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# Safety of lignosulphonate for all animal species

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## Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of lignosulphonate, when used as a technological additive, functional group: binders. In a previous opinion, the FEEDAP Panel could not conclude on the safety of the additive for target species and for the environment. The applicant provided additional information that was assessed in the current opinion. As regards the safety for the target species, the maximum recommended content of lignosulphonate of 10,000 mg/kg complete feed is considered safe in weaned piglet. A safe concentration of lignosulphonate in feed for salmonids and dairy cows could not be identified. The FEEDAP Panel reiterates also its previous conclusions that '10,000 mg lignosulphonate/kg complete feed is safe for chickens for fattening, laying hens and cattle for fattening but a margin of safety cannot be identified. Therefore, this conclusion cannot be extended to all animal species/categories'. Considering the absence of adverse effects confirmed by all the ecotoxicity studies up to very high concentrations, no concerns for the environment are expected from the use of this additive in animal nutrition according the conditions of use. Lignosulphonate is efficacious as pellet binder.



**Keywords:** technological additive, binders, lignosulphonate, safety, environment

**Requestor:** European Commission

**Question Number:** EFSA-Q-2017-00213

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# 1. Introduction

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

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additives for animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Borregaard Industries Limited, is seeking a Community authorisation of Lignosulphonate as a feed additive for all animal species (Table 1).

**Table 1:** Description of the substances

| | |
|---|---|
| **Category of additive** | Technological additive |
| **Functional group of additive** | Binders |
| **Description** | Lignosulphonate |
| **Target animal category** | All animal species |
| **Applicant** | Borregaard Industries Limited |
| **Type of request** | New opinion |

On 17 June 2015, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product, could not conclude on the safety of Lignosulphonate for all animal species/categories and the environment.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of Authority's opinion. The new data have been received on 24 February 2017. The same data have been sent to EFSA directly from the applicant.

In view of the above, the Commission asks the Authority to deliver a new opinion on Lignosulphonate as technological additive for all animal species based on the additional data submitted by the applicant.

## 1.2. Additional information

Lignosulphonate is currently authorised for use as technological additives (functional groups: binders, anti-caking agents and coagulants) in feedingstuffs for all species and categories of animals, with no maximum feed inclusion limit, and without a time limit, and foreseen for re-evaluation according to the provisions set in Regulation (EC) No 1831/2003. The applicant is seeking the re-evaluation of lignosulphonate as a technological additive, functional group: binders, in feedingstuffs for all animal species.

The European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted, in 2015, an opinion on the safety and efficacy of lignosulphonate for all animal species (EFSA FEEDAP Panel, 2015). The Panel could not conclude on the safety of lignosulphonate for all animal species/categories and the environment and concluded on an efficacious concentration higher than the minimum content proposed by the applicant.

The applicant has submitted additional information related to the characterisation, the safety of lignosulphonate for the target species and the environment and on its efficacy.

# 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[1] following a previous application on the same product.[2]

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of lignosulphonate is in line with the principles laid down in Regulation (EC) No 429/2008[3] and the relevant guidance documents: Technical

[1] FEED dossier reference: FAD-2017-0012.
[2] FEED dossier reference: FAD-2010-0209.
[3] 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.

Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017).

# 3. Assessment

The present opinion deals with the re-evaluation of the use of lignosulphonate as a technological additive, functional group: binders, in feedingstuffs for all animal species.

Lignosulphonates are amorphous branched polymers of lignin, containing sulphonated covalently linked phenyl propane monomers (C9). The polymers have an average molecular weight ranging from 1,000 to 250,000 Da. Depending on the type of pulping process, different salts, including calcium lignosulphonate, sodium lignosulphonate and magnesium lignosulphonate, can be obtained. Since the cations are not considered relevant for the efficacy of the additive as a pellet binder and the nutrition of target animals, lignosulphonates are treated in the assessment of the FEEDAP Panel as a single additive.

In its previous opinion, the FEEDAP Panel concluded on the safety of the additive for consumers, for users/workers; concerning efficacy, the Panel concluded that the additive is efficacious as pellet binder at the minimum concentration of 7,500 mg/kg complete feed – this level was not the one proposed by the applicant of 1,000 mg/kg complete feed (EFSA FEEDAP Panel, 2015). The data provided, however, did not allow the Panel to conclude on the safety of the additive for the target species and the environment. In the same opinion, the Panel proposed updated specifications regarding the content of lignosulphonate and reducing sugars in the additive.

The applicant submitted additional information to support new specifications, the safety of the additive for the target species and the environment and the efficacy at concentrations lower than 7,500 mg/kg complete feed.

## 3.1. Characterisation of the additive

The additive is manufactured from the sulfite pulping of lignocellulosic biomasses. After pulping, the remaining reducing sugars could either be filtrated of fermented to ethanol. In the latter case, their concentration in the final additive is drastically reduced.

In the original application, the additive lignosulphonate was specified to contain 45–100% lignosulphonate and < 30% reducing sugars. In its previous opinion (EFSA FEEDAP Panel, 2015), the Panel described the result of the analysis on calcium lignosulphonate, sodium lignosulphonate and magnesium lignosulphonate from five batches manufactured with the fermentation process only, and recommended updated specifications regarding lignosulphonate with ≥ 70% (dry Matter, DM), and reducing sugars with ≤ 10%.

The applicant is now proposing the following new specifications, to cover both the production process:

- Lignosulphonate ≥ 55%.
- Reducing sugars: ≤ 20%.

The analysis of five batches each non-fermented magnesium lignosulphonate and calcium lignosulphonate showed compliance with the specifications: magnesium lignosulphonate 94.4–95.2% on DM; lignosulphonate 72.4–73.8% on DM; reducing sugars 18.5–20.3% on DM; calcium lignosulphonate: DM 94.1–95.6%; lignosulphonate 73.4–75.8% of DM and reducing sugars 12.4–14.5% of DM).[4]

## 3.2. Conditions of use

Lignosulphonate is intended to be used as a pellet binder in feedingstuffs for all animal species. The applicant proposes a maximum content of 10,000 mg/kg feed. Typical inclusion levels in feed would range between 5,000 and 10,000 mg/kg complete feed, and up to 15,000 mg/kg compound/ complementary feed.

[4] Technical dossier/Annex I.

## 3.3. Safety

### 3.3.1. Safety for the target species

In its previous opinion (EFSA FEEDAP Panel, 2015), the FEEDAP Panel concluded, based on the outcome of a literature search, that 10,000 mg lignosulphonate/kg complete feed is safe for chickens for fattening, laying hens, pigs for fattening and cattle for fattening, but a margin of safety could not be identified. Therefore, this conclusion could not be extended to all animal species/categories. To support the safety of lignosulphonate for all animal species, the applicant provided two tolerance studies (one in weaned piglets and another in trout) and a review of the literature which covered dairy cows.

#### 3.3.1.1. Safety for weaned piglets

A total of 144 piglets (Piétrain × (Landrace × Large White), half of each sex, 40 days of age, average body weight 11.4 kg) were fed diets supplemented with 0, 10,000 (1× the maximum recommended dose), 25,000 (2.5× the maximum recommended dose) or 50,000 (5× the maximum recommended dose) mg calcium lignosulphonate/kg complete feed. Each treatment comprised nine replicates (pens) with four pigs each (two males and two females). The basal diets (pre-starter, from day 1 to day 14; starter, from day 15 to day 42), consisting mainly of barley, maize and soybean meal, were calculated to be isonitrogenous (pre-starter: 20.2% crude protein (CP), lysine (Lys) 1.40%; starter: 17.5% CP, Lys 1.25%) and isocaloric (pre-starter: 10.2 MJ net energy (NE)/kg; starter: 10.3 MJ NE/kg). The concentration of the additive in the diets was analytically confirmed. Feed (pellet form) and water were offered for *ad libitum* access. The duration of the study was 42 days.

Health status and mortality were monitored daily; where applicable, the most probable cause of death was determined by necropsy. Body weight (BW) was recorded at the beginning, and on days 14, 28 and 42 of the experiment; feed intake was measured daily; average daily body weight gain and feed to gain ratio were calculated for the respective pre-starter and starter periods. Prior to the start of the experiment, a blood sample was taken from eight piglets. At the end of the experiment, a blood sample was taken from one piglet per pen (i.e. a total of nine piglets per treatment) for routine blood haematology and biochemistry[5] ; the animals subsequently were killed for post-mortem examinations. Statistical analysis was done by analysis of variance (ANOVA) considering the pen as the experimental unit, and group means were compared by Tukey's test. Significance level was set at $p < 0.05$.

The main results are reported in Table 2.

**Table 2:** Main results of the tolerance study with lignosulphonate on weaned piglets

| Groups (mg/kg feed) | Daily feed intake (g) | Final body weight (kg) | Feed to gain ratio | Haematocrit (%) | Haemoglobin (g/dL) | Red blood cell count ($10^{12}$/L) | White blood cell count ($10^{9}$/L) | Serum albumin (g/dL) |
|---|---|---|---|---|---|---|---|---|
| 0 | 873$^{a}$ | 35.1$^{a}$ | 1.55$^{b}$ | 36.5$^{a}$ | 11.9$^{a}$ | 7.48$^{a}$ | 24.8$^{b}$ | 26.8$^{a}$ |
| 10,000 | 857$^{a}$ | 34.6$^{a}$ | 1.55$^{b}$ | 36.0$^{a}$ | 11.9$^{a}$ | 7.35$^{a}$ | 21.7$^{b}$ | 26.9$^{a}$ |
| 25,000 | 869$^{a}$ | 34.4$^{a}$ | 1.58$^{b}$ | 33.9$^{ab}$ | 11.2$^{a}$ | 6.68$^{b}$ | 23.7$^{b}$ | 25.8$^{ab}$ |
| 50,000 | 763$^{b}$ | 28.9$^{b}$ | 1.86$^{a}$ | 32.1$^{b}$ | 10.4$^{b}$ | 6.75$^{b}$ | 33.5$^{a}$ | 23.1$^{b}$ |

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

Mortality/culling was low: one pig was culled for the control group and one pig died in the 5× group. Performance parameters were significantly adversely affected in the 5× dose group. The animals in the highest dose group also showed significant differences in haematocrit and haemoglobin (lower), white blood cell count (higher) and serum albumin (lower) compared to all the other groups. The highest and the intermediate dose groups showed statistically lower red blood cell count compared to the other groups. The decrease in haematocrit and haemoglobin, being significant at the highest dose group, could already be seen, however without reaching statistical significance, at the

[5] Haematology: haematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) erythrocytes and leucocytes count; blood biochemistry: aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, alkaline phosphatase, protein, albumin, urea and glucose.

intermediate dose. Small differences were observed in other parameters; however, they did not show a clear pattern and were likely not treatment related.

The above-mentioned significant changes are considered adverse effects of the supplementation with 50,000 mg lignosulphonate/kg complete feed. The decrease in red blood cell count, haematocrit and haemoglobin is considered dose related, starting already at a concentration of 25,000 mg lignosulphonate/kg complete feed.

The FEEDAP Panel concludes that the additive is safe for weaned piglets at the maximum content of 10,000 mg/kg complete feed; however, a margin of safety cannot be determined.

#### 3.3.1.2. Safety for trout

A total of 660 rainbow trout fingerlings (*Oncorhynchus mykiss*, 57 g body weight, 6 months of age) were randomly distributed to five treatments corresponding to a basal diet without lignosulphonate and diets with 5,000 mg (0.5×), 10,000 mg (1× the maximum recommended concentration), 25,000 (2.5×) and 50,000 (5×) mg lignosulphonate/kg complete feed. The diets were calculated to be isonitrogenous (42% CP) and isolipidic (25% fat). The group size per treatment consisted on four replicate tanks with 33 fish per tank. Study duration was 97 days.

Mortality was monitored during the study. Fish were individually weighed at the beginning of the trial; afterwards the mean body weight of the (anesthetised) fish was determined after tank bulk weighing every 2 weeks. Feed consumption was recorded daily per tank. Total and daily weight gain, specific growth rate and feed to gain were calculated. At the end of the study, a total of 10 fish per tank were randomly selected from each treatment for collecting blood samples[6] and for necropsy. Body weight and length were measured and condition factor was calculated; the following organs were examined: stomach, global viscera, liver (also weighted, relative weight calculated), spleen and kidney.

The performance parameters (with the tank as experimental unit) were analysed by analysis if variance (ANOVA), group differences by Newman–Keuls multiple comparison test and student's t-test. Data from macroscopic examination were analysed by one-way parametric and non-parametric ANOVA. Individual statistical differences between groups were further analysed using Bonferroni's multiple comparison correction (both for comparisons vs. control and for all comparisons). Blood parameters were analysed by a one-way ANOVA and the post hoc test of Dunnett was applied for group differences. Data showing not normal distribution and heterogeneity of variance were tested by the non-parametric ANOVA and Dunnett non-parametric multiple comparison tests to reveal differences.

No mortality occurred during the study. No significant differences in performance between treatments were observed. Average mean body weight was 346 g, specific growth rate was 1.86 and feed to gain was 0.73.

No significant differences were seen in the necropsy endpoints, haematology and biochemistry, except for two parameters: haematocrit and uric acid. Haematocrit was lowest in the control (38%), and significantly different to all lignosulphonate-treated groups (39%, 40%, 42% and 41%, respectively, in ascending order of dose supplementation); small but significant differences were observed for uric acid, with the two highest dose groups with values higher than the control, and the highest dose group also higher than the lowest dose group (uric acid: 0.81, 0.82, 0.83, 0.86 and 0.86 mmol/L, in the control and in the 0.5×, 1×, 2.5× and 5× groups, respectively).

Considering the significant effect observed in haematocrit at all doses tested and the lack of any further information on other essential haematological parameters (e.g. haemoglobin, red blood cell count), a safe concentration of lignosulphonate in feed for trout could not be identified.

#### 3.3.1.3. Safety for dairy cows

The applicant submitted some publications with lignosulphonate aiming to support safety of the additive for dairy cows. They are shortly summarised below, in ascending chronological order.

The first publication (Petit et al., 1999) was an *in vitro* study on the influence of different physical treatments (including the use of lignosulphonate) of full fat soybeans on the digestibility of that feed material. This *in vitro* study does not allow to draw any conclusion on the safety of the additive in dairy cows.

Wright et al. (2005) assessed the effects of heat and lignosulphonate treatment of canola meal when fed to dairy cows. Commercially available solvent-extracted canola meal was either left untreated, processed with the addition of water and heated or processed with the addition of 5% lignosulphonate and heated. Eighteen multiparous Holstein cows, blocked according to expected level

[6] Haematocrit, Leucocytes/erythrocytes ratio, Alanine Aminotransferase, Gamma glutamyl transpeptidase, Protein and Uric Acid.

of milk production, calving date, BW and parity were randomly assigned to one of three treatment groups in a 3 × 3 Latin square, replicated six times. Experimental periods were 42 days. Lignosulphonate/heat-treated canola meal (LCM) was supplemented with 5% lignosulphonate and was included at a concentration of 21.5% in the daily ration; lignosulphonate amounted to 10,750 mg/kg DM. No adverse findings were reported. Milk production was significantly greater for cows fed the LCM diet (36.6 kg/days) than for cows fed the untreated canola meal (34.8 kg/days) but not different from the group fed the heat-treated canola meal diet (35.3 kg/days). Digestibility of crude protein was lower for LCM cows, they had reduced concentrations of ruminal ammonia nitrogen (N), blood urea N and milk urea N compared with control cows. No negative impact on other relevant parameters was reported. Lignosulphonate at a dietary level of 10,750 mg/kg DM (approximately 9,460 mg/kg standardised complete feed with 88% DM) was tolerated.

Neves et al. (2007) followed a similar study design with eight Holstein cows in a double 4 × 4 Latin square design with 20-day experimental periods. The authors compared the effects of feeding extruded vs. non-extruded soybeans as such or treated with 30,000 mg lignosulphonate/kg on apparent whole tract digestibility, feed intake, milk production, milk composition and milk fatty acid profile. Lignosulphonate treated extruded full fat soybean meal (LSB) amounted to 25.6% of the daily ration and was supplemented with 5% lignosulphonate-DM, corresponding to about 7,680 mg lignosulphonate/kg daily ration (approximately 12,000 mg/kg standardised complete feed with 88% DM). Extrusion and lignosulphonate treatment of full fat soybeans in corn silage-based diet for dairy cows had no effect on DM intake, on whole tract digestibility of CP and ether extract and on milk production. Concentrations of cis9, trans11 CLA and polyunsaturated fatty acids in milk fat tended to be increased by lignosulphonate treatment of full fat soybeans. No negative impact on other relevant parameters was reported.

Rosa dos Santos et al. (2012) assessed the effects of feeding extruded versus non-extruded canola seed, with or without 50,000 mg lignosulphonate/kg on rumen fermentation, nutrient flow to the omasum and degradability of DM and N of each diet. Four multiparous Holstein cows were used in a Latin square design with four 21-day experimental periods. The DM effective degradability increased with extrusion, whilst lignosulphonate treatment had no effect. Lignosulphonate treatment of extruded vs. non-extruded canola seeds decreased ruminal and total tract apparent digestibility of organic matter. Lignosulphonate treatment and extrusion had no effect on pH and concentrations of ammonia N and volatile fatty acids in the rumen. Standard performance parameters were not reported. No negative impact on other relevant parameters was reported.

Ferreira Figueiroa et al. (2013) studied the effect of feeding four different corn silage-based diets to four lactating cows. Diets differed in the mode ground sunflower seeds (13.4%) were included in the diets, i.e. non-pelleted or pelleted, each with or without 50,000 mg lignosulphonate/kg DM. The authors applied a Latin square design of 21 days each. The supplemented diets contained about 6,700 mg lignosulphonate/kg, corresponding to 10,900 mg/kg standardised complete feed with 88% DM. The parameters monitored were: milk production, milk fatty acids and blood biochemistry endpoints (glucose, total cholesterol, HDL, LDL, VLDL, triglycerides and urea). None of these parameters was adversely affected by the addition of lignosulphonate in the diets. The authors also identified that the pelleting process and/or lignosulphonate supplementation were not effective in protecting the unsaturated fatty acids of the diet or improving the milk fatty acid profile. No negative impact on other relevant parameters was reported.

Among the five studies submitted, two cannot be considered since one (Petit et al., 1999) is an *in vitro* study and the other (Rosa dos Santos et al., 2012) lacks to report any performance parameters. The remaining three are not tolerance studies since only one lignosulphonate dose level was introduced and the duration was very short (20 and 21 days in two studies). Although no adverse findings were reported at concentrations of 9,460, 10,900 and 12.000 mg/kg complete diet, which are approximately equal to the highest use level applied (10,000 mg/kg), the data available alone are not sufficient to allow the FEEDAP Panel to conclude on the safety of the additive for dairy cows.

#### 3.3.1.4. Conclusions on the safety for the target species

The maximum recommended content of lignosulphonate of 10,000 mg/kg complete feed is considered safe in weaned piglets, based on the results of the tolerance study assessed; this conclusion confirms the former one for pigs for fattening. A safe concentration of lignosulphonate in feed for salmonids could not be identified in the tolerance study. The data on the use of lignosulphonate in dairy cows did not allow to conclude on a safe concentration in complete feed.

Considering the previous opinion and the new information provided in the current submission, the FEEDAP Panel concludes that supplementation of diets with lignosulphonate at 10,000 mg/kg complete

feed is safe for weaned piglets, pigs for fattening, chickens for fattening, laying hens and cattle for fattening. Since a margin of safety cannot be identified, the Panel cannot conclude on the safety of lignosulphonate for the other target species/categories.

### 3.3.2. Safety for the environment

In a previous opinion (EFSA FEEDAP Panel, 2015), lignin was considered a major component of plant materials and it is also excreted by plant-eating animals. Lignin is the most abundant natural aromatic substance present in the environment. It is degraded by a variety of soil bacteria and fungi. However, lignosulphonate is a chemical different from lignin. It does not naturally occur in the environment, and therefore, an environmental risk assessment was considered necessary. The available information did not allow a complete risk assessment. Although the Panel acknowledged that the use of lignosulphonate as a feed additive probably accounts for only a small fraction compared with other uses (e.g. cellulose industry), in the absence of additional information no conclusion on the safety for the environment could be drawn.

The applicant has provided additional information to support the safety of the additive for the environment: a report on biodegradation of lignosulphonate based on a review of the scientific literature; and a series of ecotoxicity studies (a nitrogen transformation test, a seedling emergency and growth test, an earthworm reproduction test, two algal growth inhibition tests, a *Daphnia magna* acute immobilisation test and a fish acute toxicity test). This additional information is assessed below.

#### 3.3.2.1. Effect assessment

**Ecotoxicity studies for the terrestrial compartment**

The three studies provided were performed using 'DP 2657' as test item, consisting on lignosulphonate of a purity of 95% and considered worst case.

Toxicity to soil microorganisms

In a good laboratory practice (GLP) nitrogen transformation test performed in accordance with OECD Guideline 216,[7] biologically active agricultural soil (loamy sand/loam, pH 6.6, 1.45% Corg, WHC 37.39% dry soil) was either left untreated (control) or amended with lignosulphonate at concentrations of 17 mg, 170 mg and 3,333 mg test item/kg soil dry weight. The effects on $NO_3$-nitrogen-production were measured after 98 days of exposure. The test item caused no adverse effects on soil nitrogen transformation (measured as $NO_3$-N production) at the end of the 98-day incubation period. No toxicity for microorganisms was identified.

Toxicity to terrestrial plants

In a GLP seeding emergence and seedling growth test,[8] six terrestrial plants [two monocotyledons: wild oat (*Avena fatua*), onion (*Allium cepa*) and four dicotyledons: oilseed rape (*Brassica napus*), tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*), soybean (*Glycine max*)] were exposed to a concentration of 1,700 mg lignosulphonate/kg soil dry weight. The results were compared with a water control under greenhouse conditions and using 10 pots with two seeds per replicate for tomato, cucumber and soybean, seven pots with three seeds per replicate for oilseed rape as well as five pots with five seeds for wild oat and onion. The validity criteria were met. Endpoints observed at the end of the study were seedling emergence, survival of emerged seedlings, visual toxicity and biomass (shoot fresh weight). For all species tested, the endpoints resulted in an $EC_{10}$, $EC_{20}$ and $EC_{50}$ estimated to be higher than 1,700 mg test item/kg soil dry weight. No toxicity was identified for terrestrial plants.

Earthworm reproduction test

A GLP study on sublethal effects of DP 2657 on the earthworm *Eisenia andrei* in artificial soil was performed in compliance with the OECD Guideline 222 (test duration 56 days).[9] Lignosulphonate was tested at concentrations of 176, 318, 572, 1,029, 1,852 and 3,333 mg/kg soil dry weight in seven treatment groups (one control group was untreated) with four replicates (eight for the control) each containing 10 worms. The assessment of adult worm mortality, behavioural effects and biomass development was done after 28 days and reproduction after an additional 28 days. The validity criteria

[7] Technical dossier/Annex XII Nitrogen transformation.
[8] Technical dossier/Annex XIII Seedling.
[9] Technical dossier/Annex XIV Earthworm.

for the control group were met. No statistically significant adverse effects on mortality, biomass and reproduction of the earthworm *Eisenia andrei* in artificial soil were determined up to and including 3,333 mg test item/kg soil dry weight, i.e. the highest concentration tested. The NOEC for mortality, biomass and reproduction was determined to be 3,333 mg test item/kg soil dry weight. The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were estimated to be > 3,333 mg test item/kg soil dry weight. No toxicity was identified for the earthworms.

Conclusions on effects on soil compartment

No adverse effects were identified from the use of lignosulphonate in the terrestrial compartment irrespectively of the dose used.

**Ecotoxicity studies for freshwater compartment**

Ecotoxicity studies for freshwater compartment submitted used either a solid product (containing 95% dry matter)[10] or a liquid product (50% of dry matter)[11] as test items, both containing 69% lignosulphonate on DM basis.

Toxicity to aquatic algae

The applicant provided two studies on toxicity to freshwater algae. The first study was performed in 1996, according to ISO 8692 method (and also a reference to OECD Guideline 201, 1984).[12] The effect of lignosulphonate on growth inhibition of green algae *Raphidocelis subcapitata* (formerly known as *Selenastrum capricornutum*) was assessed in aqueous solution (without solvents/carriers) in seven treatments of nominal concentrations 250, 400, 630, 1,000, 1,600, 2,500 and 4,000 mg/L and compared to the control growth. Growth rate and area under the curve (according to the valid ISO standard method at a time of a study) were selected as endpoints. The results were calculated on nominal concentration basis. No data on measurement of the substance have been provided. Validity criteria (growth rate in the controls and variability within control treatments) were fulfilled. The results indicated that the 72 h $EC_{50}$ values for growth rate (2,600 mg/L) as well as the area under the curve (460 mg/L) were far above 100 mg/L, a limit concentration set by the current OECD Guideline 201 (2006, corrected 2011), and therefore, the substance should be considered as harmless to freshwater algae.

The second, more comprehensive study following the OECD Guideline 201 (1984) was performed in 1997 with green algae *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) testing lignosulphonate.[13] The study included range finding test (in nominal concentrations of 1, 10 and 100 mg/L) and the definitive test (nominal concentrations: 50, 100, 200, 400 and 800 mg/L). Validity criteria were met in both tests. The median growth rate inhibition concentration ($E_rC_{50}$) as well as no effect concentration (NOEC, $E_rC_{10}$) based on growth rate were determined upon the 72 h exposure in definite test on nominal concentrations basis, as no measurements of the test substance were performed. It should be emphasised that the range finding test resulted with no significant differences between growth rate in any treatment (including the highest tested, 100 mg/L) vs. control. In definitive test, the complete (100%) growth inhibition has not been reached even at the highest tested concentration (800 mg/L). At the lowest tested concentration (50 mg/L), the growth rate did not differ significantly from the control, so the no observed effects concentration (NOEC) was determined to be 50 mg/L. The $E_rC_{50}$ of lignosulphonate to the green alga *Desmodesmus subspicatus* was estimated to be 604 mg/L after 72 h of exposure.

From the studies mentioned above, no adverse effects for algae were identified.

Short-term toxicity to aquatic invertebrates

The toxicity of Dustex 50F towards the aquatic invertebrate *Daphnia magna* was reported from a study performed in 1997.[14] Test guidelines at that time have been considered, OECD test 202 (4 April 1984) and EEC Directive 84/449. Per concentration level, 40 individual Daphnids were tested in duplicates over a test period of 48 h in a 16–8 h light–dark cycle, nominal concentrations of lignosulphonate ranged from 50 to 800 mg/L. Nominal concentrations of the tested compound have

[10] Technical dossier/Supplementary information September 2019/Attachment 1.
[11] Technical dossier/Supplementary information September 2019/Attachment 2.
[12] Technical dossier/Supplementary information February 2019/Annex E Dustex – acute tox algae.
[13] Technical dossier/Annex XV acute tox algae.
[14] Technical dossier/Annex XVI acute tox Daphnia.

not been confirmed analytically. This appears acceptable due to the challenging chemical characterisation of lignosulphonate. More recent OECD Guidelines allow for so-called Limit tests, to demonstrate that the $EC_{50}$ is larger than 100 mg/L or above the solubility limit of the compound (EC greater than 100 mg/L means that the substance is not harmful, let alone toxic or very toxic). Oxygen and pH levels were measured and reported without any abnormalities. Validity criteria of the OECD test (< 10% control mortality and oxygen saturation > 60%) were fulfilled. The reported endpoint from this study is an $EC_{50}$ of 800 mg/kg, but it has to be noted that in the test no mortality or immobility at all was observed, so that a true $EC_{50}$ value would be even larger. No adverse effects were identified for *Daphnia magna*.

Short-term toxicity in fish

The toxicity of Dustex 50F towards the freshwater fish *Poecilia reticulata* (Guppy) was reported from a study performed in 1997.[15] Test guidelines at that time have been considered, OECD test 203 (17 July 1992) and EEC Directive 92/69. Per concentration level, seven individual Guppies of 1–3 cm length were tested over a test period of 96 h in a 14–10 h photoperiod in a static design, without feeding, nominal concentrations of lignosulphonate ranged from 150 to 2400 mg/L. Nominal concentrations have not been determined analytically. This appears acceptable due to the challenging chemical characterisation of Dustex 50F. Oxygen and pH levels were measured and reported without any abnormalities. Except for the missing analytical confirmation of exposure levels, the test report does not mention why the design was static, not semi-static or flow-through. Other validity criteria of the OECD 203 test (< 10% control mortality and oxygen saturation > 60%) were fulfilled. The reported endpoint from this study is an $LC_{50}$ of 2,400 mg/kg, but it has to be noted that in the test no mortality or immobility at all was observed, so that a true $LC_{50}$ values would be even larger. No adverse effects were identified for fish.

Conclusions on effects on aquatic compartment

No adverse effects were identified in the aquatic compartment irrespectively of the dose used.

#### 3.3.2.2. Conclusions on safety for the environment

Considering the absence of adverse effects confirmed by all the ecotoxicity studies (terrestrial and aquatic compartments) up to very high concentrations of lignosulphonate, the FEEDAP Panel estimates that the risk is unlikely and calculation of exposure is therefore considered unnecessary. No concerns for the environment are expected from the use of lignosulphonate in animal nutrition according the conditions of use.

## 3.4. Efficacy

In the previous opinion (EFSA FEEDAP Panel, 2015), three efficacy studies were assessed. In two of the studies, lignosulphonate was efficacious at inclusion level of 5,000 mg/kg feed, and in one at 7,500 mg/kg (the lowest dose tested). The Panel concluded that lignosulphonate is efficacious as a pellet binder at a minimum level of 7,500 mg/kg complete feed.

To support the efficacy of the additive at lower concentrations, the applicant has provided three additional studies, in which pellet durability (measured with Pfost tumbling method (PDI) and/or New Holmen Pellet tester (NHPT)) of different feeds was measured after pelleting at different conditions and with different lignosulphonate inclusion levels.

In a first study (Corey et al., 2014), maize/soybean-based finisher diets for chickens for fattening, containing 10,000 or 30,000 mg fat/kg feed, were pelleted at approximately 82°C with the addition of lignosulphonate at 0, 5,000 or 10,000 mg/kg feed (12 replicates of 445 kg each). The inclusion of lignosulphonate at any concentration improved significantly ($p = 0.001$) pellet durability (PDI %: 86.4%, 89.9% and 91.0%, respectively, in the three feeds). The lignosulphonate effect was not influenced by the fat concentration in the diets.

In a second study,[16] a maize/soybean (70:30) mixture was pelleted at approximately 80°C with the addition of two forms of lignosulphonate (solid and liquid, inclusion level equivalent in terms of dry matter)) at 0, 2,500, 5,000 or 10,000 mg/kg compound feed. A fourth group in which another pellet binder was included at the same concentrations was used a positive control. Feeds were pelleted in 16

[15] Technical dossier/Annex XVII acute tox fish.
[16] Technical dossier/Annex XIX.

runs with batched of 4 kg each. The solid and liquid form of lignosulphonate showed comparable results. The PDI significantly increased from approximately 88% (control) to 92%, 93% and 95% by the addition of lignosulphonate at 2,500, 5,000 and 10,000 mg/kg, respectively.

In a third study,[17] a maize-based complete feed for poultry was pelleted at approximately 82–85°C with the addition of lignosulphonate at 0, 3,000 or 5,000 mg/kg feed (23 runs, for a total of 24, 45 and 30 tons for the three groups, respectively). The NHPT increased from approximately 58% (control) to 67% for both the lignosulphonate inclusion levels.

### 3.4.1. Conclusions on the efficacy

The results of three studies showed that lignosulphonate is efficacious as pellet binder in a dose-dependent manner, effects were seen in range of 2,500–7,500 mg/kg complete feed.

## 4. Conclusions

The maximum recommended content of lignosulphonate of 10,000 mg/kg complete feed is considered safe in weaned piglets, based on the results of the tolerance study assessed; this conclusion confirms the former one for pigs for fattening. A safe concentration of lignosulphonate in feed for salmonids and dairy cows could not be identified. Considering the previous opinion and the new information provided in the current submission, the FEEDAP Panel concludes that supplementation of diets with lignosulphonate at 10,000 mg/kg complete feed is safe for weaned piglets, pigs for fattening, chickens for fattening, laying hens and cattle for fattening. Since a margin of safety cannot be identified, the Panel cannot conclude on the safety of lignosulphonate for the other target species/categories.

No concerns for the environment are expected from the use of the additive under assessment in animal nutrition according to the conditions of use.

Lignosulphonate is efficacious as pellet binder in a dose-dependent manner, effects were seen in range of 2,500–7,500 mg/kg complete feed.

## 5. Recommendation(s)

The FEEDAP Panel recommends that the specifications for the additive should contain lignosulphonate $\geq$ 55% and reducing sugars $\leq$ 20%.

Since the effect of lignosulphonate as a pellet binder depends on feed composition and on the feed processing conditions applied, the FEEDAP Panel recommends not to establish a minimum effective concentration.

## Documentation as provided to EFSA/Chronology

| Date | Event |
|---|---|
| 01/03/2017 | Dossier received by EFSA. Lignosulphonate for all animal species. Submitted by Borregaard Industries Limited |
| 10/03/2017 | Reception mandate from the European Commission |
| 21/03/2017 | Application validated by EFSA – Start of the scientific assessment |
| 22/01/2019 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the environment* |
| 25/02/2019 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 28/08/2019 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the environment* |
| 27/09/2019 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 10/01/2020 | Opinion adopted by the FEEDAP Panel by written adoption. End of the Scientific assessment |

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## Abbreviations

| | |
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| ANOVA | Analysis of variance |
| BW | body weight |
| CP | Crude protein |
| Da | Dalton |
| DM | dry matter |
| $EC_{10}$ | Concentration of a test substance which results in 10% of the exposed organisms being adversely affected, i.e. both mortality and sub-lethal effects |
| $E_rC_{50}$ | The concentration of a test substance which results in 50% of inhibition of algal growth rate |
| FEEDAP | EFSA Panel on additives and products or substances used in animal nutrition |
| GLP | Good laboratory practice |
| NE | Net energy |
| OECD | The Organisation for Economic Co-operation and Development |