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Safety of cassia gum as a feed additive for cats and dogs based on a dossier submitted by Glycomer GmbH

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Abstract

The additive cassia gum consists mainly of high-molecular weight polysaccharides composed primarily of a linear chain of 1,4-β-D-mannopyranose units with 1,6-linked α-D-galactopyranose units. In 2014, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) delivered an opinion on the safety and efficacy of cassia gum in cats and dogs. The Panel concluded, based on positive findings observed in a bacterial reverse mutation test with a semi-refined cassia gum (about 70 mg anthraquinones/kg) but not with purified semi-refined cassia gum that meets the specification as a food additive (< 0.5 mg anthraquinones/kg), that only purified semi-refined cassia gum that meets the specifications of cassia gum as a food additive can be considered safe for cats and dogs, at a maximum content of 1.5% cassia gum (15,000 mg/kg feed) in dry matter, corresponding to 1.32% (13,200 mg/kg feed) in a standardised complete feed with 12% water content. The conclusion was confirmed in an opinion delivered by the Panel in 2017. Following this opinion, the European Commission gave the possibility to the applicant to submit complementary information on the safety for cats and dogs. The semi-refined cassia gum under application was mutagenic in a bacterial reverse mutation test in *Salmonella* Typhimurium strain TA100. Positive results were also observed after heat sterilisation of the test item. Isopropanol purified semi-refined cassia gum did not show mutagenic effect. Examination of various other cassia gum extracts did not identify any source of mutagenicity. The mutagenicity of the semi-refined cassia gum under application cannot be excluded. Therefore, the FEEDAP Panel cannot establish the safety of semi-refined cassia gum for cats and dogs.

Keywords: cassia gum, safety, genotoxicity, mutagenicity, anthraquinone, cats, dogs

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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 and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Glycomer, is seeking a Community authorisation of Cassia Gum as a technological additive for dogs and cats (Table 1).

Table 1: Description of the substances

Category of additive	Technological additives
Functional group of additive	Gelling agent
Description	Cassia Gum
Target animal category	Dogs and cats
Applicant	Glycomer GmbH
Type of request	New opinion

On 25 January 2017, 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,[2] could not conclude on the safety of semi-refined cassia gum as a feed additive for dogs and cats due to the potential of the additive to exert mutagenic effects.

The European Commission (EC) 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 22 January 2018.

In view of the above, the EC asks the Authority to deliver an opinion on cassia gum as a feed additive for dogs and cats based on the additional data submitted by the applicant.

1.2. Additional information

The additive cassia gum is currently authorised as a technological additive, functional groups gelling agent, thickeners, emulsifying and stabilising agent for use in food for dogs and cats, with a maximum content of 17,600 mg/kg complete feed.[3]

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued in 2014 four opinions on the safety and efficacy of cassia gum for dogs and cats (EFSA FEEDAP Panel, 2014a–d). In these opinions, the FEEDAP Panel concluded that only purified (isopropanol extraction) semi-refined cassia gum that meets the specifications of cassia gum as a food additive (< 0.5 mg anthraquinones/kg) can be considered safe for cats and dogs. No conclusion was possible for the additive under assessment (semi-refined cassia gum). Two additional opinions were delivered in 2017 on the safety of cassia gum for dogs and cats (EFSA FEEDAP Panel, 2017a,b), in which the previously drawn conclusions were reiterated by the Panel.

The EFSA Panel on Food additives, flavourings, processing aids and materials in contact with food (AFC) issued an opinion on cassia gum as a food additive (EFSA, 2006) and concluded that the use of cassia gum complying with the newly defined specifications (anthraquinones content < 0.5 mg/kg) as an additive for the proposed food uses is not of safety concern.

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) issued an opinion on the safety of hydroxyanthracene derivatives for use in food (EFSA ANS Panel, 2017).

[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] EFSA FEEDAP Panel (2017a).
[3] COMMISSION DIRECTIVE 93/55/EEC of 25 June 1993 amending Council Directive 70/524/EEC concerning additives in feedingstuffs. OJ L 206, 18.8.93, p. 11.

2. Data and methodologies

2.1. Data

The present assessment is based on the data submitted by the applicant in the form of additional information[4] following two previous applications on the same product.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of cassia gum 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 target species (EFSA FEEDAP Panel, 2017c) and Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017d).

3. Assessment

3.1. Characterisation of the additive and conditions of use

Cassia gum is described in Regulation (EC) No 231/2012, which lays down the specification for food additives.[7] It is the ground, purified endosperm of the seeds of *Cassia tora* and *Cassia obtusifolia* (Leguminosae) containing less than 0.05% *Cassia occidentalis*. It consists mainly of high-molecular weight polysaccharides composed primarily of a linear chain of 1,4-β-D-mannopyranose units with 1,6-linked α-D-galactopyranose units. The ratio of mannose to galactose given is about 5:1; the content of galactomannans is > 75%, of acid-insoluble matter is < 2%, of protein is < 7%, of total ash is < 1.2% and of lead is < 1 mg/kg; the viscosity is < 500 mPa·s. Specifications of cassia gum as a food additive (purified semi-refined cassia gum) give a maximum content of total anthraquinones of 0.5 mg/kg (detection limit).

The additive under assessment, semi-refined cassia gum, is intended to be used as a gelling agent in complete feed for cats and dogs with moisture content higher than 20%. The applicant proposes (i) maximum content of 4,000 mg cassia gum/kg complete feed (moisture ≤ 12%) and (ii) a maximum concentration of anthraquinones (as sum of the four referenceable 1,8-dihydroxyanthraquinones rhein, emodin, chrysophanic acid and physcion after acid cleavage of the glycosides) of 60 mg/kg or, alternatively, a maximum concentration of the sum of emodin and chrysophanic acid (after acid cleavage of their glycosides) of 20 mg/kg.

In its previous opinions (EFSA FEEDAP Panel, 2014b, 2017a), the FEEDAP Panel could not conclude on the safety of semi-refined cassia for cats and dogs. The applicant has now provided additional data to support the safety of semi-refined cassia gum for the target species.

3.2. Safety

In its previous opinions (EFSA FEEDAP Panel, 2014b, 2017a), the FEEDAP Panel noted that positive findings were observed in a bacterial reverse mutation test with a semi-refined cassia gum (about 70 mg anthraquinones/kg) but not with the purified semi-refined cassia gum (obtained by isopropanol extraction and following the specifications of cassia gum as a food additive: < 0.5 mg anthraquinones/kg). The Panel, therefore, concluded that only purified semi-refined cassia gum that meets the specifications of cassia gum as a food additive (< 0.5 mg anthraquinones/kg) can be considered safe for cats and dogs, at a maximum content of 1.5% cassia gum (15,000 mg/kg feed) in dry matter (DM), corresponding to 1.32% (13,200 mg/kg feed) in a standardised complete feed with 12% water content.

The applicant submitted a series of genotoxicity studies done with the additive under assessment, semi-refined cassia gum, alone and/or in combination with other gums or with a cassia gum product comparable to the purified semi-refined cassia gum (isopropanol extraction), to support the safety of the additive for the target species.

[4] Dossier reference: FAD-2016-0043.
[5] Dossier reference: FAD-2010-0186 and FAD-2016-0065.
[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] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council.

3.2.1. Genotoxicity

The additive under assessment, semi-refined cassia gum, was tested in the *Salmonella* Typhimurium reverse mutation assay with one strain of *Salmonella* Typhimurium (TA100).[8] The test was performed in the presence and absence of metabolic activation (S9-mix) up to a maximum concentration of 5,000 μg/plate. Only one *Salmonella* strain was tested, since the study was designed as a follow up of the study previously assessed (EFSA FEEDAP Panel, 2014a–d, in which positive findings were observed). Two independent experiments were performed, the first using the plate incorporation method and the second using the pre-incubation method. In the plate incorporation assay, none of the tested concentrations showed a significant increase in the number of revertants. In the pre-incubation assay, a concentration-related increase over the tested range was found. In the absence of S9, an increase in the number of revertant colonies was observed, reaching a plateau (> 1,000) at concentrations of 1,250 μg/plate and above. In the presence of S9, a typical dose–response was observed reaching the double of the revertant frequency (about 200) at the highest concentration, compared to the negative controls. The applicant attributes the atypical high increase of revertants observed in absence of S9 to the release of histidine or histidine-related derivatives by mesophilic microorganisms during the incubation phase of 48 h of the matrix of the test item. The Panel notes that, based on the hypothesis proposed by the applicant, a comparable response was to be expected in the presence and in the absence of metabolic activation. This comparable response was not observed.

In order to address the hypothesis of the production of histidine and histidine-related derivatives by mesophilic microorganisms, the applicant repeated the reverse mutation assay with the same test item used in the study described above but treated at 121°C for 3 h.[9] The treatment aimed to deactivate the mesophilic microorganisms. The experiment was conducted only in the TA100 strain, without metabolic activation using the pre-incubation method. Although in these conditions, the atypical increase of revertant colonies at the top treatment concentrations was not observed, the test item showed a dose-related increase in the number of revertant colonies, reaching the twofold at the highest concentration of 5,000 μg/plate. This increase is considered indicative for a mutagenic effect.

The applicant also tested isopropanol-purified cassia gum (compliant with the specifications set for cassia gum authorised as a food additive (total anthraquinone < 0.5 mg/kg). A reverse mutation assay in TA100 strain was made with and without metabolic activation, using both the plate incorporation and the pre-incubation method.[10] The test item was clearly negative in all the experimental conditions.

The applicant submitted several additional studies made with various blends of unpurified and isopropanol-purified cassia gum and locust bean gum (free from anthraquinones or their metabolites). These studies could not be evaluated due to heavy microbiological contamination of the test item.

The applicant submitted a mutagenicity experiment with *Salmonella* Typhimurium TA100, in which semi-refined cassia gum had been suspended in dimethyl sulfoxide (DMSO) and filtered through a 0.2-μm filter to remove microorganisms as well as other insoluble organic matter.[11] The test was run using the pre-incubation method. The test item showed no increase in the number of revertants in the absence and presence of metabolic activation up to a maximum concentration of 5,000 μg/plate.

In another test, cassia gum was Soxhlet extracted (6 cycles) with isopropanol. The isopropanol was removed and the dry matter of the isopropanol extracted matter was dissolved in DMSO at a final concentration of 1% w/v, considered by the applicant to represent approximately a 1,000-fold concentration of potential anthranoid metabolites compared to the 5% test item suspension of cassia gum used for the mutagenicity test described above. This test item was tested in the reverse mutation assay in *Salmonella* Typhimurium strains TA97a, TA98, TA100, TA102 and TA1535 with and without metabolic activation, applying both the plate incorporation method and the pre-incubation method, in compliance with OECD guideline 471.[12] No mutagenic effect was reported in any experimental condition.

3.2.2. Conclusions on genotoxicity

The semi-refined cassia gum under application was mutagenic in a bacterial reverse mutation test in *Salmonella* Typhimurium strain TA100. Positive results were also observed after heat sterilisation of the test

[8] Technical dossier/Annex_6.
[9] Technical dossier/Annex_12.
[10] Technical dossier/Annex_14.
[11] Technical dossier/Annex_16.
[12] Technical dossier/Annex_23.

item. Isopropanol purified cassia gum did not show mutagenic effect. Examination of various cassia gum extracts, not representative of the additive under assessment, did not identify any source of mutagenicity.

4. Conclusions

The mutagenicity of the semi-refined cassia gum under application cannot be excluded. Therefore, the FEEDAP Panel cannot establish the safety of semi-refined cassia gum for cats and dogs.

Documentation provided to EFSA

1) Cassia gum for dogs and cats. Supplementary Information February 2018. Submitted by Glycomer GmbH.

Chronology

Date	Event
20/2/2018	Dossier received by EFSA
27/11/2018	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to an application on the use of cassia gum as a food additive. EFSA Journal 2006;4(12):389, 16 pp. https://doi.org/10.2903/j.efsa.2006.389

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2017. Scientific opinion on the Safety of hydroxyanthracene derivatives for use in food. EFSA Journal 2018;16(1):5090, 97 pp. https://doi.org/10.2903/j.efsa.2018.5090

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014a. Scientific Opinion on the safety and efficacy of cassia gum for dogs and cats based on a dossier submitted by Intercolloid (UK) Ltd. EFSA Journal 2014;12(11):3901, 15 pp. https://doi.org/10.2903/j.efsa.2014.3901

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014b. Scientific Opinion on the safety and efficacy of cassia gum for dogs and cats based on a dossier submitted by Glycomer GmbH. EFSA Journal 2014;12(11):3899, 16 pp. https://doi.org/10.2903/j.efsa.2014.3899

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017b. Scientific Opinion on the safety of cassia gum as a feed additive for dogs and cats based on a dossier submitted by Intercolloid (UK) Ltd. EFSA Journal 2017;15(2):4709, 7 pp. https://doi.org/10.2903/j.efsa.2017.4709

EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), 2017c. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Products or Substances used in Animal Feed), 2017d. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

Abbreviations

AFC	EFSA Panel on Food additives, flavourings, processing aids and materials in contact with food
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
DM	dry matter
DMSO	dimethyl sulfoxide
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
OECD	The Organisation for Economic Co-operation and Development
WHO	World Health Organization