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## Evaluation of calcium lignosulfonate as a acceptable previous cargo for edible fats and oils

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### Abstract

Shipping of edible fats and oils into Europe is permitted in bulk tanks, provided that the previous cargo is included in a positive list. The European Commission requested EFSA to evaluate the acceptability of calcium lignosulfonate as previous cargo for fats and oils. The evaluation was based on the same criteria as those used for the evaluation of the substances currently on the list in the Annex to Commission Directive 96/3/EC as a acceptable previous cargoes for edible fats and oils. In 2017, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that calcium lignosulfonate did not meet the acceptability criteria, due to uncertainties as regards the composition and toxicity of its low-molecular weight fraction (LMWF) below 1,000 Da. In the current evaluation, new information, showing lack of genotoxicity of the LMWF isolated from a technical grade of calcium lignosulfonate was provided. Due to uncertainties regarding the presence of lignosulfonate components below 200 Da in this LMWF tested for genotoxicity, the CONTAM Panel concluded that the information provided was insufficient to assess the acceptability of calcium lignosulfonate as previous cargo. The Panel recommends a better analysis of the LMWF and a new genotoxicity test using this LMWF, including components < 200 Da, and evidence that the tested material is representative of the LMWF in products intended to be shipped as previous cargo for edible fat and oils.

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## Summary

Shipping of edible fats and oils into Europe is permitted in bulk tanks, provided that the previous cargo is included in a positive list. The European Commission requested the European Food Safety Authority (EFSA) to evaluate the acceptability of calcium lignosulfonate as previous cargo for fats and oils. The evaluation was based on the criteria adopted by EFSA in 2009 and also used for the evaluation of the substances currently on the list of acceptable previous cargoes for edible fats and oils in the Annex to Commission Directive 96/3/EC.

Calcium lignosulfonate was re-evaluated on the basis of new information provided after the recommendations made by the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) in 2017 on the need for additional information, mainly regarding the composition and toxicity of the low-molecular weight fraction (LMWF) in the grades of lignosulfonate intended as previous cargo.

New data on the genotoxicity of a LMWF, isolated from a technical calcium lignosulfonate product as intended for shipping, were provided by the producer Borregaard. The tests comprised a reverse mutation assay in bacteria and an *in vitro* micronucleus assay in human peripheral lymphocytes. In conclusion, the LMWF was negative in the two genotoxicity tests.

Borregaard provided also data on the molecular mass distribution for: the LMWF used in the genotoxicity testing; the raw calcium lignosulfonate product from which this LMWF was isolated; a typical product intended for the previous cargo; and the lignosulfonate 45–60 product purified by ultrafiltration, that was evaluated in the past as food and feed additives. The molecular mass distribution of the LMWF deviated from that of the reference products; components < 200 Da were largely absent in the LMWF.

In addition, Borregaard provided an Ames test and an acute toxicity tests on some commercial lignosulfonate products. In the absence of (a) further information of the product tested and a demonstration of its similarity with the products intended to be shipped and (b) adequate set of *in vitro* genotoxicity assays, the relevance of these toxicity data for the evaluation of calcium lignosulfonate was questionable.

The Panel concluded that a better analysis of the, toxicologically most relevant, LMWF including components < 200 Da, is needed for calcium lignosulfonate to meet the criteria for acceptance as previous cargo. In particular, mass calibration should be performed with lower molecular mass standards, and a more comprehensive detection, should be used. Genotoxicity tests in accordance with EFSA guidance (Ames test and *in vitro* micronucleus test) (EFSA Scientific Committee, 2011, 2017, 2019) should be performed using the LMWF, including components < 200 Da. The tested material should be representative of the LMWF in products intended to be shipped as previous cargo for edible fat and oils.

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## 1. Introduction

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

#### 1.1.1. Background

Reg. (EC) 852/2004 on the hygiene of foodstuffs foresees that bulk food has to be transported in containers/tanks reserved for food. However, Reg. (EU) No 579/2014 foresees for some exemptions. It indicates that the bulk transport in seagoing vessels of liquid oils or fats, shall be permitted in tanks that are not exclusively reserved for the transport of foodstuffs, provided that the previous cargoes are listed in the Annex to the Regulation on the basis of an EFSA opinion.

In its opinion of 24 November 2016<sup>1</sup> on the evaluation of substances as a acceptable previous cargoes for edible fats and oils, EFSA evaluated 4 substances for their acceptability as previous cargoes for edible fats and oils. It concluded that calcium lignosulfonate does not meet the acceptability criteria as there are uncertainties regarding the composition and toxicity of the low molecular mass fraction. On 16 January 2019, the company Borregaard submitted to the Commission and EFSA two new genetic toxicity studies on a low molecular weight fraction of calcium lignosulfonate and requested a re-evaluation of calcium lignosulfonate for acceptability as previous cargoes for fats and oils. EFSA is now asked to evaluate this new information in order to verify whether the conclusions on calcium lignosulfonate in the previous opinion need to be updated.

#### 1.1.2. Terms of Reference

In accordance with Art 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority for an updated scientific opinion on the evaluation of substances as a acceptable previous cargoes for edible fats and oils taking into account the new information submitted by Borregaard or, in case the conclusions of the opinion would remain the same, to publish an opinion on the evaluation of the new data and the confirmation of the previous conclusions.

### 1.2. Interpretation of the Terms of Reference

No new information has been retrieved or provided that would change the previous conclusions of the CONTAM Panel (EFSA CONTAM Panel, 2017) in relation to other substances accepted as previous cargoes, except for calcium lignosulfonate. Therefore, the present opinion of the CONTAM Panel should comprise the re-evaluation of the substance calcium lignosulfonate (CAS No 8061-52-7) following the additional information and data provided to EFSA.

The evaluation is based on the criteria used for the Scientific Opinions on the evaluation of the substances currently on the list in the Annex to Commission Directive 96/3/EC as a acceptable previous cargoes for edible fats and oils.

### 1.3. Additional information

#### 1.3.1. Criteria set for the evaluation of substances proposed as a acceptable previous cargoes

In 2009, the CONTAM Panel review the criteria for acceptable previous cargoes for edible fats and oils set by the Scientific Committee on Food (SCF) (EFSA CONTAM Panel, 2009). The conclusions of the CONTAM Panel are summarised in Annex A.

#### 1.3.2. Legislation

Council Directive 93/43/EEC<sup>2</sup> on the hygiene of foodstuffs laid down the general rules of hygiene for foodstuffs and the procedures for verification of compliance with these rules. Chapter IV of the Annex considered the hygienic transportation of foodstuffs. Commission Directive 96/3/EC<sup>3</sup> derogated certain provisions of Council Directive 93/43/EEC on the hygiene of foodstuffs as regards the transport

<sup>1</sup> EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on the evaluation of substances as acceptable previous cargoes for edible fats and oils. EFSA Journal 2017;15(1):4656.

<sup>2</sup> Council Directive 93/43/EEC of 14 June 1993 on the hygiene of foodstuffs. OJ L 175, 19.7.1993, p. 1–11.

<sup>3</sup> Commission Directive 96/3/Euratom, ECSC, EC of 26 January 1996 granting a derogation from certain provisions of Council Directive 93/43/EEC on the hygiene of foodstuffs as regards the transport of bulk liquid oils and fats by sea. OJ L 21, 27.1.1996, p. 42–46.

of bulk oils and fats by sea, permitting the transport by sea of bulk oils and fats in tanks which have previously been used to transport substances listed in the Annex to this Directive and subject to conditions which ensure the protection of public health and safety of foodstuffs concerned.

In order to take account of scientific developments and based on the evaluations carried out by the SCF (1997, 2003), the list of acceptable previous cargoes was amended by Commission Directive 2004/4/EC<sup>4</sup>. Council Directive 93/43/EEC was repealed by Regulation (EC) No 852/2004<sup>5</sup> on the hygiene on foodstuffs, which laid down general hygiene requirements relating to transport of food applicable to all food business operators. Annex II of Chapter IV states, among others, that 'receptacles in vehicles and/or containers are not to be used for transporting anything other than foodstuffs where this may result in contamination'.

The outcome of the EFSA scientific opinions on the evaluation of the substances on their acceptability as previous cargoes for fats and oils published from 2009 to 2012 was taken into account for the subsequent repeal of Directive 96/3/EC and its replacement by Commission Regulation (EU) No 579/2014<sup>6</sup>. This Regulation provides for derogation from point 4 of Chapter IV of Annex II to Regulation (EC) No 852/2004 as regards the transport on seagoing vessels of oils and fats intended for or likely to be used for human consumption under certain conditions and includes a revised list of acceptable previous cargoes. As the combined entry for 'ammonium nitrate solution and calcium nitrate (CN-9) solution and their double salt' created confusion to ship charterers and competent authorities, the Commission replaced this entry in the list of acceptable previous cargoes by the separate entries of 'ammonium nitrate solution' and 'calcium ammonium nitrate'. Further, having identical hazard profiles and only differing in the amount of crystal water, 'calcium ammonium nitrate', 'calcium (II) nitrate dehydrate' and 'calcium nitrate tetrahydrate' were inserted. At its 68th plenary meeting,<sup>7</sup> the EFSA CONTAM Panel confirmed that these changes have no impact on the toxicological properties and chemical reactivity. Therefore, the list of acceptable previous cargoes in the Annex to Regulation (EU) No 579/2014 has been amended by Commission Regulation (EU) 2016/238<sup>8</sup>. On the basis of the EFSA scientific opinion of 4 November 2016,<sup>9</sup> by means of Regulation (EU) No 2019/978<sup>10</sup>, 2-ethylhexanol and methylacetate were added to the Annex to Regulation (EU) No 579/2014. However, EFSA concluded that calcium lignosulfonate does not meet the acceptability criteria, as there are uncertainties as regards the composition and toxicity of the low molecular mass fraction. Therefore, calcium lignosulfonate was not included in the Annex to Regulation (EU) No 579/2014 by means of Regulation (EU) 2019/978.

## 2. Data and methodologies

### 2.1. Data

#### 2.1.1. Documents submitted to EFSA for the re-evaluation of calcium lignosulfonate

Borregaard AS, one of the main producers of lignosulfonate products, provided the CONTAM Panel with information on the lower molecular weight fraction (LMWF) < 1,000 Da of a calcium lignosulfonate product estimated to be representative of the products intended to be shipped as previous cargo (see Documentation provided to EFSA No 1–3).

<sup>4</sup> Commission Directive 2004/4/EC amending Directive 96/3/EC granting a derogation from certain provisions of Council Directive 93/43/EEC on the hygiene of foodstuffs as regards the transport of bulk liquid oils and fats by sea. OJ L 15, 22.1.2004, p. 25–30.

<sup>5</sup> Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–54.

<sup>6</sup> Commission Regulation (EU) No 579/2014 of 28 May 2014 granting derogation from certain provisions of Annex II to Regulation (EC) No 852/2004 of the European Parliament and of the Council as regards the transport of liquid oils and fats by sea. OJ L 160, 29.5.2014, p. 14–20.

<sup>7</sup> Minutes of the 68th CONTAM Plenary meeting available online: <http://www.efsa.europa.eu/en/events/event/141125b-m.pdf> 10Commission

<sup>8</sup> Regulation (EU) 2016/238 of 19 February 2016 amending the Annex to Regulation (EU) No 579/2014 granting derogation from certain provisions of Annex II to Regulation (EC) No 852/2004 of the European Parliament and of the Council as regards the transport of liquid oils and fats by sea. OJ L 45, 20.2.2016. p. 1–2.

<sup>9</sup> EFSA Journal 2017;15(1):4656.

<sup>10</sup> Commission Regulation (EU) 2019/978 of 14 June 2019 amending the Annex to Regulation (EU) No 579/2014 granting derogation from certain provisions of Annex II to Regulation (EC) No 852/2004 of the European Parliament and of the Council as regards the transport of liquid oils and fats by sea. OJ L 159, 17.6.2019, p. 26–28.



### 2.1.2. Data retrieved by search

The CONTAM Panel considered the previous assessments (EFSA CONTAM Panel, 2011, 2017) as comprehensive, covering all relevant publications up to 18 April 2016. For the present evaluation of the substances as a acceptable previous cargoes, the CONTAM Panel considered literature made publicly available after 18 April 2016. No new information was retrieved from PubMed, Web of Science or the European Chemicals Agency (ECHA).

## 2.2. Methodologies

In the years 2009–2016, the CONTAM Panel evaluated the acceptability of the substances listed in the Annex to Commission Directive 96/3/EC as a acceptable previous cargoes for edible fats and oils, based on its review of the criteria set by SCF (EFSA CONTAM Panel, 2009) and the experience gained in its subsequent evaluations, which highlighted the importance of addressing impurities that might be present (EFSA CONTAM Panel, 2011, 2012a,b, 2017).

The present opinion is based on the criteria established by the EFSA CONTAM Panel (EFSA, 2009):

- The substance is transported/stored in an appropriately designed system, with adequate cleaning routines, including the verification of the efficacy of cleaning between cargoes, effective inspection and recording procedures. The CONTAM Panel was of the opinion that records of the three previous cargoes should be kept, in accordance with the Codex Recommended International Code of Practice for the Storage and Transport of Edible Fats and Oils in Bulk. The CONTAM Panel noted that the choices made with respect to design of the transport system and the cleaning methods are part of the responsibility of those managing the transport of previous cargoes. It was the nature and amount of substances that might be carried over into a subsequent cargo of edible fats and oils that was taken into account by the CONTAM Panel in its evaluation of previous cargoes.
- Residues of the substance in the subsequent cargo of fat or oil should not result in adverse human health effects. The acceptable daily intake (ADI) (or total daily intake (TDI)) of the substance should be greater than or equal to 0.1 mg/kg body weight (bw) per day. Substances for which there is no numerical ADI (or TDI) should be evaluated on a case-by-case basis. For non-genotoxic substances, their transport as second and third previous cargoes is not of concern, taking into account their very limited carry over. However, genotoxic substances would not be acceptable as second and third previous cargoes.
- The substance should not be or contain a known allergen, unless the identified allergen can be adequately removed by subsequent processing of the fat or oil for its intended use. This criterion covers all allergens, not only food allergens.
- If the substance reacts with edible fats and oils, reaction products must comply with the above two criteria. Reactions may be promoted by the acidity from free fatty acids and may occur over many months or at high temperatures during refining after the transport; they do not need to result in high yields to be potentially relevant.
- The development of analytical methods of sufficient sensitivity to check for residues in fats and oils should be feasible, e.g. for control authorities, but such methods are seldom available, since most substances used as previous cargoes are not commonly analysed in fats and oils. The CONTAM Panel therefore evaluated the feasibility of developing such methodology as part of its assessment of each substance. In those cases where, due to the nature or composition of the substance to be evaluated as previous cargo, the feasibility of developing suitable analytical methods was considered questionable, this was indicated when discussing the substance and was used as an argument for the rejection of a substance as previous cargo.
- It is unrealistic to assume that chemical analysis would regularly be applied to check the purity of a substance used as previous cargo or the efficiency of the cleaning procedure. Therefore, the substances were evaluated under worst case assumptions with regard to cleaning efficiency and material composition (in particular the potential presence of toxic impurities or the formation of reaction products with edible fats and oils).
- Potentially relevant impurities in the previous cargo should be taken into account, since they may be toxicologically more important than the substance itself. As most products exist in different grades, a reasonable worst-case product within the specification provided was assumed, the concentration of the impurity estimated from available sources and evaluated in

the same way as a listed substance. Impurities are often specified for fine chemicals and highly purified products. However, these are unlikely to be shipped in bulk. Those more commonly encountered are likely to be of intermediate to low purity grade and no specific information about impurities is publicly available (sources and methods of synthesis are usually confidential). Due to this lack of information, the source and most probable way (or ways) of synthesis of the substance are investigated to determine potentially relevant impurities, such as unreacted starting substances or products of side reactions.

### 3. Assessment

#### 3.1. Production and use of calcium lignosulfonate

Calcium lignosulfonate is obtained from sulfite pulping of wood (Toledo and Kuznesof, 2008). From the three main components of wood, namely cellulose, hemicellulose and lignin, cellulose is isolated by digesting the wood chips with acidic calcium bisulfite solution for 6–10 h at approximately 130°C. Bisulfite ions react with the native lignin, cleaving it to smaller sulfonated units. By this process lignosulfonate is formed, the water solubility of which enables its removal. Further reactions may cause elimination of water (Rydholm, 1965; Hoyt and Goheen, 1971). The hemicellulose is largely hydrolysed to monomeric sugars. The water-insoluble cellulose is separated by filtration. The brownish filtrate, the crude lignosulfonate, consists of a complex mixture of breakdown products, but also includes natural components of wood, such as lipids, fatty acids, wax esters, sterols and their degradation products, resin acids and long-chain alcohols, as well as sulfite, inorganic salts, sugars and reaction products.

Technical grade products of lignosulfonates are steam-stripped, a process that removes the excess of sulfur dioxide (SO<sub>2</sub>) as well as volatile substances such as formaldehyde, furfural, hydroxymethyl furfural (HMF), acetic acid and formic acid. Fermentation followed by distillation of ethanol is a process that may be used to remove the fermentable sugars. Subsequent water evaporation to solutions of 50% lignosulfonate, the commercial products shipped, further reduces the volatile components. Precipitated salts are removed by filtration. Purification to obtain food- and feed-grade products involves ultrafiltration through a semi-permeable membrane in order to further reduce the low molecular weight species (EFSA ANS Panel, 2010).

The largest use of lignosulfonates is as plasticiser in concrete to improve flow properties and slow solidification. Lignosulfonates allow concrete to be made with less water (giving stronger concrete), while maintaining the flow properties. They are also applied during the production of cement, where they act as grinding aids. Similarly, lignosulfonates serve in the production of plasterboard to reduce the amount of water required to make the stucco flow and form the layer between two sheets of paper. The reduction in water content allows lower temperatures for drying, saving energy.

Calcium lignosulfonates are also used in petroleum drilling (blocking agent, improvement of mud fluidity), for asphalt emulsification, tanning leather, as a dispersant of chemicals and pesticides, as an additive of slurry mixture of water and coal, and as an additive for feedstuff processing (deflocculant).

Further purified lignosulfonate products (calcium, sodium or magnesium salts) are also used as feed additives (EFSA FEEDAP Panel, 2015).

#### 3.2. Previous evaluations

Calcium lignosulfonate has been evaluated by the SCF in 1996 and considered acceptable as previous cargo, being toxicologically inert and easily removable by tank cleaning. It was also evaluated as an acceptable additive to animal feedstuff (SCF, 1997).

In 2008, calcium lignosulfonate 40–65 (a purified lignosulfonate product with an average molecular weight range of 40,000–65,000 Da) was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) as a food additive, intended to be used as carrier of encapsulated food ingredients (JECFA, 2009). JECFA established an ADI of 0–20 mg/kg bw based on a no-observed-effect level (NOEL) of 2,000 mg/kg bw per day from a 90-day dietary rat study and applying a safety factor of 100 (JECFA, 2009).

In 2010, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) evaluated the use of calcium lignosulfonate 40–65 as a carrier for vitamins and carotenoids added to foods for colouring and nutrient purposes (EFSA ANS Panel, 2010). It was specified that more than 90% of this calcium lignosulfonate ranged between 1,000 and 250,000 Da, which was in agreement with JECFA (2008). The ANS Panel concluded that the available data were insufficient to establish an ADI and that long-term toxicity studies were needed to determine whether with time the histiocytosis



in the mesenteric lymph nodes of the rats observed in the 90-day study may progress into a more adverse state (EFSA ANS Panel, 2010).

In 2011, the ANS Panel considered that new information provided by the petitioner did not address the questions raised by the Panel in 2010 and that the requested chronic toxicity study of at least 12 months was still needed (EFSA ANS Panel, 2011).

In 2011, the CONTAM Panel evaluated calcium lignosulfonate (unspecified, i.e. including less purified grades) as a acceptable previous cargo. Several data gaps (long-term toxicity, carcinogenicity, and limited data on reproductive toxicity) were noted. No evidence of genotoxicity or concern regarding allergenicity was reported in the limited data provided. The CONTAM Panel considered that the available information was sufficient to conclude that the exposure to the evaluated grade of calcium lignosulfonate (calcium lignosulfonate 40–65) would not give rise to toxicological concern when used as a previous cargo. However, there were doubts that this evaluation would also cover the less purified grades mainly intended as previous cargoes, and no information on its potential reactivity with fats and oils was available. Therefore, the CONTAM Panel concluded that calcium lignosulfonate does not meet the criteria for acceptability as a previous cargo (EFSA CONTAM Panel, 2011).

In 2015, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of EFSA delivered an opinion on the safety of lignosulfonate for target animals, consumers, users and the environment, when used as a technological additive in feeds belonging to the functional group binders (EFSA FEEDAP Panel, 2015). It concluded that there was no concern for consumer safety from the use in feeds at up to 1% in complete feed. Since no margin of safety could be identified, this conclusion was not extended to all animal species/categories. Lignosulfonate was neither considered as an irritant to the skin and eyes nor as a skin sensitiser. Exposure to dust by inhalation was considered a hazard. The FEEDAP Panel could not conclude on the safety of lignosulfonate for the environment.

In 2017, the CONTAM Panel re-evaluated calcium lignosulfonate as previous cargo, following additional information provided to EFSA. It still concluded that it does not meet the criteria as previous cargo due to data gaps, mainly regarding the composition and toxicity of the LMWF < 1,000 Da in the grades of lignosulfonate intended as previous cargo.

### 3.3. Current evaluation

#### 3.3.1. Additional information received

New data on genotoxicity were provided for a LMWF isolated from a technical product as intended for shipping. Later it became clear from the molecular mass distribution of the tested LMWF that this did not contain a fraction of < 200 Da. The tests comprised a reverse mutation assay in bacteria and an *in vitro* micronucleus assay in human peripheral lymphocytes. In addition, Borregaard provided an Ames test and an acute toxicity tests on some commercial lignosulfonate products.

Borregaard clarified that the calcium lignosulfonate products intended to be shipped as previous cargo are steam-stripped, but usually not further purified. Borregaard provided also data on the molecular mass distribution for the LMWF, the raw calcium lignosulfonate product from which the LMWF was isolated, a typical product intended for previous cargo, and the lignosulfonate 45–60 purified by ultrafiltration, evaluated in the past as food and feed additive.

#### 3.3.2. Composition of the low molecular weight fraction used for toxicity studies

According to Borregaard, the LMWF tested in the genotoxicity studies was isolated from a representative product of the grades intended to be shipped as previous cargo. It was obtained by ultrafiltration through a membrane with molecular weight cut-off of 1,000 g/mol and freeze-drying.

This LMWF was obtained and characterised as follows:

A technical grade calcium lignosulfonate product was subjected to an ultrafiltration procedure using a membrane with a MW cut-off of 1,000 Da followed by freeze-drying of the permeate. From a total weight of 617 g lignosulfonate dry matter content a low molecular weight fraction of 35 g was isolated as a brown powder. This corresponds to an isolated LMWF of 6% relative to the source material.

The purity of this LMWF was calculated using an indirect determination based on inorganic material and total sugars according to the following formula, with all values expressed on a dry matter basis):

$$\% \text{ lignosulfonate} = 100\% - (\% \text{ total sugars}) - (\% \text{ inorganic material})$$

For total sugars, a High-Performance Anion-Exchange Chromatography method with Pulsed Amperometric Detection (HPAEC-PAD) was used, determining arabinose, galactose, rhamnose, glucose, xylose, fructose and mannose and expressing the total sugar content as the sum of these 7 sugars, For inorganic material, the sample was combusted at 900°C and the ash determined gravimetrically.

Using this approach the LMWF was found to contain 56.8% calcium lignosulfonate with the remainder, by definition, being sugars and inorganic material. The doses (concentrations) applied in the toxicity tests were increased by this factor of 100/56.8 (i.e.  $\times 1.76$ ) so that the doses (concentrations) applied refer to the calcium lignosulfonate and not the total dry matter content'.

The LMWF was isolated from a raw (i.e. non-purified) lignosulfonate from a spruce raw material. It was not clear whether the product corresponds to the typical product intended for previous cargo, as in Table 1 the two products differ somewhat. However, it was stated that the products intended to be shipped are all manufactured from spruce as raw material and will have similar composition as the ones presented and used for toxicity testing.

Specifically, the products intended to be shipped as previous cargo have similar molecular weight distributions, with typical weight average molecular weight in the range of 30–40 kDa. However, it was not clearly specified in which respect these products differ (e.g. method of production, composition, etc.).

Borregaard provided mass distribution data for the isolated LMWF used in the genotoxicity testing, the raw lignosulfonate product from which the LMWF was isolated, a typical product intended for previous cargo and the lignosulfonate 45–60 (Table 1). The raw material used to isolate the tested LMWF was intended to represent the least purified grades of product and had not been ultrafiltrated (the procedure used to reduce the amount of low molecular components for some lignosulfonate products).

The molecular weight distribution of the LMWF and the commercial grades of lignosulfonate was determined using a size exclusion chromatography method with ultraviolet detection at 280 nm (SEC-UV). Chromatograms with absorbance vs. elution time were obtained and the relationship between retention time and MW was calculated from a calibration curve/equation made using lignosulfonate standards of MW 6,000 and 33,500 Da. The provided interpretation of the chromatograms is shown in Table 1.

**Table 1:** Molecular mass distribution obtained by SEC-UV (280 nm) of the LMWF used for genotoxicity testing (LMWF), the raw lignosulfonate from which the LMWF was isolated, a lignosulfonate typical for the products intended as previous cargo and a lignosulfonate purified by ultrafiltration (LS 40-65)

MW g/mol	LMWF	Raw material	Typical product	LS 40-65
< 6,000	100.0	45.3	42.9	30.6
< 2,000	83.9	20.6	18.7	7.8
< 1,000	25.3	9.1	8.1	2.8
< 500	3.2	3.1	2.8	1.2
< 200	0.1	0.7	0.4	0.2

MW: molecular weight; LMWF: low molecular weight fraction; SEC-UV: size exclusion chromatography method with ultraviolet detection.

According to this analysis, the LMWF tested contained 25.3% material below 1,000 Da, which corresponds to an enrichment by a factor of 2.8 and 3.1 compared to the raw lignosulfonate and the typical product, respectively, used as reference products. However, the molecular mass distribution within the fraction < 1,000 Da deviated from that of the reference products: it mainly contained compounds between 500 and 2,000 Da. The proportion of the fraction < 500 Da was only similar to the two reference products and again with a distribution shifted towards the higher masses: the fraction < 200 Da was largely absent in the LMWF (0.1%).

The Panel concluded that the fraction tested for genotoxicity has not been shown to be sufficiently representative for the LMWF in the products intended to be shipped as previous cargo due to an apparent severe loss of constituents of lowest molecular mass. It was also noted that this analysis only refers to components detected in UV at 280 nm, which mainly records phenol-type compounds, such as lignin degradation products. According to Borregaard, 23 and 20% of the fraction tested consisted of inorganic salts and sugars, respectively, derived from cleaved hemicellulose. However, as this material was not detected by UV at 280 nm and the mass distribution of these undetected constituents is probably different from that of the material detected, it is unknown how this influenced the percentages reported above. The Panel assumed that also the undetected compounds below 200 Da were largely missing.

Table 1 also provides data on the molecular mass distribution of a lignosulfonate product purified by ultrafiltration (LS 40-65 evaluated in the past). According to the SEC-UV (280 nm) analysis, this product still included 2.8% material < 1,000 Da, which corresponds to a reduction by a factor of around 3 compared to the raw lignosulfonate and the typical product. Furthermore, the data suggests that the molecular mass distribution within the fraction < 1,000 Da was similar to the raw lignosulfonate and the typical product, i.e. no selectivity in the removal by ultrafiltration is observed. However, not all purification steps were disclosed that may have altered the composition of the LS 40-65.

### 3.3.3. Toxicity testing

The data available on the toxicity of calcium lignosulfonate are limited to the grade 40–65 product that was more refined than the technical grades expected to be shipped in large amounts.

No new relevant data have been identified in the literature since the publication of the previous opinions of the CONTAM Panel (EFSA CONTAM Panel, 2011, 2017) regarding absorption, distribution, metabolism and elimination (ADME), acute toxicity and subacute, subchronic, chronic toxicity, *in vitro* and *in vivo* genotoxicity, carcinogenicity, developmental and reproductive toxicity, immunotoxicity and allergenicity.

In reaction to the previous evaluation of calcium lignosulfonate as a previous cargo (EFSA CONTAM Panel, 2017), Borregaard provided additional data on *in vitro* genotoxicity of a LMWF as described in Section 3.3.2. The genotoxicity testing strategy was in accordance with the recommendations of the EFSA Scientific Committee (2011).

#### *In vitro* genotoxicity with the LMWF

- *Reverse mutation assay in bacteria*

The LMWF has been tested, at doses of 0, 31.6, 100, 316, 1,000, 3,160 and 5,000 µg per plate in a set of five *Salmonella* Typhimurium strains TA98, TA100, TA102, TA1535 and TA 1537 in presence or absence exogenous metabolic activation with S9 mix (rat liver induced by Aroclor 1254). No increase in revertant colony numbers as compared with control numbers was observed in any of the five test strains with and without metabolic activation. The positive controls showed a significant increase in the number of revertant colonies of the respective test strain (for details see Appendix A).

- *Micronucleus assay*

The capacity of LMWF to induce structural and/or numerical chromosome aberrations was tested in cultured freshly isolated human peripheral lymphocytes (unspecified number of donors), in presence or absence of metabolic activation, at concentrations of 0, 250, 500, 1,000 and 2,000 µg/mL. No increase in the frequency of micronucleated PCE was observed in presence or absence of S9 mix (rat liver induced by Aroclor 1254). No signs of cytotoxicity were noted up to the highest concentration tested with or without metabolic activation. The positive controls MMC and CP gave the expected positive results (for details see Appendix A).

In conclusion, the low molecular weight fraction (< 1,000 g/mol) of a standard commercial grade calcium lignosulfonate was negative in two *in vitro* genotoxicity tests including a reverse mutation assay in bacteria and a micronucleus test in mammalian cells.

#### Other toxicity tests with technical grades of lignosulfonate

Borregaard also provided some additional toxicity data on certain of its products summarised in Appendix B.

Out of the five additional tests provided, only the Ames test might be of some relevance for the genotoxicity testing of calcium lignosulfonate. However, in the absence (a) of further information of the product tested and a demonstration of its similarity with the products intended to be shipped and (b) of adequate set of *in vitro* genotoxicity assays (EFSA Scientific Committee, 2011, 2017, 2019), the relevance of this toxicity data for the evaluation of calcium lignosulfonate is questionable.

## 4. Conclusion

The data provided did not show the presence of the fraction < 200 Da in the material tested for genotoxicity. The Panel concluded that the information provided was insufficient to assess the acceptability of lignosulfonate as previous cargo.

## 5. Recommendations

To meet the criteria for acceptance as previous cargo, a better analysis of the toxicologically most relevant LMWF < 1,000 Da, including components < 200 Da, is needed. In particular, mass calibration should be performed with lower molecular mass standards, and a more comprehensive detection should be used.

Genotoxicity tests in accordance with EFSA guidance (Ames test and *in vitro* micronucleus test) (EFSA Scientific Committee, 2011, 2017, 2019) should be performed using the LMWF including components < 200 Da.

The tested material should be representative of the LMWF in products intended to be shipped as previous cargo for edible fat and oils.

### Documentation as provided to EFSA (if appropriate)

- 1) LPT Report No 36604 and No 36605. January 2019. Submitted by Borregaard.
- 2) Letter to EFSA. August 2019. Submitted by Borregaard.
- 3) Additional information. September 2019. Submitted by Borregaard.
- 4) Reply to EFSA's request. September 2016. Submitted by Borregaard.

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- OECD TG404 Acute dermal irritation/corrosion. *OECD Guidelines for the Testing of Chemicals*, Section, 4.
- OECD TG405 Acute eye irritation/corrosion *OECD Guidelines for the Testing of Chemicals*, Section, 4.
- OECD TG429 Skin sensitisation: local lymph node assay. *OECD Guidelines for the Testing of Chemicals*, Section, 4.
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## Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and elimination
ANS	EFSA Panel on Food Additives and Nutrient Sources Added to Food
CAC	Codex Alimentarius Committee
CCFO	Codex Committee for Fats and Oils
CONTAM	EFSA Panel on Contaminants in Food
ECHA	European Chemicals Agency
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
HPAEC-PAD	high-performance anion-exchange chromatography method with pulsed amperometric detection
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD <sub>50</sub>	lethal dose, 50%
LMWF	low-molecular weight fraction
NOEL	no-observed-effect level
OECD	Organisation for Economic Co-operation and Development
SCF	Scientific Committee on Food
SEC-UV	size exclusion chromatography method with ultraviolet detection
TDI	total daily intake
WHO	World Health Organization

## Appendix A – *In vitro* genotoxicity with the LMWF

**Table A.1:** *In vitro* genotoxicity tests on calcium lignosulfonate low molecular weight fraction (< 1,000 g/mol) (LMWF)

Type of test	Experimental test system	Test substance	Exposure conditions	Results	Comments
<b>Reverse mutation assay</b>	<i>Salmonella</i> Typhimurium TA98, TA100, TA1535, TA1537 and TA102	Calcium lignosulfonate LMWF (< 1,000 g/mole) Batch No. DP 3678 56.8% (calcium lignosulfonate fraction) Solvent = negative control: aqua ad injectabilia Positive controls: positive substances as recommended in TG 471	With and without S9 mix 3 plates/conc. Preliminary test: plate incorporation assay in TA100 10 conc.: 0.316–5,000 µg/plate Main test: 1st exp.: plate incorporation assay 2nd exp.: pre-incubation test 6 conc.: 31.6–5,000 µg/plate	No signs of cytotoxicity up to 5,000 µg/plate Negative	Bacteria colonies were counted using the Biosys Biocount 5000 system GLP-compliant assay in accordance to OECD 471 (1997)
<b>Micronucleus test</b>	<b>Human lymphocytes</b>	Calcium lignosulfonate LMWF (< 1,000 g/mol) Batch No. DP 3678 56.8% (calcium lignosulfonate fraction) Solvent = negative control: aqua ad injectabilia Positive controls: –S9: MMC and colchicine, +S9: CP	With and without S9 mix (rat liver induced by Aroclor 1254) Preliminary cytotoxicity test: 7 conc.: 3.16–2,000 µg/mL, expo: –S9: 4 h and 24 h, +S9: 4 h Main test: 1st exp.: – S9: 4 h expo., harvesting 20 h later, 250, 500 and 2,000 µg/mL; + S9 (2% v/v): 4 h expo., harvesting 20 h later, 250, 500 and 2,000 µg/mL 2nd exp.: –S9: 24 h expo., harvesting at end of treatment Duplicate cultures	No signs of cytotoxicity up to 2,000 µg/mL Negative	No relevant changes on pH or osmolality Cytokinesis block technique applied (Cyto B) GLP-compliant assay in accordance with OECD 487 (2016)

OECD: Organisation for Economic Co-operation and Development; GLP: Good laboratory practice.



## Appendix B – Other toxicity tests with technical grades of lignosulfonate

**Table B.1:** Acute toxicity

Test	Substance tested	Result	Comments
<b>Acute oral toxicity Rat (Wistar) 5 M + 5 F/group</b>	Sodium lignosulfonate	LD <sub>50</sub> > 2,000 mg/kg bw	According to OECD TG401 GLP
<b>Skin irritation Rabbit (4)</b>	Calcium lignosulfonate	Not irritant	According to OECD TG404 GLP
<b>Eye irritation Rabbit (4)</b>	Calcium lignosulfonate	Not irritant	According to OECD TG405 GLP
<b>Skin sensitisation Local lymph node assay in mouse</b>	Calcium lignosulfonate (composition provided)	Not sensitiser	According to OECD TG429 GLP

LD<sub>50</sub>: lethal dose, 50%; bw: body weight; OECD: Organisation for Economic Co-operation and Development; GLP: Good Laboratory Practice.

**Table B.2:** Ames test

Type of test	Experimental test system	Test substance	Exposure conditions	Result	Comments
<b>Reverse mutation assay</b>	<i>Salmonella</i> Typhimurium TA98, TA100, TA1535, TA1537	Calcium lignosulfonate Positive controls: B(α)P and Na azide	With and without S9 mix (rat liver induced by Aroclor 1254) 1st test: 0, 0.01, 0.1, 1 and 10 mg/plate 2nd test: 0, 0.1, 0.5, 1 and 5 mg/plate Plate incorporation assay	<b>Negative</b>	Not according to GLP Before OECD 471 was developed

OECD: Organisation for Economic Co-operation and Development; GLP: Good laboratory practice.

## Annex A – Review of the criteria for acceptable previous cargoes for edible fats and oils (EFSA, 2009)

In 2009, EFSA received a request from the European Commission to review the criteria for acceptable previous cargoes for edible fats and oils set by the SCF (Table A.1). The CONTAM Panel assessed the appropriateness of the four criteria of the Codex Committee for Fats and Oils (CCFO) (Table A.2) by comparing them with those set by the SCF in 1996 (EFSA, 2009).

**Table A.1:** Criteria for the inclusion of substances in the list of acceptable previous cargoes according to the SCF (1997, 2003)

SCF Criteria <sup>(a)</sup>	
1.	No toxicological concerns, particularly with regard to their genotoxic and carcinogenic potential, for which a threshold is difficult to establish
2.	Efficacy of procedures used to clean ships' tanks between cargoes
3.	Dilution factor in relation to the potential amount of residue of the previous cargo and any impurity which the previous cargo might have contained and the quantity of oil or fat transported
4.	Subsequent application of refining processes and solubility relevant to the occurrence of possible contaminating residues
5.	Availability of analytical methods to verify the presence of trace amounts of residues or the absence of contamination of oils and fats

(a): The SCF criteria have no numbering in the original reference.

**Table A.2:** Criteria proposed for immediate previous cargoes by the Codex Committee for Fats and Oils (CCFO) during their 21st meeting (CCFO, 2009) and adopted by the Codex Alimentarius Committee (CAC) (FAO/WHO, 2011)

CCFO Criteria (adopted at Step 5)	
1.	The substance is transported/stored in an appropriately designed system; with adequate cleaning routines, including the verification of the efficacy of cleaning between cargoes, followed by effective inspection and recording procedures
2.	Residues of the substance in the subsequent cargo of fat or oil should not result in adverse human health effects. The ADI (or TDI) of the substance should be greater than or equal to 0.1 mg/kg bw/day. Substances for which there is no numerical ADI (or TDI) should be evaluated on a case by case basis
3.	The substance should not be or contain a known food allergen, unless the identified food allergen can be adequately removed by subsequent processing of the fat or oil for its intended use
4.	Most substances do not react with edible fats and oils under normal shipping and storage conditions. However, if the substance does react with edible fats and oils, any known reaction products must comply with criteria 2 and 3

ADI: acceptable daily intake; TDI: total daily intake; bw: body weight.

The CONTAM Panel concluded that the criteria for evaluation of acceptable previous cargoes for edible fats and oils as proposed by the CCFO are not in conflict with any of the five criteria developed by SCF. SCF criteria 1 to 4 are either explicitly or implicitly covered by the CCFO criteria. SCF criterion 5, dealing with the availability of analytical methods, is not explicitly addressed in the CCFO criteria. The CONTAM Panel considers that SCF criterion 5 is still important. The CCFO criteria also cover food allergens and compounds that may react with oil and fats. The CONTAM Panel considers these additions relevant. In addition, the CONTAM Panel made the following remarks:

- The CCFO criteria specifically apply to the immediate previous cargo. The CCFO criterion 1, which addresses, among other issues, documentation procedures, does not specify for how many previous cargoes records should be kept. This might be particularly important in the event that earlier previous cargoes consist of substances for which an acceptable daily intake (ADI) (or tolerable daily intake, TDI) has not been established. The CONTAM Panel was of the opinion that records of the three previous cargoes should be kept, in accordance with the Codex Recommended International Code of Practice for the Storage and Transport of Edible Oils and Fats in Bulk.
- The CCFO criteria specifically apply to the immediate previous cargo. The CCFO criterion 1, which addresses, among other issues, documentation procedures, does not specify for how

many previous cargoes records should be kept. This might be particularly important in the event that earlier previous cargoes consist of substances for which an acceptable daily intake (ADI) (or tolerable daily intake, TDI) has not been established. The CONTAM Panel was of the opinion that records of the three previous cargoes should be kept, in accordance with the Codex Recommended International Code of Practice for the Storage and Transport of Edible Oils and Fats in Bulk.

- With respect to CCFO criterion 2, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) agreed with the proposed threshold of an ADI (or TDI) of  $\geq 0.1$  mg/kg body weight (bw). For substances for which there is no numerical ADI (or TDI) a case by case evaluation is needed. The Panel also considered the situation of second and third previous cargoes and concluded that for non-genotoxic substances their transport as second and third previous cargoes is not of concern, taking into account their very limited carry over. However, the CONTAM Panel noted that genotoxic substances would not be acceptable as previous cargoes. Also, in relation to CCFO criterion 2, the CONTAM Panel noted that as consequence of the above some substances will turn out to be unacceptable as previous cargoes. This could include substances with ADI (or TDI)  $< 0.1$  mg/kg bw or substances with genotoxic activity. The Panel was of the opinion that the exclusion of such substances as previous cargoes is appropriate.
- CCFO criterion 3 is sufficient to cover "known food allergens". However, the CONTAM Panel considered that the scope of the CCFO criterion is too narrow, and should apply to all known allergens, not just to known food allergens, given the fact that the same cargo may be sold for cosmetic use.
- The CONTAM Panel endorsed CCFO criterion 4 without further remarks'.