepiolite in animal feed. The Executive Summary of the EURL report can be found in Annex A.[5]

[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] Sepiol, S.A. Ctra. A-2 km 38.600, 19200 Azuqueca de Henares, Spain and Tolsa, S.A., Ctra. Vallecas-Mejorada del Campo km 1.600, 28031 Madrid, Spain.
[3] COMMISSION REGULATION (EC) No 2439/1999 of 17 November 1999 on the conditions for the authorisation of additives belonging to the group 'binders, anti-caking agents and coagulants' in feedingstuffs. OJ L 297, 18.11.1999, p. 8.
[4] FEED dossier reference: FAD-2010-0365.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2010-0365-sepiolite.pdf

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Sepiolite is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

The products under assessment are mixtures of clay and non-clay fractions (hereby referred to as Sepiolite) containing a minimum of 60% of sepiolite (mineral). The applicant is seeking a re-evaluation of the authorisation for the use of the products as technological additives (functional groups (e) thickeners, (g) binders and (i) anticaking agents) at a maximum concentration of 20,000 mg/kg in complete feed for all species.

3.1. Characterisation

3.1.1. Characterisation of the additive

The feed additive Sepiolite is specified as hydrated magnesium silicate of sedimentary origin, containing at least 60% sepiolite, no more than 30% montmorillonite and being free of asbestos. The CAS number of sepiolite is 63800-37-3 and the EC number is 264-465-3.

The Sepiolite under application originates from Spanish sepiolite deposits. Its major component (min 60%) is sepiolite and it further contains other clays (smectite (e.g. montmorillonite), attapulgite, illite), and non-clay fractions as calcite, dolomite, feldspars and quartz.

The applicant provided results of the mineralogical analysis performed by X-ray diffraction (XRD) and elemental analysis by X-ray fluorescence spectroscopy (XRF) on four products from Company A (Table 1) and from three products from Company B (Table 2).[7] The sepiolite content [redacted] The latter product is referred to as rheological sepiolite [redacted] and is specified by the applicant to be used as thickener only.

Table 1: Mineralogical composition measured on three batches of Sepiolite product from Company A, by X-ray diffraction. Values reported as mean (min–max)

[redacted]

[6] 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.

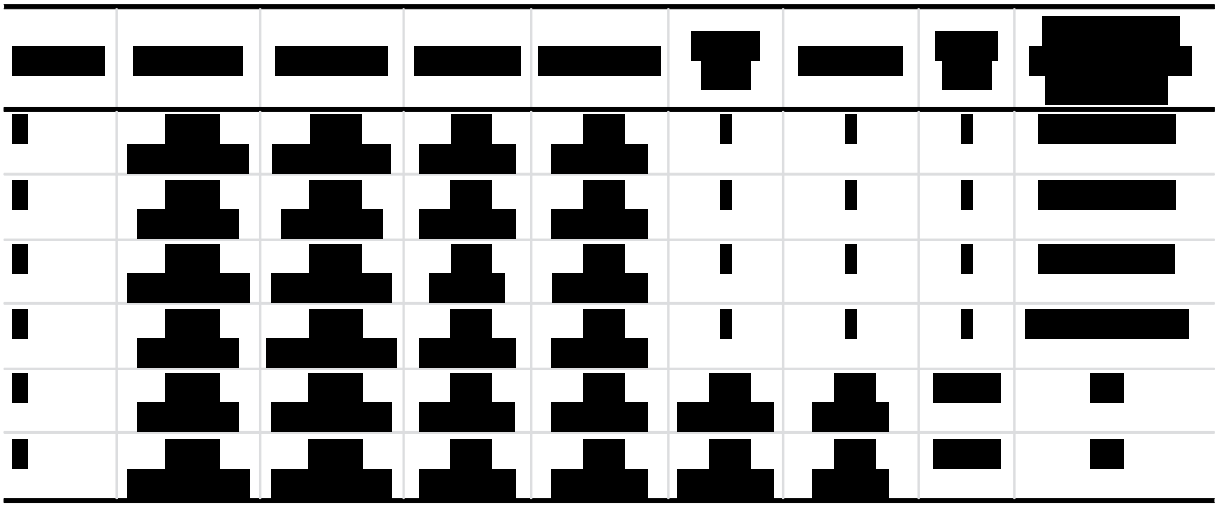
[7] Technical dossier/Supplementary information (June 2021)/Annex Sin_1_1 and Technical dossier/Section II/Annex_II_5.

Table 2: Mineralogical composition measured on three batches of Sepiolite products from Company B, by X-ray diffraction. Values reported as mean (min–max)

*: Analysed in four batches.

Chemical composition analysis was provided for six (three batches each)[8] of the above-mentioned seven products. The main elements that constitute the additive are [redacted] They are expressed as oxides and are reported in Table 3.

Table 3: Results of elemental analysis measured on three batches of six Sepiolite products



Cadmium, lead, mercury,[9] arsenic, nickel, dioxins and PCBs were analysed in three batches of the seven products. Lead content, based on analysis of seven products, ranged [redacted] The level of cadmium in all samples was [redacted] The analysed values for [redacted] Mercury in all samples was [redacted] The content of fluorine was analysed in three batches of four products (products 1, 2, 3 and 4) and ranged [redacted]

The content of nickel was analysed by inductively coupled plasma mass spectrometry (ICP-MS) in three batches of each product under assessment and ranged between [redacted][10]

The content of dioxins[9] (sum of polychlorinated dibenzo-paradioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)) [redacted]

The levels of the detected impurities do not raise a safety concern.

Sepiolite is a free-flowing solid presented in a dry, granular or powder form. The bulk density of the different sepiolite products [redacted] and the moisture [redacted] The loss on ignition was measured on three batches of six products, resulting in average values [redacted] The water absorption capacity measured in three or four batches of four Sepiolite products (products 1, 5, 6 and 7) [redacted][11]

The dusting potential of the additive was measured in three batches of the seven products of the additive according to the Stauber Heubach method.[12] The particle size distribution analysis was

[8] Technical dossier/Section II/Annex_II_5.
[9] Technical dossier/Section II/Annex_II_5–6.
[10] Technical dossier/Supplementary information (June 2021)/Annex_Sin_2_1, Annex_Sin_2_2.
[11] Technical dossier/Section II II/Annex_II_5.
[12] Technical dossier/Supplementary information (June 2021)/ Annex_Sin_3_1, Annex_Sin_3_2.

performed by laser diffraction analysis in the same batches.[13] The results of both analyses are reported in Table 4.

Table 4: Average dusting potential and particle size distribution of Sepiolite products

The applicant also submitted data on the content of fibre-shaped particles and asbestos [redacted] No asbestos nor fibre-shaped particles were detected.[14]

3.1.2. Manufacturing process

The product is obtained by mining, drying and milling sedimentary strata in mines in Spain. It undergoes a first trituration and once dried, minerals with > 60% sepiolite are selected and triturated again in the manufacturing plant, then are dried and granulometrically classified to get the commercial presentations.

The rheological Sepiolite has a sepiolite content of [redacted] and it is used as thickener for liquid feeding. It is manufactured following four steps: (i) crushing of the lumps below 20 mm, (ii) soaking in water, (iii) wet micronisation by wet milling, pressing, conforming and drying and (iv) final milling, grounding down the conformed pellets to the final product particle size.

3.1.3. Stability and homogeneity

3.1.3.1. Stability in premixture and feeding stuffs

For technological additives, stability could be demonstrated by analytical follow-up of the active substance or with the demonstration of persistence of the technological effect (efficacy). The applicant provided a stability study in premixtures (measuring the presence of sepiolite and following up the anticaking properties) and one study in feedingstuff measuring the persistence of the effect as a pellet binder.

In a first experiment,[15] two batches of Sepiolite [redacted] The persistency of the anticaking effect is described in Section 3.3.2.

In the second experiment,[16] two complementary feedingstuffs for cattle and two for lambs, and three complete feedingstuffs for pigs were pelleted with the inclusion of 20,000 mg/kg of the additive (approximately 70% sepiolite) at different pelleting conditions (not detailed). Pellet durability was measured with the Pfost method after pelleting and after 3 months of storage (in three batches for all the ruminants' feeds and two in the pigs' feed). The results showed no loss of durability (> 97%) in any of the samples analysed.

[13] Technical dossier/Supplementary information (June 2021)/Annex_Sin_4_1, Annex_Sin_4_2.
[14] Technical dossier/Supplementary information (June 2021)/Annex_Sin_5_2, Annex_Sin_5_3.
[15] Technical dossier/Section II/Annex_II_11.
[16] Technical dossier/Section II/Annex_II_12.

3.1.3.2. Homogeneity

Homogeneity of sepiolite was studied[17] in four different samples of complete feedingstuffs (starter, 21–42 days) for piglets (two replicated analyses of 10 subsamples each) using the loss on ignition method. The percentage of ashes was measured in each sample (averages: 9.6–10.3%) and the coefficient of variation (CV) per batch calculated. The CV ranged from 2.4% to 4.3%.

In another study, three samples of a premixture for pigs containing 200,000 mg/kg sepiolite and one control feed were analysed using the loss on ignition method. The analysed sepiolite content from the ashes (range 19.76–19.88) corresponds with the theoretical content of inclusion and the CV was 0.37%.

Homogeneity of rheological Sepiolite in liquid feeds was studied[18] in six subsamples of a liquid feed supplemented with 0 and 10,000 mg/kg Sepiolite. The data were statistically analysed and showed that homogeneity indexes of dry matter (DM), ash, crude fibre, fat and crude protein were significantly higher when sepiolite was added to the feed.

A fourth study[19] reports the results of the analysis of homogeneity of rheological Sepiolite in liquid feeds for pigs for fattening and gestating and lactating sows. Liquid feeds with different DM content, supplemented with 0 or 7,000 mg/kg rheological Sepiolite, were analysed for homogeneity index. The results showed that the homogeneity index was improved in the supplemented feed with rheological Sepiolite compared to the control, at any DM content.

3.1.4. Physico-chemical incompatibilities or interactions

The applicant submitted a study to demonstrate that Sepiolite has no masking effects for mycotoxins.[20] [redacted] indicating that sepiolite does not interfere in the analytical determinations of mycotoxins.

3.1.5. Conditions of use

The applicant proposes the use of Sepiolite products as thickener, binder and anticaking agent under the category technological additive with a maximum level of 20,000 mg/kg complete feed for all animal species.

3.2. Safety

3.2.1. Absorption, distribution, metabolism and excretion

The FEEDAP Panel considers it unlikely that Sepiolite and the other mineral components of the additive, in common with other clays, will be degraded during their passage through the gastrointestinal tract of target animals or absorbed. Clays are essentially not absorbed, and carry-over to animal tissues/products is therefore not expected.

Sepiolite chemical structure shows the presence of fluorine (F) as $(SiO_2)_nOMgF$ fragments (Santaren et al., 1990) the stability of which could be critical in terms of possible release of fluorine in the gastrointestinal tract and subsequent deposition in edible tissues/products, as a consequence of the use of the additive.

The applicant has submitted studies in support of the stability and low bioavailability of F bound to sepiolite.

Rats were distributed in three groups (15 animals per group) given different diets: control (basal diet containing 56 mg F/kg), diet with sepiolite containing 258 mg total F/kg feed and a diet with 258 mg fluorine as NaF/kg feed, for 226 days (Álvarez et al., 1987). Potentiometric analysis of fluorine was conducted in bone and urine on six animals (two animals per each group) on day 70 and in all

[17] Technical dossier/Section II/Annex_II_14.
[18] Technical dossier/Section II/Annex_II_15.
[19] Technical dossier/Section II/Annex_II_16.
[20] Technical dossier/ Supplementary information (June 2021)/Annex_5_2, Annex_Sin_7_1.
[redacted]

remaining rats on day 226 (end of experiment). There were no differences in the content of fluorine in bone and urine between the control group and the group of rats given sepiolite at both times of analysis. A significant increase was noted in the animals fed NaF compared to the control and to the group receiving sepiolite.

Bioavailability and distribution of F in tissues were investigated in rats given sepiolite (Suárez et al., 2008)[22]. Three groups of rats were administered by gastric intubation with three different treatments: a solution of 0.45 mg NaF/kg body weight (bw), a solution of 4.5 mg NaF/kg bw or a suspension of sepiolite-feed in water at 1 g sepiolite/kg bw. Samples of plasma, liver, kidney and muscle from six rats (three males and females) were taken at eight different time points during a period of 24 h for measurement of fluorine by potentiometry. Maximum levels of fluorine (3.13 and 0.77 μg/mL for the high and the low level, respectively) in plasma of animals given NaF were reached at 1 h. Fluorine plasma levels of animals given 1 g sepiolite in suspension were 0.12 μg/mL at 1 h. A similar fluorine profile was observed in liver (0.55 μg/g) and in kidney (3.09 μg/g) in the highest dose of NaF group 1 h after administration, elimination being almost complete after 8 h. In the sepiolite group, levels of fluorine in liver and kidney after 1 h from administration were 0.13 and 0.33 μg/g, respectively, and were maintained low over the time. In muscle samples, fluorine levels were similar to the limit of detection (LOD, 0.048 μg/g) in all the samples analysed. This study demonstrated that fluorine present in sepiolite is not absorbed when administered to rats.

The bioavailability of sepiolite (maximum fluorine content of 9,800 mg/kg) was studied in laying hens by comparing the absorption of fluorine given in diet as NaF or as sepiolite (Nogareda et al., 1990). Three groups of animals were administered with a control diet (basal diet containing 21 mg F/kg), a diet supplemented with NaF at 217 mg fluorine/kg or a diet containing sepiolite at 2% corresponding to 217 mg fluorine/kg. The experiment started when the animals aged 27 weeks until 64 weeks of age. Fluorine was determined by potentiometry analysis conducted in the tibial bone in five animals from each group at weeks 27, 42 and 63. The same analyses were also conducted on eggshell at weeks 42, 55 and 73. No significant differences were found in the fluorine contents in tibial bone and eggshell in the sepiolite group compared to the control. The content of fluorine in the animals given NaF was four times higher compared to the control ($p < 0.001$).

One hundred and twenty White Leghorn male chicks were divided in five experimental groups fed from day 3 to day 90 of age with a basal diet alone as control group (content of fluorine 39 mg/kg), the basal diet supplemented with NaF at 100, 200 or 300 mg/kg or the basal diet supplemented with sepiolite at 20 g/kg (content of fluorine 180 mg/kg) (Tortuero, 1992). On day 40 of the experiment, two animals from the control group, the group fed with NaF at 200 mg/kg, and the group fed with sepiolite were separated in individual cages for evaluation of ileal digestibility of fluorine. On days 60 and 90, six animals from each group were killed for the evaluation of fluorine availability in tibial bone. The ileal digestibility of fluorine in animals given sepiolite was lower (15%) compared to animals in the control and NaF at 200 mg/kg groups (around 90%). Mean fluorine concentrations in tibial bone of animals given NaF at 100, 200, 300 mg/kg or sepiolite was significantly higher (2,910; 4,827; 5,921; 1,585 mg/kg, respectively) compared to the control (1,095 mg/kg). All the groups treated with NaF showed a tibial content fluorine significantly higher than that in the sepiolite group.

A comparative bioavailability of fluorine given in the diet of lambs (12 animals in total, 6 animals each experimental group) twice per day either as NaF (214 mg/kg) or as sepiolite at 212 mg fluorine/kg feed was evaluated (Chaso et al., 1991). Animals were fasted 24 h before starting the experiment. Blood samples were collected for plasma fluorine analysis by potentiometry. Peak plasma concentration of fluorine in lambs fed with NaF was 0.75 μg/mL while in animals fed sepiolite was 0.35 μg/mL. The relative bioavailability of fluorine from sepiolite was significantly lower (3%) compared to fluorine from NaF (100%).

3.2.1.1. Conclusions on ADME

Clays are essentially not absorbed, and carry-over to animal tissues/products is therefore not expected. Based on the studies available, the FEEDAP Panel concluded that fluorine remains tightly bound to the Sepiolite during gastrointestinal passage of monogastric animals and ruminants. Fluorine absorption, as measured by the leached fluorine, is consistently low and being almost completely excreted in faeces.

[22] Technical dossier/Section III/Annex_III_29.

3.2.2. Toxicological studies

3.2.2.1. Genotoxicity studies

Bacterial reverse mutation test

In order to investigate the potential of Sepiolite to induce gene mutations in bacteria, an Ames test was performed according to OECD Test Guideline (TG) 471 and following the Good Laboratory Practice (GLP) principles in *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and *E. coli*.[23,24] Preliminary solubility test showed that the test item was soluble in water at 50 mg/mL. Based on the results of a cytotoxicity assay, sepiolite was tested at five concentration levels ranging from 60 to 5,000 μg/plate. Two independent experiments were performed applying the plate incorporation and pre-incubation methods both in the presence and absence of the metabolic activation. Appropriate positive and negative controls were evaluated concurrently. All positive control chemicals induced significant increases in revertant colony number, confirming the sensitivity of the test system and efficacy of the metabolic activation system. No toxicity was observed at any tested concentration. No increase in the mean number of revertant colonies was observed at any tested condition in any tester strain. Therefore, the Panel concluded that Sepiolite did not induce gene mutations in bacteria under the experimental conditions applied in the study.

In vitro micronucleus test

To evaluate the potential of a suspension obtained from Sepiolite to induce chromosome damage [redacted] an *in vitro* micronucleus test was carried out according to OECD TG 487 and following GLP.[25] [redacted] The Panel concluded that Sepiolite did not induce micronuclei in CHO cells under the experimental conditions employed in this study.

In vivo micronucleus test

In order to investigate the potential of Sepiolite to induce chromosomal damage in bone marrow cells, an *in vivo* micronucleus test was performed in Sprague-Dawley rats, according to OECD TG 474 (OECD, 1997) and following GLP.[24,26] Animals were treated twice by oral gavage, 24 h apart, at 500, 1,000 and 2,000 mg/kg bw. The highest dose corresponded to the top dose recommended by OECD TG 474 (1997) for not toxic substances. No exposure of the target tissue was demonstrated, since comparable frequencies of polychromatic erythrocyte (PCE) were reported between negative controls and treated animals. No increase in the frequency of micronuclei was observed in treated animals. The FEEDAP Panel considered that this study would not be useful for the risk assessment as the additive is not absorbed and consequently only possible effects at the level of site of contact should be investigated.

3.2.2.2. Subchronic repeated dose oral toxicity studies

In a non-GLP and non-guideline compliant study, two groups of Wistar rats (15 animals/sex per group) were administered 1.5% Sepiolite or 1% lignosulfite (control) in pelleted feeds for 12 weeks. In order to investigate a sepiolite effect on the gastrointestinal speed transit, additional 14 animals/sex per group were administered 20 mg sepiolite incorporated in 0.5 mL colloid carbon suspension, by gavage.[27,28] Animals were observed twice daily for mortality and weekly for clinical signs of toxicity throughout the study. Body weights were recorded at study initiation, weekly and at study termination. At study termination, gross necropsy was performed on all animals. Organ weights were recorded for

[23] Technical dossier/Section III/Annexes_III_32.
[24] Technical dossier/Section III/Supplementary_Information (November 2021)/Annex_SIn2_Q6_1.
[25] Technical dossier/Section III/Supplementary_Information (June 2021)/Annexes_Add SIn_2_1.
[26] Technical dossier/Section III/Annexes_III_33.
[27] Technical dossier/Section III/Annexes_III_35.
[28] Technical dossier/Section III/Supplementary_Information (November 2021)/Annex_SIn2_Q6_2.

liver, kidneys, lung and heart, spleen, small intestine and stomach. At necropsy, all animals were subject to macroscopic examination on stomach, intestine, colon, liver, kidneys, spleen, lung, heart, central nervous system (CNS), lymph nodes, subcutaneous fat, blood and muscles. Tissues from five animals/sex per group were processed for microscopic examination. Histopathology examination was conducted on liver, kidney, stomach and large intestine. No mortality was observed during the study. The final mean body weights and changes in mean body weights, feed intake and water intake of rats receiving sepiolite were similar to those of the controls and no significant differences were detected. No significant differences in the absolute and relative organ weights were reported. There were no gross or microscopic lesions attributable to sepiolite administration. No effects on the gastrointestinal speed transit attributable to sepiolite administration were observed.

The FEEDAP Panel noted that the study shows several deviations from the current OECD TG 408. These include a shorter exposure duration, limited experimental protocol and results reporting. Owing to that the additive is not absorbed, the FEEDAP Panel considered the conclusions of the study acceptable and concluded that oral administration of Sepiolite at a level of 1.5% in feed in rats for 12 weeks did not induce any adverse effect under the reported experimental conditions.

3.2.2.3. Conclusion on biological and toxicological studies

The FEEDAP Panel concluded that Sepiolite and the other mineral components of the additive, as other clays, are essentially not absorbed, and carry-over to animal tissues/products is therefore not expected. Sepiolite is not genotoxic and does not induce any adverse effects following oral administration.

3.2.3. Safety for the target species

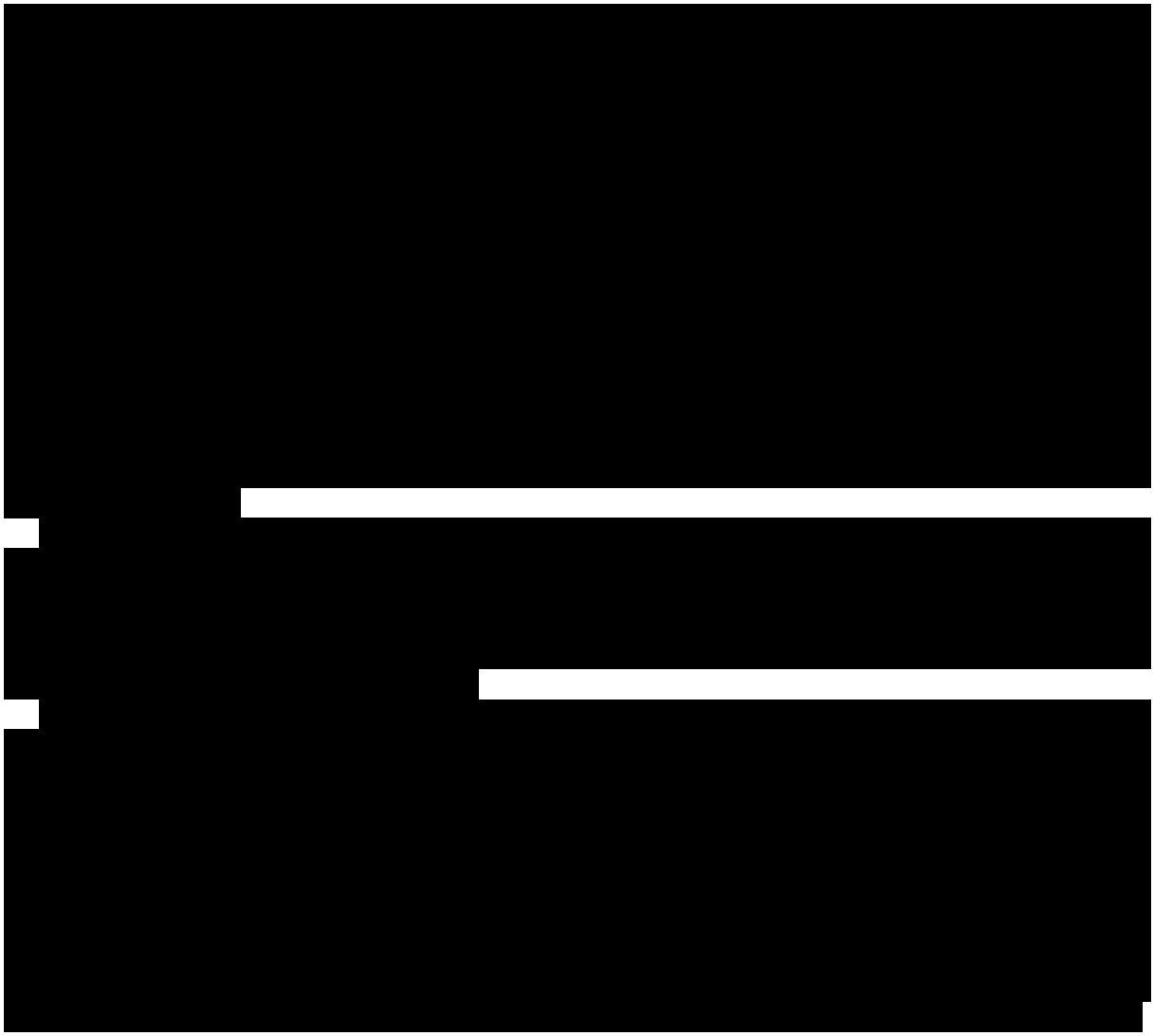
The applicant provided several publications in journals and efficacy studies conducted in different species/categories including chickens, laying hens, pigs, rabbits, cattle/calves for fattening and dairy cows. These studies were not designed to assess the safety of the additive: (i) The levels were not higher than the maximum use level proposed and/or (ii) the parameters measured did not include some of those considered relevant to the safety assessment such as blood haematology and biochemistry or necropsy of the animals under study. Therefore, the conclusions from the results in those studies are not considered sufficient to support the safety of the additive for the different target species.

Four tolerance trials were submitted, two in chickens, one in weaned piglets and one in cows.

The two tolerance trials in chickens and the one in weaned piglets showed some limitations. [redacted][29][redacted][30][redacted] Considering all the limitations identified, these trials were not considered further in the assessment.

In the tolerance trial in dairy cows, [redacted][31][redacted][32][redacted]

[29] Technical dossier/Section III/Annex III_1.
[30] Technical dossier/Supplementary information (June 2021)/Annex 1.3 and Supplementary information (November 2021)/ Annex_Q5_I.
[31] Technical dossier/Supplementary information (June 2021)/Annex_1_1. The days in milk are higher than the one expected for a tolerance trial but may be acceptable considering the nature of the additive and the impact of its inclusion in the diets.
[32] The definition of complete feed in the study report did not include the forages.



Conclusions on the safety for the target species

Based on the study in dairy cows, the FEEDAP Panel concludes that the additive is safe for dairy cows at the maximum use level of 20,000 mg/kg complete feed with a safety factor of 2.5. The conclusion is extrapolated to other dairy ruminants. Owing to the lack of sufficient data, no conclusions can be drawn on the safety of the additive for the other target species/categories.

3.2.4. Safety for the consumer

The Panel considers that Sepiolite and the other mineral components of the additive are not absorbed, and that it is unlikely that harmful amounts of residues of any chemical component would occur in edible tissues/products as a consequence of the use of sepiolite in animal nutrition. The additive is therefore considered safe for the consumers.

[34] The data included: haemoglobin (Hb), red blood cell count (RBC), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), platelets, white blood cell counts (WBC), white blood cell differentials (segmented neutrophils, banded neutrophils, monocyte, lymphocytes, Eosinophils), prothrombin time, fibrinogen; alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine phosphokinase (CPK), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), glutamate dehydrogenase (GLDH), glutathione peroxidase (GSH-Px), bilirubin, creatinine, cholesterol, albumin, globulin, total protein, glucose, urea/uric acid, sodium, potassium, chloride, calcium, phosphate and magnesium.

3.2.5. Safety for user

3.2.5.1. Effects on the respiratory system

Based on the dusting potential data available (up to [redacted]), the FEEDAP Panel considered that the exposure of users through inhalation is very likely.

The applicant provided an acute inhalation toxicity study with Sepiolite[35] following GLP principles and according to OECD TG 436. [redacted] Based on the results of this study, the FEEDAP Panel considered that Sepiolite should not be regarded as a potential hazard by inhalation.

In addition, the applicant has provided a publication (Wagner et al., 1987) evaluating the inhalation effects of sepiolite dust obtained from the same area as the sepiolite used in the acute inhalation study described above. A group of 20 male and 20 female Fischer 344 rats, 6 weeks of age, were exposed to 10 mg/m^3 sepiolite dust for 6 h per day, 5 days per week for 12 months. The respirable dust contained 1.15 $\times$ 10^6 fibres/μg. After 3, 6 and 12 months, two animals per sex per group were killed and their lungs examined for the presence and severity of fibrosis. After 12-month exposure ceased, and the remaining rats were maintained for their normal life span (except two males and two females killed after 24 months). A full necropsy was performed on all animals and lungs, liver, spleen, kidneys and any other relevant organs (not further specified) were examined for histopathology. Animals killed up to 24 months of age showed a grade 3 of fibrosis (early interstitial reaction). Based on the presence of pulmonary early interstitial reaction, the FEEDAP Panel concluded that sepiolite is a respiratory irritant.

The FEEDAP Panel also notes that the additive contains crystalline silica (range [redacted]). Inhalation of silica is known to be hazardous and is associated with increased risk of lung cancer and the industrial disease, silicosis. The European Directive 2017/2398 set an occupational exposure limit of 0.1 mg/m^3 of air for respirable crystalline silica dust. The FEEDAP Panel noted that data on respirable fraction of the dust were not available, therefore used as a worst-case scenario the highest dusting potential data to calculate the content of silica present in the dust. The dust fraction of sepiolite was up to [redacted], corresponding to 398 mg crystalline silica/m^3 dust.

The highest nickel content analysed in the additive was [redacted] The highest dusting potential of the product was [redacted], corresponding to about 0.035 mg Ni/m^3 which is above the occupational exposure limit (OEL) for the inhalable fraction of water-soluble nickel (0.03 mg Ni/m^3; ECHA, 2018). Due to the presence of nickel in the additive, it should be considered as a respiratory sensitiser.

3.2.5.2. Effects on the eyes and skin

The skin irritation potential of Sepiolite was tested in a study performed according to OECD TG 404, which showed that it is not a skin irritant.[36]

The eye irritation potential of Sepiolite was tested in a study performed according to OECD TG 405[37] using three female New Zealand White rabbits. The initial test demonstrated some hyperaemic blood vessels in the treated eye, 1 h after test item instillation. On day 2 of the study, corneal damage was observed in both eyes, which was of similar nature in the treated and untreated eye. The authors considered these corneal injuries related to physical damage inflicted by the animal itself. In the confirmatory test, no major ocular reactions were recorded in one animal. In the second animal, the treated eye showed some hyperaemic blood vessels 1 h after instillation of the test item, which had disappeared on day 9 of the study. No gross lesions were observed at the necropsy. Based on the results of the test, Sepiolite was not considered irritant or corrosive to eyes.

No data on skin sensitisation were provided by the applicant. The nickel content of the additive is up to [redacted] given its well-known sensitisation potential (European Commission, 2011; ECHA, 2018), the additive is considered as a skin sensitiser.

3.2.5.3. Conclusions on safety for the user

Sepiolite poses a risk by inhalation. It is not irritant to the skin or eyes but should be considered as skin and respiratory sensitiser.

[35] Technical dossier/ Section III/Annex_III_39.
[36] Technical dossier/ Section III/Annex_III_41.
[37] Technical dossier/ Section III/Annex_III_40.

3.2.6. Safety for the environment

Sepiolite is a naturally occurring clay widely distributed in the environment. Therefore, it is not expected that the use of the additive in animal nutrition would adversely affect the environment.

3.3. Efficacy

3.3.1. Efficacy as a binder

To support the efficacy of the additive as a binder, the applicant provided five published studies. Three of these publications showed several shortcomings (e.g. lack of control group and appropriate replicates, lack of statistical analysis), the fourth one was done with an uncharacterised sepiolite from a different manufacturer. Therefore, these four publications were not further considered in the assessment. The applicant also provided a summary table of the analysis of durability of feeds supplemented with the additive at different inclusions levels; however, the lack of statistical analysis excludes these data from further assessment.

In a study (Angulo et al., 1995) in which the effect of Sepiolite on pellet durability was studied using the Pfost durability test, three types of complete feed (pigs, sows and rabbits), each of them with two levels of fat (5,000 or 40,000 mg/kg) were supplemented with sepiolite at 0 and 20,000 mg/kg feed. Pellet durability, percentage of broken pellets and fines collected in the cooler and cyclone were measured in four replicates for each feed. The results were statistically analysed considering the contribution of the main factors and their corresponding interactions to the sum of squares. The detailed results are summarised in Table 5.

Table 5: Results of pellet durability, presence of broken pellets and fines in feeds supplemented with 0 and 20,000 mg/kg Sepiolite and 5g/kg and 40 g/kg of fat

Type of feed	Fat inclusion level (mg/kg)	Sepiolite inclusion level mg/kg)	Durability (%)	Broken pellets (%)	Fines (%)
Pig grower	5,000	0	93.8	1.54	1.95
		20,000	96.0	0.84	2.95
	40,000	0	81.9	3.96	6.89
		20,000	92.9	1.36	3.76
Sow	5,000	0	96.1	0.96	2.04
		20,000	97.1	0.76	1.41
	40,000	0	85.7	3.24	5.65
		20,000	92.0	1.80	4.31
Rabbit	5,000	0	97.4	0.82	1.51
		20,000	98.0	0.55	1.01
	40,000	0	91.8	2.15	3.70
		20,000	95.9	1.06	2.36

The inclusion of Sepiolite improved pellet durability reduced the percentage of broken pellets and the amounts of fines in all the three feedingstuffs ($p < 0.0001$, contribution to total variability 19.2%). The interaction between sepiolite and fat content of the feed ($p < 0.0001$, contribution to total variability 10.6%) suggests that sepiolite is more effective in feeds with higher fat concentrations.

3.3.2. Efficacy as anti-caking agent

To support the efficacy of the additive as an anticaking agent, the applicant provided a published study that was not considered relevant for the assessment because no parameters relevant to the effect as anticaking were measured.

Five *in vitro* studies were provided to support the efficacy as an anticaking in premixture and feedingstuffs. However, two were not further considered as one showed several shortcomings (e.g. lack of appropriate replicates, lack of statistical analysis) and the other did not measure parameters relevant for the effect as anticaking. The other three relevant studies are described below.

In the first study, the flowability of a premixture [redacted]

[redacted]15

In a second study,[38]

In a third study,[39]

[38] Technical dossier/ Supplementary information (June 2021)/Annex_Sin_8_1, Annex_SIn_8_5 and Annex_SIn_8_6.
[39] Technical dossier/ Supplementary information (June 2021)/Annex_SIn_8_7, Annex SIn_8_8 and Annex SIn_8_9.

3.3.3. Efficacy as thickener

The applicant proposed that only rheological Sepiolite ([redacted]) is to be used as thickener. To support the efficacy of the additive as a thickener in liquid feeds, the applicant provided four published studies. Two of these publications were studies *in vivo* and the properties of the additive in feed were not studied, while a third publication showed several shortcomings (e.g. lack of appropriate replicates, lack of statistical analysis). Therefore, these three publications were not further considered. In addition, the applicant submitted an *in vitro* study with two feedingstuffs.

The standard endpoint to measure the efficacy of an additive as a thickener is viscosity. In some of the studies provided, syneresis was measured. Syneresis measures the clear supernatant liquid volume at the top of the suspension overtime. This parameter is correlated to viscosity, since increasing viscosity reduces syneresis. Therefore, the analysis of this parameter was considered appropriate when evaluating the efficacy of the additive as a thickener.

In a published study (Hoppenbrock et al., 1998), syneresis, viscosity and fluidity were measured in pigs' liquid feed supplemented with 0 and 10,000 mg/kg Sepiolite (97% sepiolite). Syneresis was measured in a graduated cylinder at different times after setting, and viscosity was measured using a Brookfield viscometer. Both were measured in duplicate samples of one batch of the pig feed at different DM content (24%, 25%, 26%, 27% and 28%) and the results were statistically analysed. Fluidity, calculated as the percentage of feed flowing out of a cylinder with an inclination of 5° for 3 min, was measured in the same feed with 25% DM content, before pumping through the circuit and after the first trough in the circuit. The results were not statistically analysed. The detailed results are summarised in Table 8.

Table 8: Results of viscosity, syneresis and fluidity in feeds supplemented with 0 and 10,000 mg/kg Sepiolite

DM (%)	Viscosity (cP)		Syneresis (%)		Fluidity (%) in 25% DM content		
	Control	10,000 mg/kg sepiolite	Control	10,000 mg/kg sepiolite		Control	10,000 mg/kg Sepiolite
24	–	560	20[a]	4[b]	Before pumping	30	62
25	480[b]	630[a]	20[a]	4[b]			
26	950[b]	1,160[a]	7[a]	0[b]	After first trough	39	82
27	715[b]	1,830[a]	2.7[a]	0[b]			
28	1830[a]	1,860[a]	0.8[a]	0[b]			

a,b: Mean values within a row with a different superscript are significantly different ($p < 0.05$).

The addition of sepiolite decreased syneresis of all feeds, while viscosity was increased in feeds with a DM content up to 27%. Sepiolite also (significantly) increased fluidity of the feed with 25% DM both before pumping and after first trough.

In a study,[40] [redacted]

[40] Technical dossier/ Supplementary information (June 2021)/Annex_9_3.

[redacted]

3.3.4. Conclusions on efficacy

Based on the data available covering a range of premixtures, feed materials and complete feeds, the FEEDAP Panel concludes that Sepiolite is efficacious as a binder and anticaking agent. Rheological Sepiolite ([redacted]) is efficacious as a thickener of liquid feeds.

4. Conclusions

The FEEDAP Panel concludes that the additive is safe for dairy cows at the recommended use level. The conclusion is extrapolated to other dairy ruminants. Owing to the lack of sufficient data, no conclusions can be drawn on the safety of the additive for the other target species/categories.

The additive, at the proposed conditions of use, is considered safe for the consumers and the environment.

For the user, Sepiolite poses a risk by inhalation. It is not irritant to the skin or eyes but should be considered as skin and respiratory sensitiser.

Based on the data available covering a range of premixtures, feed materials and complete feeds, the FEEDAP Panel concludes that Sepiolite is efficacious as a binder and anticaking agent. Rheological Sepiolite is efficacious as a thickener of liquid feeds.

5. Documentation as provided to EFSA/Chronology

Date	Event
27/06/2019	Dossier received by EFSA. Sepiolite for all animal species. Submitted by Tolsa S.A. and Sepiol S.A.
27/07/2019	Reception mandate from the European Commission
08/08/2019	Application validated by EFSA – Start of the scientific assessment
23/10/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: conditions of use, characterisations and efficacy*.
05/11/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
11/11/2019	Comments received from Member States
03/12/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: toxicology and safety for the target species and user*
24/06/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
26/10/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the target species and toxicology*.
26/11/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
23/03/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ALT	alanine transaminase
AST	aspartate aminotransferase
BW	Body weight
CHO	Chinese Hamster Ovary
CNS	Central nervous system
Cp	Centipoise
CPK	creatine phosphokinase
CV	coefficient of variation
DM	dry matter
ECHA	European Chemicals Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GGT	Gamma-glutamyl transpeptidase
GLP	good laboratory practices
LC-MS/MS	Liquid Chromatography with tandem mass spectrometry
LDH	Lactate dehydrogenase
LOD	Limit of detection
MCH	mean corpuscular haemoglobin
MCV	mean cell volume
OECD	Organisation for Economic Co-operation and Development
OEL	proposed occupational exposure limit
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzo-paradioxins
PCDFs	polychlorinated dibenzofurans
PCE	polychromatic erythrocyte
PCV	packed cell volume
RBC	Red blood count
SCC	Somatic cell count
TEM	Transmission Electron Microscopy
TG	Test guidelines
XRD	X-ray diffraction
XRF	X-ray fluorescence spectroscopy

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

In the current application, an authorisation is sought under Article 10 for sepiolite 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. Specifically, the authorisation is sought for the use of the feed additive for all animal species. The feed additive is a hydrated magnesium silicate of sedimentary origin, containing a minimum of 60% (w/w) of sepiolite. The feed additive is intended to be used in premixtures and feedingstuffs. The Applicant suggested a maximum inclusion level of sepiolite of 40 g/kg complete feedingstuffs. For the characterisation of the feed additive, the Applicant suggested to determine its mineralogical and elemental composition. For the determination of the mineralogical composition of the feed additive, the Applicant submitted an X-ray diffraction (XRD) method. Furthermore, the feed additive was characterised by the Applicant using X-ray fluorescence (XRF) and atomic absorption spectrometry (AAS). Based on the experimental evidence provided, the EURL recommends for official control of the feed additive the mineralogical characterisation by X-ray diffraction (XRD) together with the elemental analysis by X-ray fluorescence (XRF) or atomic absorption spectrometry (AAS). The Applicant provided no analytical method or experimental data for the determination of sepiolite in premixtures and feedingstuffs, as the unambiguous determination of the feed additive added to the matrices is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control for the determination of sepiolite in premixtures and feedingstuffs. Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.