Safety and efficacy of a feed additive consisting of an essential oil from *Cinnamomum cassia* (L.) J. Presl (cassia leaf oil) for use in all animal species (FEFANA asbl)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Mojca Fasdmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Andrew Chesson, Josef Schlatter, Dieter Schrenk, Johannes Westendorf, Paola Manini, Fabiola Pizzo and Birgit Dusemund

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 an essential oil from leaves, stalks and twigs of *Cinnamomum cassia* (L.) J. Presl (cassia leaf oil) when used as a sensory additive (flavouring) in feed and water for drinking for all animal species. Owing to the presence of styrene in cassia leaf oil, the FEEDAP Panel is not in the position to conclude for long-living animals and animals for reproduction. For ‘short-living’ animals, the FEEDAP Panel concluded that cassia leaf oil is considered as safe up to the maximum proposed use levels in complete feed of 28.5 mg/kg for chickens for fattening and other minor poultry, 38 mg/kg for turkeys for fattening, 51 mg/kg for piglets and other minor Suidae, 61 mg/kg for pigs for fattening, 100 mg/kg for veal calves (milk replacer), 60 mg/kg for cattle for fattening and other ruminants for fattening, 30 mg/kg for horses, 25 mg/kg for rabbits, 125 mg/kg for salmonids and other fin fish. For the other minor species, the additive is considered as safe at 28.5 mg/kg complete feed. For ‘short-living’ animals, the FEEDAP Panel considered the use in water for drinking as safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed. No concerns for consumers were identified following the use of the additive at the use levels considered safe in feed for the target species. When handling the essential oil, exposure of unprotected users to styrene cannot be excluded. Therefore, to reduce the risk, the exposure of the users should be minimised. The use of the additive under the proposed conditions in animal feed was not expected to pose a risk for the environment. Cassia leaf oil was recognised to flavour food. Since its function in feed would be essentially the same as that in food, no further demonstration of efficacy was considered necessary.

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Keywords: sensory additives, flavouring compounds, *Cinnamomum cassia* (L.) J. Presl, cassia leaf oil, styrene, coumarin, safety

Requestor: European Commission

Question number: EFSA-Q-2010-01296 (new EFSA-Q-2022-00104)

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Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output (in alphabetical order of the last name): Montserrat Anguita, Jaume Galobart, Frank Verdonck and Maria Vittoria Vettori.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Fašmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brantom P, Chesson A, Schlatter J, Schrenk D, Westendorf J, Manini P, Pizzo F and Dusemund B, 2022. Scientific Opinion on the safety and efficacy of a feed additive consisting of an essential oil from Cinnamomum cassia (L.) J. Presl (cassia leaf oil) for use in all animal species (FEFANA asbl). EFSA Journal 2022;20(10):7600, 29 pp. https://doi.org/10.2903/j.efsa.2022.7600

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.
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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003\(^1\) establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7 and in addition, Article 10(2) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of seven years after the entry into force of this Regulation.

The European Commission received a request from the Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)\(^2\) for authorisation/re-evaluation of 18 preparations (cassia oil, cassia bark extract (sb), camphor oil, cinnamon oil, cinnamon bark oleoresin, cinnamon tincture, laurel leaves oil, laurel leaves extract/oleoresin, litsea berry oil, boldo extract (wb), boldo tincture, ylang-ylang oil, mace oil, nutmeg oil, nutmeg oleoresin, kawakawa tincture, pepper oil and pepper oleoresin) belonging to botanically defined group (BDG) 6 – Laurales, Magnoliales, Piperales, when used as a feed additive for all animal species (category: sensory additives; functional group: flavouring compounds). During the assessment, the applicant withdrew the applications for eight preparations.\(^3\) These preparations were deleted from the register of feed additives.\(^4\) In addition, during the course of the assessment, the application was split, and the present opinion covers only one out of the 10 remaining preparations under application: an essential oil from the leaves, stalks and twigs of *Cinnamomum cassia* (L.) J. Presl\(^5\) (cassia leaf oil) for all animal species.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 3 January 2011.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of an essential oil from *C. cassia* (cassia oil), when used under the proposed conditions of use (see Section 3.2.4).

The remaining nine preparations belonging to botanically defined group (BDG) 6 - Laurales, Magnoliales, Piperales under application are assessed in separate opinions.

1.2. Additional information

‘Cassia oil’ from *Cinnamomum aromaticum* Nees is currently authorised as a feed additive according to the entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined) for use in all animal species. It has not been assessed as a feed additive in the EU.

There is no specific EU authorisation for any *C. cassia* preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008\(^6\), flavourings preparations produced from food or food ingredients with flavouring properties, may be used without an evaluation and approval

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\(^1\) 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, pp. 29.

\(^2\) On 13/03/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1,050 Brussels, Belgium.

\(^3\) On 8 October 2020, EFSA was informed about the withdrawal of the applications on cassia bark extract (sb), cinnamon bark oleoresin, laurel leaves extract/oleoresin, mace oil, nutmeg oleoresin, boldo extract (wb), boldo tincture and kawakawa tincture.

\(^4\) Register of feed additives, Annex II, withdrawn by OJ L162, 10.5.2021, p. 5.

\(^5\) Accepted name: *Cinnamomum cassia* (L.) J. Presl; synonyms: *Cinnamomum aromaticum* Nees, *Cinnamomum cassia* Blume (*C. aromaticum* Nees).

\(^6\) Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Regulation (EC) No 1601/91 of the Council, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34.
as long as ‘they do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer, and their use does not mislead the consumer’.

For ‘Cinnamomi cassiae aetheroleum’, the essential oil obtained from twigs and leaves of *Cinnamomum aromaticum* Nees, there is an EMA summary report for veterinary use (EMA, 1998).

‘Cassia oil (Cinnamomi cassiae aetheroleum)’ obtained by steam distillation of the leaves and young branches of *C. cassia* (L.) J. Presl (syn. *Cinnamomum aromaticum* Nees) is described in a monograph of the European Pharmacopoeia 10.0 (PhEur, 2020).

Many of the individual components of cassia leaf oil have been already assessed as chemically defined flavourings for use in feed and food by the FEEDAP Panel, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) and the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). The list of flavouring compounds currently authorised for food and feed uses together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000\(^9\) and the corresponding EFSA opinion is given in Table 1.

**Table 1:** Flavouring compounds already assessed by EFSA as chemically defined flavourings, grouped according to the chemical group (CG) as defined in Commission Regulation (EC) No 1565/2000, with indication of the EU Flavour Information System (FLAVIS) number and the corresponding EFSA opinion.

| CG  | Chemical Group                                                                 | Product – EU register name (common name) | FLAVIS No | EFSA opinion,(*), Year |
|-----|---------------------------------------------------------------------------------|------------------------------------------|-----------|------------------------|
| 01  | Straight-chain primary aliphatic alcohols/ aldehydes/ acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes | Tetradecanal | 05.032 | 2013 |
| 06  | Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers | α-Terpineol | 02.014 | 2012a |
|     |                                                                                  | (E)-Nerolidol | 02.018 | |
|     |                                                                                  | l-α-Bisabolol(a) | 02.129 | 2011a, CEF |
| 08  | Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols | d,l-Borneol | 02.016 | 2016a |
| 15  | Phenyl ethyl alcohol, phenylacetic acid, related esters, phenoxyacetic acids and related esters | 2-Phenylethan-1-ol | 02.019 | 2012b |
|     |                                                                                  | Phenethyl benzoate | 09.774 | |
| 18  | Allylhydroxybenzenes                                                                 | Eugenol | 04.003 | 2011 |
| 21  | Aromatic ketones, secondary alcohols and related esters                           | Acetophenone | 07.004 | 2016b |
|     |                                                                                  | 1-Phenethyl acetate | 09.178 | |
| 22  | Aryl-substituted primary alcohol, aldehyde, acid, ester and acetal derivatives    | Cinnamyl alcohol | 02.017 | 2017a |
|     |                                                                                  | 3-Phenylpropan-1-ol | 02.031 | |
|     |                                                                                  | Cinnamaldehyde(b) | 05.014 | |
|     |                                                                                  | 3-Phenylpropanal | 05.080 | |
|     |                                                                                  | Cinnamyl acetate | 09.018 | |

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7 Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1.

8 European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online: [https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03.pdf](https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03.pdf)

9 Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8.
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 in support of the authorisation request for the use of cassia leaf oil from *C. cassia* as a feed additive.

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

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings. The applicant submitted a written agreement to use the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 6, including the current one under assessment.11

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phytochemical markers in botanically defined flavourings from Group 06 – Laurales, Magnoliales, Piperales. During the assessment, upon request from European Commission and EFSA, the EURL issued two amendments of the original report.12 For the additive under assessment, cassia oil, the evaluation of the method of analysis is included in the second

| CG | Chemical Group | Product – EU register name (common name) | FLAVIS No | EFSA opinion, (*) | Year |
|----|----------------|-----------------------------------------|-----------|------------------|------|
| 23 | Benzyl alcohols/aldehydes/esters/esters/acetals | Benzaldehyde | 05.013 | | 2012c |
| | | Salicylaldehyde | 05.055 | | |
| | | 2-Methoxybenzaldehyde | 05.129 | | |
| | | Benzyl benzoate | 09.727 | | |
| 31 | Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes | Limonene | 01.001 | 2008a, AFC | |
| | | 1-Isopropyl-4-methylbenzene (p-cymene) | 01.002 | 2015 | |
| | | Pin-2(10)-ene (β-pinene) | 01.003 | 2016c | |
| | | Pin-2(3)-ene (α-pinene) | 01.004 | | |
| | | β-Caryophyllene | 01.007 | | |
| | | Camphene | 01.009 | | |
| | | δ-Cadinene | 01.021 | 2011b, CEF | |
| | | β-Bisabolene | 01.028 | | |
| | | α-Muurolene | 01.052 | | |
| 32 | Epoxides | β-Caryophyllene epoxide | 16.043 | 2014, CEF | |

(*): FEEDAP opinion unless otherwise indicated.

(a): Evaluated for use in food. According to Regulation (EC) 1565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.

(b): EFSA evaluated cinnamaldehyde [05.014] (EFSA FEEDAP Panel, 2017a). The configuration of the double bond in cinnamaldehyde [05.014] has not been specified. However, the substance is anticipated to contain more than 97% trans-cinnamaldehyde (EFSA, 2009).

(c): JECFA and EFSA evaluated d-limonene [01.045] (EFSA, 2008a). d-Limonene [01.045] and l-limonene [01.046] were also evaluated for use in feed (EFSA FEEDAP Panel, 2015).

(d): Evaluated applying the ‘Procedure’ described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010).

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EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phytochemical markers in botanically defined flavourings from Group 06 – Laurales, Magnoliaceae, Piperales. During the assessment, upon request from European Commission and EFSA, the EURL issued two amendments of the original report.12 For the additive under assessment, cassia oil, the evaluation of the method of analysis is included in the second

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10 FEED dossier reference: FAD-2010-0218.
11 Technical dossier/Supplementary information/Letter dated 29/04/2021.
12 Preparations included in the first amendment: ylang ylang oil, camphor white oil and cinnamon tincture; preparations included in the second amendment: nutmeg oil, laurel leaves oil, pepper oil black, cinnamon oil, cassia oil and pepper oleoresin black.
amendment. The Executive Summary of the second amendment of the EURL report can be found in Annex A.  

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of cassia leaf oil from *C. cassia* is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA SC, 2009), Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012d), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012e), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017d), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA SC, 2019b), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA SC, 2019c) and General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP, 2021).

3. Assessment

The additive under assessment, cassia leaf oil, is an essential oil obtained by steam distillation of the leaves, stalks and twigs from *C. cassia* (L.) J. Presl. It is intended for use as a sensory additive (functional group: flavouring compounds) in feed and water for drinking for all animal species.

3.1. Origin and extraction

*C. cassia* (L.) J. Presl (synonyms: *C. aromaticum* Nees, *C. cassia* Nees ex Blume) is an evergreen tree belonging to the Lauraceae. It is native to south-west China, and is cultivated in China, Vietnam and other tropical regions.

The essential oil is obtained from leaves, stalks and twigs by steam distillation and then separated from water by decantation.

3.2. Characterisation

3.2.1. Characterisation of cassia leaf oil

The essential oil under assessment is a yellow to reddish brown mobile liquid with a characteristic aroma of cinnamaldehyde. In three batches of the additive, the specific optical rotation (20°C) was 0°, the refractive index (20°C) ranged between 1.606 and 1.611 (specification: 1.600 to 1.614) and the density (20°C) between 1,052 and 1,056 kg/m³ (specification: 1,052–1,070 kg/m³). Cassia oil is identified with the single Chemical Abstracts Service (CAS) number 8007-80-5, the European Inventory of Existing Chemical Substances (EINECS) number 284-635-0, the Council of Europe (CoE) number 131 and the Flavor Extract Manufacturers Association (FEMA) number 2258. In the ECHA website, a different CAS number 84961-46-6 is associated with extracts obtained from *C. cassia* (such as cinnamon bark oil from *C. zeylanicum* Nees. http://echa.europa.eu/substance-information/-/substanceinfo/100.132.785

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13 The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0218_en
14 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.
15 https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf
16 Technical dossier/Supplementary information January 2020/Annex II_SIn_reply_cassia oil_batch_COA_chromatograms.
17 The FEEDAP Panel notes that the CAS number 8007-80-5 is associated to cinnamon bark oil from *C. zeylanicum* Nees. http://echa.europa.eu/substance-information/-/substanceinfo/100.132.785
as tinctures, concretes, absolutes, essential oils, oleoresins, terpenes, terpenes-free fractions, distillates, residues, etc.) and the EINECS number 926-879-9 is associated with *C. cassia* leaf oil.

For cassia leaf oil, the product specifications used by the applicant are based on those developed by the International Organisation for Standardization (ISO) 3216:1997 for essential oil of cassia, Chinese type, adapted to reflect the concentrations of the main volatile components of the essential oil. Four components contribute to the specification as shown in Table 2, with (E)-cinnamaldehyde selected as the phytochemical marker. Analysis of eight batches of the additive showed compliance with these specifications when analysed by gas-chromatography with flame ionisation detection (GC-FID) and expressed as % of gas chromatographic peak area (% GC area).

The applicant provided the full characterisation of the volatile constituents in eight batches (five from China and three from Vietnam) obtained by gas chromatography-mass spectrometry (GC-MS). The four compounds account for 93.8% on average (range 88.9–95.8%) of % GC area (Table 2).

### Table 2:

Major constituents of the essential oil from the leaves, leaf stalks and twigs of *Cinnamomum cassia* (L.) J. Presl (Chinese type) as defined by the ISO standard (3216:1997): specifications and batch to batch variation based on the analysis of eight batches. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%.

| Constituent                        | CAS No.  | FLAVIS No. | % GC Area Specifications | Mean (a) | Range         |
|-----------------------------------|----------|------------|--------------------------|----------|---------------|
| (E)-Cinnamaldehyde                | 14371-10-9 | 05.014(b) | 70.89                    | 83.7     | 78.8–89.0     |
| (E)-2-Methoxycinnamaldehyde(c)    | 60125-24-8 | –         | 0.15                     | 5.97     | 0.82–10.3     |
| Cinnamyl acetate                  | 103.54-8 | 09.018     | 0.5                      | 1.98     | 0.44–3.33     |
| Coumarin(e)                       | 91-64-5  | –         | 0.5                      | 2.18     | 0.80–3.96     |
| Total                             |          |           |                          | 93.8     | 88.9–95.8     |

*CAS no.: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.*

(a): Mean calculated on eight batches.

(b): EFSA evaluated cinnamaldehyde [05.014] (EFSA FEEDAP Panel, 2016c). The configuration of the double bond in cinnamaldehyde [05.014] has not been specified. However, the substance is anticipated to contain more than 97% (E)-cinnamaldehyde (EFSA, 2009).

(c): EFSA evaluated 2-methoxycinnamaldehyde [05.048]: Mixture of (E)- and (Z)-isomers (FGE. 214).

(d): Cinnamyl acetate [09.018]: Mixture of (E)- and (Z)-isomers, composition of stereoisomeric mixture not specified.

(e): Substance which shall not be added as such to food (Annex III), maximum level in food is set by Regulation (EC) No 1334/2008, including traditional and/or seasonal bakery ware containing a reference to cinnamon in the labelling (50 mg/kg), bread and bakery ware containing a reference to cinnamon in the labelling (15 mg/kg) and desserts (5 mg/kg).

In total, 47 constituents were detected and accounted on average for 100% (98.5–100.1%) of the % GC area. Besides the four compounds indicated in the product specifications, 14 other compounds were detected at individual levels ≥ 0.1% and are listed in Table 3. These 18 compounds ≥ 0.1% together account on average for 98.4% (98.0–99.1%) of the % GC area. The remaining 29 compounds (ranging between 0.01% and 0.1%) and accounting for 1.44% (0.46–1.48%) are listed in the footnote. Based on the available data on the characterisation, cassia leaf oil is considered a fully defined mixture.

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18 https://echa.europa.eu/it/substance-information/-/substanceinfo/100.076.913
19 https://echa.europa.eu/it/substance-information/-/substanceinfo/100.240.061
20 Technical dossier/Supplementary information January 2020/Annex III_SIn_reply_cassia oil_ISO_3.216_1997.
21 Technical dossier/Supplementary information January 2020/SIn_reply_cassia oil/GC-FID analysis: (E)-cinnamaldehyde (76.9–87.4%), (E)-2-methoxycinnamaldehyde (3.14–5.3%), cinnamyl acetate (1.8–5.3%) and coumarin (0.25–2.43%).
22 Additional constituents: constituents (n = 15) between < 0.1 and ≥ 0.05%: cinnamyl alcohol, vinylbenzene (hereinafter referred to as styrene), alloaromadendrene, 3-phenylpropan-1-ol, (E)-nerolidol, pin-2(3)-ene (α-pinene), β-cadinene, tetradecal, viridiflorene, α-murolene, spathulenol, γ-cadinene, β-caryophyllene epoxide, (E)-β-bergamotene and α-curcumene; constituents (n = 14) between < 0.05 and ≥ 0.01%: camphene, (i)-α-bisabolol, borneol, 13-epi-dolabadiene, benzyl benzoate, 1-isopropyl-4-methylbenzene, pin-2(10)-ene (β-pinene), phenethyl benzoate, limonene, rimuene, acetylsphenone, eugenol, 1,5,8-para-menthatriene, α-terpinel.
The applicant made a literature search for the chemical composition of Cinnamomum cassia (L.) Presl (Chinese type) accounting for ≥ 0.1% of the composition (based on the analysis of eight batches) not included in the specification. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%.

The applicant made a literature search for the chemical composition of C. cassia and its preparations and the identity of any recognised substances of concern,23 which identified coumarin and styrene (also known as vinylbenzene). Coumarin, which is part of the specification of the essential oil, is identified in the EFSA Compendium of botanicals (EFSA, 2012)24 as a substance of concern for the essential oil obtained from the leaf and young stem (1.5–4%). Coumarin is also listed in Annex III of Regulation (EC) No 1334/200825 among the substances which shall not be added as such to food. In addition, among the 29 compounds listed in footnote 23 the oil was shown to contain low concentrations of styrene in three batches of the additive (0.09% on average, range 0.06–0.11%). The occurrence of styrene in cassia leaf oil is most probably due to oxidation of cinnamaldehyde to cinnamic acid with subsequent decarboxylation to yield styrene. The literature search indicates that styrene concentrations in essential oils from various sources (C. zeylanicum Nees and C. cassia) have been evaluated to be 120–450 mg/kg (Fragniére et al., 2003).

Table 3: Other constituents of the essential oil from the leaves, leaf stalks and twigs of Cinnamomum cassia (L.) J. Presl (Chinese type) accounting for ≥ 0.1% of the composition (based on the analysis of eight batches) not included in the specification. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%.

| Constituent           | CAS No | FLAVIS No | % GC area | Mean (a) | Range         |
|-----------------------|--------|-----------|-----------|----------|--------------|
| Benzaldehyde          | 100-52-7 | 05.013 | 1.05      | 0.54-2.36 |
| 3-Phenylpropanal      | 104-53-0 | 05.080 | 0.76      | 0.47-1.70 |
| α-Copaene             | 3856-25-5 |   –     | 0.52      | 0.27-1.12 |
| 2-Phenylethan-1-ol    | 60-12-8  | 02.019 | 0.45      | 0.22-0.77 |
| (Z)-Cinnamaldehyde    | 57194-69-1 |     –  | 0.41      | 0.18-0.67 |
| 2-Methoxybenzaldehyde | 135-02-4 | 05.129 | 0.30      | 0.05-0.70 |
| 2-Methoxyacinnamic alcohol |     –  |     –   | 0.23      | 0.10-0.43 |
| Salicylaldehyde       | 90-02-8  | 05.055 | 0.22      | 0.08-0.67 |
| δ-Cadinene            | 29350-73-0 | 01.021 | 0.20      | 0.12-0.32 |
| β-Caryophyllene       | 87-44-5  | 01.007 | 0.15      | 0.07-0.40 |
| 2-Methylbenzofuran    | 4265-25-2 |     –  | 0.13      | 0.02-0.46 |
| γ-Murolene            | 30021-74-0 |     –  | 0.13      | 0.08-0.24 |
| β-Bisabolene          | 495-61-4  | 01.028 | 0.12      | 0.08-0.20 |
| 1-Phenethyl acetate   | 93-92-5  | 09.178 | 0.10      | 0.02-0.19 |
| Total                 |         |         | 4.62      | 3.16-9.05 |

CAS no.: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.

(a): Mean calculated on eight batches.

The applicant makes reference to the ‘periodic testing’ of some representative flavourings premixtures for mercury, cadmium and lead, arsenic, fluoride, dioxins and polychlorinated biphenyls (PCBs), organo-chloride pesticides, organo-phosphorous pesticides, aflatoxins B1, B2, G1, G2 and ochratoxin A. However, no data have been provided on the presence of these impurities. Since cassia leaf oil is produced by steam distillation, the likelihood of any measurable carry-over of all the above-mentioned elements is low except for mercury.

3.2.2. Impurities

The applicant makes reference to the ‘periodic testing’ of some representative flavourings premixtures for mercury, cadmium and lead, arsenic, fluoride, dioxins and polychlorinated biphenyls (PCBs), organo-chloride pesticides, organo-phosphorous pesticides, aflatoxins B1, B2, G1, G2 and ochratoxin A. However, no data have been provided on the presence of these impurities. Since cassia leaf oil is produced by steam distillation, the likelihood of any measurable carry-over of all the above-mentioned elements is low except for mercury.

23 Technical dossier/Supplementary information January 2020/Literature search_cassia oil.
24 Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals
25 Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34
3.2.3. Stability

The typical shelf-life of cassia leaf oil is stated to be at least 12 months, when stored in tightly closed containers under standard conditions (in a cool, dry place protected from light). However, no data supporting this statement were provided.

3.2.4. Conditions of use

Cassia leaf oil is intended to be added to feed and water for drinking for all animal species without a withdrawal time. The maximum proposed use level in complete feed for certain target species are reported in Table 4. No use level has been proposed by the applicant for the use in water for drinking.

Table 4: Conditions of use for the essential oil from the leaves, leaf stalks and young twigs of *Cinnamomum cassia* (L.) J. Presl (Chinese type): maximum proposed use levels in complete feed for the target species

| Animal category                  | Maximum use level (mg/kg complete feed) |
|----------------------------------|----------------------------------------|
| Chicken for fattening            | 28.5                                   |
| Laying hen                       | 42                                     |
| Turkey for fattening             | 38                                     |
| Piglet                           | 51                                     |
| Pig for fattening                | 61                                     |
| Sow lactating                    | 74.5                                   |
| Veal calf (milk replacer)        | 100                                    |
| Cattle for fattening             | 60                                     |
| Dairy cow                        | 60                                     |
| Sheep/goat                       | 95                                     |
| Horse                            | 30                                     |
| Rabbit                           | 25                                     |
| Fish                             | 125                                    |
| Dog                              | 10                                     |
| Cat                              | 10                                     |
| Ornamental fish                  | 10                                     |

3.3. Safety

The assessment of safety of cassia leaf oil is based on the maximum use levels proposed by the applicant (Table 4).

Many of the components of cassia leaf oil, accounting for about 95% (when considering (E)- and (Z)-cinnamaldehyde) of the GC peak area, have been previously assessed and considered safe for use as flavourings, and are currently authorised for use in food without limitations and for use in feed at individual use levels higher than those resulting from the intended use of the essential oil in feed. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2). The FEEDAP Panel considers that the conclusions of the assessment of cinnamaldehyde [05.014] apply to the geometric isomers (E)- and (Z)-cinnamaldehyde present in the additive (EFSA FEEDAP Panel, 2017a).

Two compounds, δ-cadinene [01.021] and α-muurolene [01.052], have been evaluated in FGE25.Rev2 (EFSA CEF Panel, 2011b) by applying the procedure described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). For these compounds, for which there is no concern for genotoxicity, EFSA requested additional subchronic toxicity data (EFSA CEF Panel, 2011b). In the absence of such toxicological data, the EFSA CEF Panel was unable to complete its assessment. For these compounds, the FEEDAP Panel applies the approach recommended in the Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a).

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26 Technical dossier/Section II.
Two additional cinnamyl derivatives, namely (E)-2-methoxycinnmaldehyde and 2-methoxycinnamic alcohol, have not been evaluated for use as flavourings, but are closely related to the flavouring compounds already assessed in CG 22 (EFSA FEEDAP Panel, 2017a). α-Copaene (0.5%) and 11 additional volatile components accounting for < 0.1% of the GC peak area (spathulenol, 1,5,8-p-menthatriene, α-curcumene, γ-murolene, alloaromadendrene, viridiflorene, β-cadinene, γ-cadinene, rimuene, 13-epi-dolabradiene, (E)-α-bergamotene) have not been previously assessed for use as flavourings. The FEEDAP Panel notes that they are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 8 and 31 and a similar metabolic and toxicological profile is expected. These lipophilic compounds are expected to be rapidly absorbed from the gastrointestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2015, 2016a,c). These compounds were screened with the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure–Activity Relationship (QSAR) Toolbox and no alert was identified for in vitro mutagenicity, for genotoxic and non-genotoxic carcinogenicity and for other toxicity endpoints.27

The following sections focus on those compounds not previously assessed for use as flavourings or considered substances of concern, namely coumarin and styrene, based on the evidence provided by the applicant in the form of literature searches.

Coumarin, as a naturally occurring flavouring substance, was evaluated in 2004 and 2008 by the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) (EFSA, 2004, 2008b). For styrene, reference is made to the most recent assessment by the International Agency for Research on Cancer (IARC, 2019) and the EFSA assessment of its impact on the safety of the substance styrene (FCM No 193) for its use in plastic food contact materials (EFSA CEP Panel, 2020).

3.3.1. Absorption, distribution, metabolism and excretion

**Coumarin**

In contrast to most of the other major components of cassia leaf oil, coumarin, which is present with a mean around 2%, shows considerable differences among species regarding metabolism and excretion. The absorption, distribution, metabolism and excretion (ADME) of coumarin has been extensively investigated by numerous authors and has been summarised in the EFSA AFC opinions on coumarin in food flavourings (EFSA, 2004, 2008b). Comparative studies on the toxicokinetics and metabolism of coumarin in humans and rodents have confirmed species differences, as reported by the Scientific Committee on Food (SCF) (EC, 1999). The most comprehensive review was published by Lake (1999). In the rat, the absorption after oral application is about 20%, whereas approximately 45% is absorbed in dogs and rhesus monkeys. In humans, coumarin is completely absorbed from the GI-tract. Coumarin undergoes very extensive metabolism along two major pathways, (i) 7-hydroxylation and (ii) epoxidation at 2,3-position with subsequent ring opening to o-hydroxyphenylacetaldehyde, which is further oxidised to o-hydroxyphenylacetic acid. There are numerous minor metabolites, many of which are secondary products from the primary metabolites. The relative extent of the two major pathways is highly variable between species. This is of toxicological importance, because the 7-hydroxylation leads to less toxic metabolites while epoxidation originates the reactive 3,4-epoxide metabolite which, by subsequent lactone ring opening, produces the toxic metabolite o-hydroxyphenylacetaldehyde. While ring-opening predominates in rodents, ferrets, dogs, marmosets, Syrian hamsters and squirrel monkeys (7-hydroxylation rate 0.3–5%), 7-hydroxylation is the most prominent pathway in humans, and baboons (rate > 60%). The 7-hydroxylation rate for cats, pigs and rabbits is 12–19%. In humans, a CYP2A6 polymorphism exists, particularly in Asia and southern Europe, with some individuals showing a reduced capacity to metabolise coumarin to 7-hydroxy coumarin (poor metabolisers). There is evidence for a human subpopulation with an increased susceptibility to coumarin-induced hepatotoxicity for which an association with a reduction of 7-hydroxylation activity is discussed (EFSA, 2004, 2008b). Metabolites formed by phase I metabolism are conjugated with glucuronic acid, sulfate, glycine or glutathione and excreted in the urine or bile. The elimination half-life of coumarin is quite similar in humans and in the different animal species studied (rat, gerbil, dog, rhesus monkey), being around 1–2 h in humans and...

27 Technical dossier/Supplementary information January 2020/Annex VI,SIn_reply_cassia_oil_QSAR. Structural alerts for α-curcumene were due to the presence of arenes. α-Curcumene (or α-curcumene has been evaluated in preparations from BDG 09).
between 1 and 4 h in other species (in Lake, 1999). No evidence of significant tissue accumulation of coumarin and/or coumarin metabolites was obtained after oral administration to rats and rabbits (Kaighen and Williams, 1961, as referenced in Lake, 1999) or intraperitoneal administration to rats (van Sumere and Teuchy, 1971 as referenced in Lake, 1999).

Styrene

The metabolism of styrene has been widely investigated in humans and experimental animals and has been summarised in the IARC assessment (IARC, 2019).

Due to the impact of styrene in occupational exposure, most published ADME studies were performed by administering styrene by inhalation and dermal routes and only few after oral administration. In a study carried out by Sbrana et al. (1983) aimed at evaluating the genotoxicity of styrene, a kinetic study was performed in parallel after administration of the compound to mice by gavage at daily doses of 200 mg/kg body weight (bw) for 70 days. Blood was collected on day one and day 70 at selected time intervals. Urine was collected at the same days over a period of 24 h after styrene administration. In a study carried out by Sbrana et al. (1983) aimed at evaluating the genotoxicity of styrene, a kinetic study was performed in parallel after administration of the compound to mice by gavage at daily doses of 200 mg/kg body weight (bw) for 70 days. Blood was collected on day one and day 70 at selected time intervals. Urine was collected at the same days over a period of 24 h after styrene administration for quantification of metabolites by GC-MS. Styrene was rapidly absorbed with a plasma Cmax of 10 μg/mL 1 h after administration by gavage. Excretion was rapid, with a t1/2 of 36 min. No differences were observed between styrene kinetics in blood on days 1 and 70 of administration. In urine, styrene metabolites derived from styrene-7,8-oxide (i.e. phenylethylene glycol, mandelic acid, benzoic acid, phenylglyoxylic acid and hippuric acid) and from styrene-7,8-oxide conjugation with glutathione (i.e. mercapturic acid) accounted for 79% and 71% of the administered dose, respectively after day one and day 70 of administration.

Mendrala et al. (1993) made an ex vivo comparative evaluation of the relevant enzymes responsible for the metabolism of styrene, measuring the activities of monoxygenase and epoxide hydrolase in the microsomal fraction and of glutathione-S-transferase in the cytosolic fraction of the liver from rats and mice not exposed or previously exposed to styrene via inhalation. The same enzymes were measured in human liver fractions prepared from accident victims submitted to liver transplantation. Mice showed the greatest and humans the lowest capacity to form styrene epoxide and the activity of epoxide hydrolase relative to monoxygenase activity was higher in the human than in the rodent liver. The data indicate that, in rodents, the formation of styrene epoxide is greater and its inactivation by hydrolysis is slower compared to humans. However, it is questionable whether this difference impacts the metabolic activation at human dietary exposure levels.

The blood levels of styrene and styrene epoxide were measured in vivo after oral administration of 500 mg styrene/kg bw to non-exposed rats or to rats previously exposed to 1,000 mg/kg bw styrene (Mendrala et al., 1993). Styrene blood levels in both groups of rats were similar during the first 6 h after administration, the mean value ranging from 22 to 53 μg/g, decreasing to 0.4 μg/g at 24 h. Mean blood levels of styrene oxide ranged from 0.07 to 0.53 μg/g during the first 10 h after dosing, being not detected after this time. The area under the curve (AUC) of styrene and styrene oxide was similar in both groups indicating that no enzymatic induction occurred from the previous exposure to styrene.

Plotnick and Weigel (1979; only the abstract is available) studied the distribution and excretion of 14C-styrene orally administered to rats at a dose of 20 mg/kg bw. Radioactivity peaked in tissues at 4 h after administration. Kidney was the organ with the highest concentration, followed by liver and pancreas. Excretion was almost complete in urine after 24 h, only 2% of the dose was excreted in faeces.

In humans, following inhalation or dermal exposure, styrene is readily absorbed and distributed throughout the body tissues. Repeated exposure to styrene leads to a gradual accumulation in the adipose tissue but not in other tissues. In humans, styrene is initially oxidised by cytochrome P450s (CYPs) through three distinct pathways: (i) epoxidation of the vinyl double bond, the major metabolic pathway; (ii) oxidation on the vinyl group; and (iii) oxidation on the phenyl ring (reviewed in IARC, 2019). Metabolites from all three pathways have been detected in humans exposed to styrene and in experimental studies in laboratory animals. The majority of absorbed styrene (about 90%) is metabolised in the liver by oxidation of the vinyl double bound to styrene 7,8-oxide, the main reactive metabolite, which, if not hydrolysed, can form adducts with DNA, leading to mutations and cancer (Vodicka et al., 2016). In the main metabolic pathway, styrene-7,8-oxide is further metabolised by epoxide hydrolase to styrene glycol and excreted in the urine mainly as mandelic acid (60–80%) and phenylglyoxylic acid (about 30%). Minor amounts of hippuric acid are also excreted. Styrene-7,8-oxide can also be conjugated with glutathione to yield glutathione conjugates, which are further catabolised to isomeric phenylhydroxyethylmercapturic acids. Minor pathways involve the oxidation of the vinyl
group resulting in the formation of 1- and 2-phenylethanol or the oxidation of the aromatic ring with the formation of vinylphenols, mainly 4-vinylphenol, which are excreted as glucuronide and sulfate-conjugates. The intermediate styrene-3,4-epoxide may also be formed (Watabe et al., 1982). About 1 to 2% of the dose is excreted unchanged in urine.

In summary, studies made in experimental animals and data from humans exposed to styrene show that styrene is rapidly absorbed, widely distributed in the organism, extensively biotransformed through similar metabolic pathways and almost completely excreted in urine.

3.3.2. Toxicological studies

Subacute or chronic toxicity studies and genotoxicity studies performed with the additive under assessment were not made available to the FEEDAP Panel. The major constituents of the additive (i.e. cinnamoyl derivatives) have been evaluated in an opinion on chemical group (CG) 22 (EFSA FEEDAP Panel, 2017a) and maximum safe levels in feed were established. Assessment reports on the toxicity of coumarin and styrene, which have been identified as substances of concern, are available in the open literature and are summarised below.

**Coumarin**

Coumarin, as a naturally occurring flavouring substance, has been evaluated in 2004 and 2008 by the EFSA AFC Panel (EFSA, 2004, 2008b). As reported in these opinions, long-term studies showed induction of liver and lung tumours at high doses in rats and mice, respectively. From the negative results of *in vivo* studies on adduct formation in rats, unscheduled DNA synthesis (UDS) in rats and a micronucleus assay in mice, the AFC Panel concluded that coumarin is not an *in vivo* genotoxic agent. Therefore, a threshold-based risk assessment approach was considered justified based on the long-term hepatotoxicity observed in dogs as the most sensitive species (EFSA, 2004). From a no observed adverse effect level (NOAEL) of 10 mg coumarin/kg bw per day in dogs and by applying an uncertainty factor of 100, the AFC Panel derived a tolerable daily intake (TDI) of 0.1 mg coumarin/kg bw per day (EFSA, 2004, 2008b).

**Styrene: carcinogenicity and genotoxicity**

In 2019, IARC updated the previous evaluation of styrene (IARC, 2002) in which the substance had been classified as ‘possibly carcinogenic to humans’ (Group 2B). In this last monograph (IARC, 2019), IARC categorised styrene and its metabolite styrene-7,8-oxide as ‘probably carcinogenic to humans’ (Group 2A). Furthermore, IARC considered that ‘there is strong evidence that both styrene and styrene-7,8-oxide are genotoxic, and that this mechanism can also operate in humans’.

The EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) evaluated the impact of the conclusions of IARC on the safety assessment of the substance styrene (FCM No 193) for its use in plastic food contact materials (EFSA CEP Panel, 2020).

As summarised by EFSA (EFSA CEP Panel, 2020), ‘The recent IARC monograph classified styrene as ‘probably carcinogenic to humans’ (Group 2A), on the basis of ‘limited evidence’ in humans and ‘sufficient evidence’ in experimental animals. Increased incidence of, or mortality, from leukaemia and lymphomas were reported in several epidemiological studies in cohorts of workers exposed to styrene by inhalation, mainly in the reinforced plastics industries; there was also a strong signal for sinonasal adenocarcinoma, a rare cancer in humans, based on a few cases observed in a single large study. Overall, IARC concluded that *the epidemiological studies provide some credible evidence that exposure to styrene causes lymphohaematopoietic malignancies in humans, but confounding, bias or chance cannot be ruled out*: (…)’

‘Nine studies of carcinogenicity of styrene in mice were reported (three by gavage, five via inhalation, one intraperitoneal). Increased incidence of bronchioloalveolar adenoma or carcinoma of the lung was described in two studies by inhalation in CD1 mice and in one study of transplacental exposure followed by gavage in O20 mice. In a study in B6C3F1 mice, styrene administered by gavage significantly increased the incidence of bronchioloalveolar adenoma or carcinoma in males, and a significant positive trend in the incidence of hepatocellular adenoma in females. Nine studies of carcinogenicity of styrene in rats were reported (four by gavage, one in drinking water, two via inhalation, one via intraperitoneal administration and one via subcutaneous injection). One study out of two carcinogenicity studies in rats exposed to styrene by inhalation described a significant increase in the incidences of malignant tumours of the mammary gland. No significant increase in the incidence of any tumour type was observed in the other rat studies’.
The IARC Monograph concluded that there is 'strong evidence' for a genotoxic mechanism of styrene, mediated by its metabolic activation to the electrophilic styrene-7,8-oxide, an epoxide that is genotoxic and directly reactive to DNA. (…)

The large majority of in vitro studies on styrene genotoxicity, described in the IARC monograph, showed positive results only in the presence of metabolic activation. Gene mutations in bacterial cells (Ames test) were found in Salmonella Typhimurium strains that detect base-pair substitutions (TA100, TA1530 and TA1535) but not in strains that detect frameshift mutations (TA98, TA1537 and TA1538) and in Escherichia coli strains. Positive results were reported for gene mutation in mammalian cells. Cytogenetic studies (chromosomal aberration test, micronucleus assay and sister chromatid exchange (SCE)) in mammalian cell lines (V79, CHO) also showed positive results. Positive results without metabolic activation were reported in cytogenetic studies in human whole blood lymphocytes. The IARC monograph also reports a large number of in vivo genotoxicity studies carried out by inhalation or intraperitoneal injection. These studies showed positive results for markers of DNA damage (DNA-adducts, single-strand breaks detected by Comet assay and SCE), while negative or weakly positive results were reported for chromosomal damage (structural chromosomal aberrations and micronuclei). Two in vivo oral studies described in the IARC Monograph reported negative results for chromosomal aberrations in bone marrow of male and female mice exposed up to the maximum tolerated doses, after single or repeated administrations (Loprieno et al., 1978; Sbrana et al., 1983). In one of these oral studies, separate experiments carried out in parallel with styrene oxide at the same range of doses showed a statistically significant dose-related increase in chromosomal aberrations (Loprieno et al., 1978). The IARC Monograph supports that the mechanism of genotoxicity of styrene observed in experimental systems is likely to operate also in humans'.

(…) 'The large majority of the human biomonitoring studies were carried out in the reinforced plastics industry, using DNA damage biomarkers, i.e. DNA adducts, oxidative DNA damage, single-strand breaks by Comet assay, chromosomal aberrations, micronucleus test and SCE. Mixed results were described in studies applying different genotoxicity biomarkers, and a lack of consistency was also shown among the studies using the same genotoxicity biomarker. DNA adducts in peripheral blood cells have been reported to be significantly higher in exposed workers than in unexposed controls in a number of studies. The majority, but not all, of the several available studies showed increased levels of DNA damage as measured by the Comet assay. Studies using the Comet assay to assess oxidative damage to DNA were negative, studies measuring 8-hydroxy-2'-deoxyguanosine in DNA were inconsistent. In the few studies on gene mutation, no clear relationship was found with occupational exposure to styrene. Mixed results were reported in the studies on chromosomal endpoints (chromosomal aberration, micronuclei frequency) in blood cells of exposed workers'.

In its assessment of the impact of the IARC Monograph Vol. 121 on the safety of the substance styrene (FCM No 193) for its use in plastic food contact materials, the EFSA CEP Panel concluded that based on the data provided in the IARC Monograph and by the industry, a concern for genotoxicity associated with oral exposure to styrene remains. The EFSA CEP Panel also recommended that 'a systematic review of genotoxicity and mechanistic data, comparative toxicokinetics and analysis of species differences is required for assessing the safety of styrene' (EFSA CEP Panel, 2020).

Styrene: Other toxicity studies

In a 2-year oral toxicity study, Charles River COBS CD (SD) rats received 0, 125, or 250 mg of styrene/L of drinking water. At 250 mg/L, females showed a significantly lower terminal body weight than control females. No other treatment-related effects were seen. The parameters studied were clinical signs, mortality, growth, food and water intake, haematology, clinical chemistry, urinalysis, gross necropsy and histopathology. The NOAEL in this study was 125 mg/L (corresponding to 7.7 mg/kg bw for males and 12 mg/kg of bw for females) (Litton Bionetics, 1980, as referenced in WHO, 2003).

3.3.3. Safety for the target species

Tolerance studies with the target species and/or toxicological studies made with the essential oil under application were not submitted.

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28 WHO (World Health Organization), 2003, ref 19: Litton Bionetics. Toxicological study on styrene incorporated in drinking water of rats for two years in conjunction with a three-generation reproduction study. Styrene. Revised final report, weeks 1–105. Vol. I. Washington, DC, Chemical Manufacturers Association, 1980.
In the absence of these data, the approach to the safety assessment of a mixture whose individual components are known is based on the safety assessment of each individual component (component-based approach). This approach requires that the mixture is sufficiently characterised. The individual components can be grouped into assessment groups, based on structural and metabolic similarity. The combined toxicity can be predicted using the dose addition assumption within an assessment group, taking into account the relative toxic potency of each component (EFSA SC, 2019a).

As the additive under assessment is a fully defined mixture (> 99% of the components were identified, see Section 3.2.1), the FEEDAP Panel applied a component-based approach to assess the safety for target species of the components of the essential oil, except styrene, which is assessed separately.

**Components other than styrene**

Based on considerations related to structural and metabolic similarities, the components were allocated to 11 assessment groups, corresponding to the chemical groups (CGs) 1, 6, 8, 14, 15, 18, 21, 22, 23, 31 and 32, as defined in Annex I of Regulation (EC) No 1565/2000. For chemical group 31 (‘aliphatic and aromatic hydrocarbons’), the application of sub-assessment groups as defined in Flavouring Group Evaluation 25 (FGE.25) and FGE.78 is applied (EFSA CEF Panel, 2015a,b). The allocation of the components to the (sub-)assessment groups is shown in Table 5 and in the corresponding footnote.

For each component in the assessment group, exposure in target animals was estimated considering the use levels in feed, the percentage of the component in the oil and the default values for feed intake according to the guidance on the safety of feed additives for target species (EFSA FEEDAP Panel, 2017c). Default values on body weight are used to express exposure in terms of mg/kg bw. The intake levels of the individual components calculated for chickens for fattening, the species with the highest ratio of feed intake/body weight per day, are shown in Table 5.

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification. For some components in the assessment group, toxicological data were available to derive NOAEL values. Structural and metabolic similarity among the components in the assessment groups were assessed to explore the application of read-across. If justified, extrapolation from a known NOAEL of a component of an assessment group to the other components of the group with no available NOAEL was made. If sufficient evidence was available for members of a (sub-)assessment group, a (sub-)assessment group NOAEL was derived.

Subchronic studies, from which NOAEL values could be derived, were available for octyl acetate [09.007], the representative compound in CG 1 (EFSA FEEDAP Panel, 2013), terpineol [02.230] in CG 6 (EFSA FEEDAP Panel, 2012a), 2-benzofurancarboxaldehyde [13.031] in CG 14 (EFSA CEF Panel, 2011c), eugenol [04.003] in CG 18 (EFSA FEEDAP Panel, 2011), 1-phenethyl acetate in CG 21 [09.178] (EFSA FEEDAP Panel, 2015b), 2-benzofurancarboxaldehyde in CG 22 (EFSA FEEDAP Panel, 2017a), salicylaldehyde [05.055] in CG 23 (EFSA FEEDAP Panel, 2012c), limonene [01.001], p-cymene [01.002], myrcene [01.008] and β-caryophyllene in CG 31 (EFSA FEEDAP Panel, 2015, 2016c) and β-caryophyllene oxide in CG 32 (EFSA CEF Panel, 2014). For benzaldehyde [05.013] in CG 23, the FEEDAP Panel concluded that the maximum proposed concentration of 25 mg/kg complete feed is safe, based on its structural and metabolic relationship with benzoic acid [02.010], which was considered safe up to 125 mg/kg complete feed (EFSA FEEDAP Panel, 2012c). In addition, for benzyl alcohol the FAF Panel established an acceptable daily intake (ADI) of 4 mg/kg bw per day based on a NOAEL of 400 mg/kg bw per day from a carcinogenicity study in rats (EFSA FAF Panel, 2019). For benzyl benzoate [09.727], the applicant provided a recent review which indicated a NOAEL of 194 mg/kg bw per day from a carcinogenicity study in rats (EFSA FAF Panel, 2019). For coumarin, the reference point of 10 mg/kg bw per day used to derive the TDI as food flavouring was selected (EFSA, 2008b).

29 Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8.

30 Terpineol is a mixture of four isomers: α-terpineol [02.014], a mixture of (R)-(+)α-terpineol and (S)-(−)α-terpineol, β-terpineol, γ-terpineol and 4-terpineol [02.072] (or δ-terpineol). The specification for terpineol [02.230] covers α, β, γ and δ-terpineol. Composition of mixture: 55-75% α-terpineol, 16–23% γ-terpineol, 1–10% cis-β-terpineol, 1–13%trans-β-terpineol and 0–1% δ-terpineol (EFSA CEF Panel, 2015c).
Considering the structural and metabolic similarities in CG 1, the NOAEL of 120 mg/kg bw per day for octyl acetate [09.007] was selected as the reference point for the group and extrapolated to tetradecanal [05.032].

For the subgroup of terpinyl derivatives in CG 6, i.e. α-terpineol [02.072] and (l)-α-bisabolol [02.129], the reference point was selected based on the NOAEL of 250 mg/kg bw per day available for terpineol [09.018], d-limonene [05.080], 2-methoxycinnamic alcohol, cinnamic acid [02.017] and 3-phenylpropan-1-ol [02.031] and selected as the reference point for CG 22.

Concerning the structural and metabolic similarities in CG 22, read-across was applied using the NOAEL of 275 mg/kg bw per day for cinnamaldehyde [05.014] to extrapolate to (E)-2-methoxy-cinnamaldehyde, cinnamyl acetate [09.018], 3-phenylpropanol [05.080], 2-methoxycinnamic acid, cinnamic acid [02.017] and 3-phenylpropan-1-ol [02.031] and selected as the reference point for CG 22.

Read-across was also applied using the NOAEL of 400 mg/kg bw per day for benzyl alcohol [02.010] to extrapolate to benzaldehyde [05.013] and the NOAEL of 10 mg/kg bw per day for salicylaldehyde [05.055] to extrapolate to 2-methoxybenzaldehyde [05.129] in CG 23. The NOAEL of 50 mg/kg bw per day for 1-phenethyl acetate [09.178] was applied to extrapolate to acetophenone [07.04] in CG 21.

The NOAELs for the representative compounds of CG 31, limonene [01.001], p-cymene [01.002] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within sub-assessment groups III, IVe and V (EFSA CEF Panel, 2015a,b).

For the remaining compounds, (E)-nerolidol [02.232], spathulenol, borneol [02.016], 2-phenylethanol-1-ol [02.019], phenethyl benzoate [09.774] and α-curcumene, toxicity studies performed with the compounds under assessment and NOAEL values derived from toxicity studies were not available and read-across was not possible. Therefore, the threshold of toxicological concern (TTC) approach was applied (EFSA FEEDAP Panel, 2012d, 2017c). All these compounds belong to Cramer class I.

As the result of the hazard characterisation, a reference point was identified for each component in the assessment group based on the toxicity data available (NOAEL from in vivo toxicity study or read across) or from the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class (i.e. 3 mg/kg bw per day for Cramer Class I compounds).

For risk characterisation, the margin of exposure (MOE) was calculated for each component as the ratio between the reference point and the exposure. For each assessment group, the combined (total) margin of exposure (MOET) was calculated as the reciprocal of the sum of the reciprocals of the MOE of the individual substances (EFSA SC, 2019a). A MOE(T) > 100 allowed for interspecies- and intra-individual variability (as in the default 10x10 uncertainty factor). The compounds resulting individually in an MOE > 50,000 were not further considered in the assessment group as their contribution to the MOE(T) is negligible.31

The approach to the safety assessment of cassia leaf oil for the chickens for fattening is summarised in Table 5.

31 Compounds included in the assessment groups but not reported in the table: tetradecanal (CG 1); α-bisabolol and α-terpineol (CG 4); eugenol (CG 18); acetoephone (CQ 21); 3-phenylpropan-1-ol (CG 22); benzyl benzoate (CG 23); limonene and 1,2,5-p-menthatriene (CG 31, III); p-cymene (CG 31, IVe); viridiflore, β-cadinene, α-pinene, α-murolene, γ-cadinene, α-bergamotene, camphene, β-pinene, rimuene, 13-epi-dolabradiene (CG 31, V); β-caryophyllene epoxide (CG 32).
Table 5: Compositional data, intake values (calculated for chickens for fattening at 28.5 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of cassia leaf oil classified according to assessment groups.

| Essential oil composition | Exposure | Hazard characterisation | Risk characterisation |
|---------------------------|----------|------------------------|----------------------|
| Assessment group          | FLAVIS-No | Highest conc. in the oil | Highest Feed conc. | Intake<sup>(a)</sup> | Cramer Class<sup>(b)</sup> | NOAEL<sup>(c)</sup> | MOE | MOET |
| Constituent               | %        | mg/kg                  | mg/kg bw/day       | mg/kg bw/day   | –                  | –                  | –   | –    |
| CG 6                      |          |                       |                     |               |                    |                    |     |      |
| (E)-Nerolidol             | 02.232   | 0.09                   | 0.043              | 0.0038       | I                  | 3                  | 782 | 418  |
| Spathulenol               | n.a.     | 0.13                   | 0.037              | 0.0033       | I                  | 3                  | 902 |      |
| MOET CG 6                 |          |                       |                     |               |                    |                    |     |      |
| CG 8                      |          |                       |                     |               |                    |                    |     |      |
| Borneol                   | 02.016   | 0.03                   | 0.009              | 0.0008       | I                  | 3                  | 3,909 | |
| CG 14                     |          |                       |                     |               |                    |                    |     |      |
| 2-Methylbenzofuran        | n.a.     | 0.46                   | 0.131              | 0.0118       | (III)              | 25<sup>(d)</sup>   | 2,124 | |
| CG 15                     |          |                       |                     |               |                    |                    |     |      |
| 2-Phenylethan-1-ol        | 02.019   | 0.77                   | 0.219              | 0.0197       | I                  | 3                  | 152 |      |
| Phenethyl benzoate        | 09.774   | 0.03                   | 0.009              | 0.0008       | I                  | 3                  | 3,909 | |
| CG 21                     |          |                       |                     |               |                    |                    |     |      |
| 1-Phenetyl acetate        | 09.178   | 0.054                  | 0.0049             | (I)          | 50                 | 10,286            |     |      |
| CG 22                     |          |                       |                     |               |                    |                    |     |      |
| (E)-Cinnamaldehyde        | 05.014   | 88.97                  | 25.36              | 2.2763       | (I)                | 275                | 121 | 147  |
| 2-Methoxyacinnamaldehyde  | n.a.     | 10.34                  | 2.947              | 0.2646       | (I)                | 275                | 1,039 | |
| Cinnamyl acetate          | 09.018   | 3.33                   | 0.949              | 0.0852       | (I)                | 275                | 3,228 | |
| 3-Phenylpropanal          | 05.080   | 1.70                   | 0.485              | 0.0435       | (I)                | 137.5             | 3,161 | |
| (Z)-Cinnamaldehyde        | 05.014   | 0.67                   | 0.191              | 0.0171       | (I)                | 275                | 16,042 | |
| 2-Methoxyacinnamyl alcohol| n.a.     | 0.41                   | 0.123              | 0.0110       | (I)                | 275                | 24,996 | |
| Cinnamyl alcohol          | 02.097   | 0.25                   | 0.071              | 0.0064       | (I)                | 275                | 42,994 | |
| MOET CG 22                |          |                       |                     |               |                    |                    | 100 |      |
| CG 23                     |          |                       |                     |               |                    |                    |     |      |
| Benzaldehyde              | 05.013   | 2.36                   | 0.673              | 0.0604       | (I)                | 200<sup>(f)</sup>  | 3,312 | |
| Salicyldehyde             | 05.055   | 0.67                   | 0.191              | 0.0171       | (I)                | 5<sup>(g)</sup>    | 279  |     |
| 2-Methoxybenzaldehyde     | 05.129   | 0.70                   | 0.200              | 0.0179       | (I)                | 5<sup>(g)</sup>    | 292  |     |
| MOET CG 23                |          |                       |                     |               |                    |                    | 137 |      |
| CG 31, III (Cyclohexene hydrocarbons) |          |                       |                     |               |                    |                    |     |      |
| β-Bisabolene              | 01.028   | 0.20                   | 0.057              | 0.0051       | (I)                | 250               | 48,856 | |
| MOET CG 31, III           |          |                       |                     |               |                    |                    | 40,714 | |
| CG 31, IVe (Benzene hydrocarbons, alkyl) |          |                       |                     |               |                    |                    | 1,163 | |
| α-Curcumene               | n.a.     | 0.1                    | 0.029              | 0.0026       | I                  | 3                 | 1,173 | |
| CG 31, V (Br-, tricyclic, non-aromatic hydrocarbons) |          |                       |                     |               |                    |                    |     |      |
| α-Copaene                 | n.a.     | 1.12                   | 0.319              | 0.0287       | (I)                | 222               | 7,747 | |
| β-Caryophyllene           | 01.007   | 0.40                   | 0.114              | 0.0102       | (I)                | 222               | 21,692 | |
| δ-Cadinene               | n.a.     | 0.32                   | 0.091              | 0.0082       | (I)                | 222               | 27,115 | |
| γ-Muurolene               | n.a.     | 0.24                   | 0.068              | 0.0061       | (I)                | 222               | 36,154 | |
| Alloaromadendrene         | n.a.     | 0.24                   | 0.068              | 0.0061       | (I)                | 222               | 36,154 | |
As shown in Table 5, for all the assessment groups, the MOE(T) was ≥ 99. Therefore, no safety concern was identified for the cassia leaf oil (without considering the presence of styrene) when used as a feed additive for chickens for fattening at the proposed use levels (28.5 mg/kg). From the lowest MOE of 99 (for coumarin) in chickens for fattening, the MOE was calculated for the other target species considering the respective daily feed intake/kg bw and conditions of use. The results are summarised in Table 6.

Table 6: Margin of exposure (MOE) for coumarin calculated for the different target animal categories at the proposed use level in feed.

| Animal category | Body weight (kg) | Feed intake (g DM/day) | Proposed use level (mg/kg feed)(a) | Lowest MOE |
|-----------------|-----------------|------------------------|-----------------------------------|------------|
| Chicken for fattening | 2 | 158 | 28.5 | 99 |
| Laying hen | 2 | 106 | 42 | 100 |
| Turkey for fattening | 3 | 176 | 38 | 99 |
| Piglet | 20 | 880 | 51 | 99 |
| Pig for fattening | 60 | 2,200 | 61 | 99 |
| Sow lactating | 175 | 5,280 | 74.5 | 100 |
| Veal calf (milk replacer) | 100 | 1,890 | 100 | 117 |
| Cattle for fattening | 400 | 8,000 | 60 | 186 |
| Dairy cow | 650 | 20,000 | 60 | 120 |
| Sheep/goat | 60 | 1,200 | 95 | 117 |
| Horse | 400 | 8,000 | 30 | 371 |
| Rabbit | 2 | 100 | 25 | 178 |
| Salmon | 0.12 | 2.1 | 125 | 99 |
| Dog | 15 | 250 | 10 | 1,311 |
| Cat(b) | 3 | 60 | 10 | 1,114 |
| Ornamental fish | 0.012 | 0.054 | 10 | 4,458 |

DM: dry matter.
(a): Complete feed containing 88% DM, milk replacer 94.5% DM.
(b): The MOET for cats is increased to 500 because of the reduced capacity of glucuronidation.
Table 6 shows that for all species the MOET is close to or exceeds the value of 100. Because glucuronidation is an important metabolic reaction to facilitate the excretion of the components of the essential oil, the use of cassia leaf oil as additive in cat feed needs a wider margin of exposure. Considering that cats have an unusually low capacity for glucuronidation (Court and Greenblatt, 1997; Lautz et al., 2021), a MOET of 500 is considered adequate. Therefore, with respect to the components other than styrene, no safety concern was identified for cassia leaf oil, when used as a feed additive at the proposed maximum use levels in the different species.

No specific proposals have been made by the applicant for the use level in water for drinking. The FEEDAP Panel considers that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed (EFSA FEEDAP Panel, 2010).

**Styrene**

Low concentrations of styrene were detected in three out of the eight batches of the additive under assessment (average: 0.09% on average, range 0.06-0.11%). The use of cassia leaf oil at the proposed use levels in feed for the different target species (ranging from 10 to 125 mg/kg complete feed, see Section 3.2.4), would result in concentrations ranging from 0.01 to 0.11 mg styrene/kg complete feed.

The average and highest daily intake of styrene for the different target animal categories is reported in Table 7, considering the analysed values of styrene reported in Section 3.2.1.

### Table 7: Target animal intake of styrene (as µg/kg bw per day) at the maximum proposed use level of the additive in feed for each species (ranging from 10 to 125 mg/kg complete feed).

| Target species                  | Daily feed intake | Body weight | Use level in feed | Intake styrene |
|---------------------------------|-------------------|-------------|-------------------|----------------|
|                                 | kg DM/day         | Kg          | mg/kg             | µg/kg bw per day |
| Chicks for fattening            | 0.158             | 2           | 28.5              | 2.30           |
| Laying hens                     | 0.106             | 2           | 42                | 2.28           |
| Turkey for fattening            | 0.176             | 3           | 38                | 2.28           |
| Piglet                          | 0.88              | 20          | 51                | 2.30           |
| Pig for fattening               | 2.2               | 60          | 61                | 2.29           |
| Sow lactating                   | 5.28              | 175         | 74.5              | 2.30           |
| Veal calf (milk replacer)       | 1.89              | 100         | 100               | 1.80           |
| Cattle for fattening            | 8                 | 400         | 60                | 1.23           |
| Dairy cows                      | 20                | 650         | 60                | 1.89           |
| Sheep/goat                      | 1.2               | 60          | 95                | 1.94           |
| Horse                           | 8                 | 400         | 30                | 0.61           |
| Rabbit                          | 0.1               | 2           | 25                | 1.28           |
| Salmon                          | 0.0021            | 0.12        | 125               | 2.24           |
| Dog                             | 0.25              | 15          | 10                | 0.17           |
| Cat                             | 0.06              | 3           | 10                | 0.20           |
| Ornamental fish                 | 0.000054          | 0.012       | 10                | 0.05           |

DM: dry matter; bw: body weight.

The use of cassia leaf oil at the proposed use level in feed would result in an average intake of styrene ranging between 0.05 µg/kg bw per day in ornamental fish and 2.3 µg/kg bw per day in chickens for fattening and pigs (highest intake 2.81 µg/kg bw per day in piglets).

Styrene is a ubiquitous air pollutant and is present in many foods as such or as a biodegradation/fermentation product. The EFSA CEP Panel estimated the dietary exposure of consumers to styrene migrating from styrene-based plastics to be in the order of 0.1 µg/kg bw per day, in the same range as exposure from styrene present in foods as such, with an exposure from food of 0.12–0.38 µg/kg bw per day. The EFSA CEP Panel estimated that the daily exposure to styrene by inhalation is in the range of 0.1–0.6 µg/kg bw for adults (EFSA CEP Panel, 2020).
Cassia leaf oil for all animal species

The FEEDAP Panel notes that cinnamic acid is widely present in feed of plant origin. It is likely that the processing of feed containing cinnamic acid or flavoured with cinnamaldehyde or cinnamic acid would produce styrene, particularly when feed is pelleted at high temperatures. Although it would be reasonable to assume that animal feed or the air inhaled by animals are also contaminated by styrene from other sources, comparable styrene intake figures are not available for target animals, which would allow a quantitative risk assessment to be performed. Therefore, the FEEDAP Panel could not evaluate whether the exposure of target animals to styrene is likely to be increased by the use of cassia leaf oil as feed additive compared to the intake from other dietary sources (as described in EFSA FEEDAP Panel, 2021).

Considering that a concern for genotoxicity associated with oral exposure to styrene remains and pending the outcome of the overall safety assessment of styrene by oral route, the FEEDAP Panel is not in the position to conclude on the safety of cassia leaf oil as feed additive for long-living animals and reproductive animals, including those animals reared for laying/breeding/reproduction.

The FEEDAP Panel noted that genotoxicity and carcinogenicity are relevant endpoints for long-living animals. For 'short-living' animals, defined as those animals raised for fattening whose lifespan under farming conditions makes it very unlikely that they develop cancer as a result of the exposure to genotoxic and/or carcinogenic substances in the diet, other non-neoplastic endpoints are considered more appropriate for the risk assessment (EFSA FEEDAP Panel, 2021). When the estimated exposure of species for fattening is compared to the NOAEL of 7.7 mg styrene/kg bw per day (see Section 3.3.2), an MOE ranging from 2,136 in chickens for fattening and 5,133 in cattle for fattening is calculated. Therefore, the FEEDAP Panel concludes that the use of the cassia leaf oil at the proposed use levels in feed is not expected to be of concern for target species for fattening.

3.3.3.1. Conclusions on safety for the target species

Owing to the presence of styrene in cassia leaf oil, the FEEDAP Panel is not in the position to conclude on the safety of the additive for long-living animals and reproductive animals including those animals reared for laying/breeding/reproduction.

For 'short-living' animals, the FEEDAP Panel considers cassia leaf oil as safe up to the maximum proposed use levels in complete feed of 28.5 mg/kg for chickens for fattening and other minor poultry, 38 mg/kg for turkeys for fattening, 51 mg/kg for piglets and other minor Suidae, 61 mg/kg for pigs for fattening, 100 mg/kg for veal calves (milk replacer), 60 mg/kg for cattle for fattening and other ruminants for fattening, 30 mg/kg for horses, 25 mg/kg for rabbits, 125 mg/kg for salmonids and other fin fish. For other minor species, the additive is considered as safe at 28.5 mg/kg complete feed.

For 'short-living' animals, the FEEDAP Panel considers the use in water for drinking as safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

3.3.4. Safety for the consumer

Cassia leaf oil is added to a wide range of food categories for flavouring purposes (Ternes et al., 2005). Fenaroli (Burdock, 2009) describes 'cassia oil' as a synonym of 'cinnamon bark oil' and 'Cinnamomum cassia oil'. Fenaroli further explains that different plant species (C. zeylanicum Nees-Ceylon cinnamon, C. loureirii Nees-Saigon cinnamon and C. cassia Nees-Chinese cinnamon) are described as 'cinnamon'. Although individual consumption figures are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites values of 3.0 mg/kg bw per day for 'cinnamon' and of 0.014 mg/kg bw per day (FEMA 2291) for the 'cinnamon bark oil'.

The majority of the individual constituents of the essential oil under assessment are currently authorised as food flavourings without limitations and have been already assessed for consumer safety when used as feed additives in animal production (see Table 1, Section 1.2).

No data on residues in products of animal origin were made available for any of the constituents of the essential oil. However, the Panel recognises that the constituents of cassia leaf oil are expected to be extensively metabolised and excreted in the target species. Also for styrene, the available data indicate that it is absorbed, metabolised and rapidly excreted and at the concentrations in feed resulting from the use of the additive (0.01 to 0.11 mg styrene/kg complete feed) is not expected to accumulate in animal tissues and products (see Section 3.3.1).

Considering the above and the reported human exposure due to the direct use of cassia leaf oil in food (Burdock, 2009), it is unlikely that the consumption of products from animals given cassia leaf oil at the proposed maximum use level would significantly increase human background exposure.

Consequently, no safety concern would be expected for the consumer from the use of cassia leaf oil up to the highest safe use level in feed for the target animals.
3.3.5. Safety for the user

No specific data were provided by the applicant regarding the safety of the additive for users. The applicant produced a safety data sheet\(^{32}\) for cassia leaf oil, where hazards for users have been identified. When handling the essential oil, exposure of unprotected users to styrene cannot be excluded. Therefore, to reduce the risk, the exposure of the users should be minimised.

3.3.6. Safety for the environment

*C. cassia* is not a native species to Europe. Therefore, the safety for the environment is assessed based on the individual components of the essential oil.

The major components (cinnamaldehyde and cinnamyl acetate), as well as nine components accounting for > 0.1% of the composition of the additive (benzaldehyde, 3-phenyl propanol, 2-phenylethan-1-ol, 2-methoxybenzaldehyde, salicylaldehyde, δ-cadinene, β-caryophyllene, β-bisabolene and 1-phenetylacetate) and 19 components accounting for < 0.1% have been evaluated by EFSA as sensory additives for animal feed, they were considered to be safe for the environment at individual use levels higher than those resulting from the use of the essential oil in feed. These compounds account together for 95% of the composition of the oil.

The remaining identified constituents of the essential oil are mainly aliphatic mono- or sesquiterpenes partially with functional groups, they are chemically related to the substances evaluated by EFSA as CG 31 for use in animal feed (EFSA FEEDAP Panel, 2015, 2016c) for which EFSA concluded that they were ‘extensively metabolised by the target species (see Section 3.2.2.1) and excreted as innocuous metabolites or carbon dioxide’. Therefore, no risk for the safety for the environment is foreseen. ‘Average feed levels of constituents of the essential oil are much lower than the use levels for CG 31 substances.

The use of the additive in animal feed under the proposed conditions of use is not expected to pose a risk for the environment.

3.4. Efficacy

Cinnamon and its preparations are listed in Fenaroli’s Handbook of Flavour Ingredients (Burdock, 2009), by the Flavour and Extract Manufactures Association (FEMA) with the reference numbers 2,289 (cinnamon), 2,290 (cinnamon bark extract) and 2,291 (cinnamon bark oil). The FEEDAP Panel notes that Fenaroli describes ‘cassia oil’ as a synonym of ‘cinnamon bark oil’ and ‘Cinnamomum cassia oil’. Fenaroli further explains that different plant species (*C. zeylanicum* Nees-Ceylon cinnamon, *C. loureirii* Nees-Saigon cinnamon and *C. cassia* Nees-Chinese cinnamon) are described as ‘cinnamon’.

Cassia leaf oil is added to a wide range of food categories for flavouring purposes (Ternes et al., 2005). In addition, the components of the leaf oil are known for their food flavouring properties. Since cassia leaf oil and ‘cinnamon’ and its preparations are universally recognised to flavour food and their function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

4. Conclusions

Cassia leaf oil from *C. cassia* (L.) J. Presl may be produced from plants of different geographical origins and by various processes resulting in preparations with different composition and toxicological profiles. Therefore, the following conclusions apply only to cassia leaf oil which contains ≤ 0.11% styrene and is produced by steam distillation of the leaves, stalks and twigs of *C. cassia*.

Owing to the presence of styrene in cassia leaf oil, the FEEDAP Panel is not in the position to conclude for long-living animals and reproductive animals including those animals reared for laying/breeding/reproduction. For ‘short-living’ animals, the FEEDAP Panel concludes that cassia leaf oil is considered as safe up to the maximum proposed use levels in complete feed of 28.5 mg/kg for chickens for fattening and other minor poultry, 38 mg/kg for turkeys for fattening, 51 mg/kg for piglets and other minor Suidae, 61 mg/kg for pigs for fattening, 100 mg/kg for veal calves (milk replacer), 60 mg/kg for cattle and other ruminants for fattening, 30 mg/kg for horses for meat production, 25 mg/kg for rabbits, 125 mg/kg for salmonids and other fin fish. For the other minor species, the

\(^{32}\) Technical dossier/Supplementary Information January 2020/Annex_X_SI reply_cassia_oil_MSDS. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1), serious eye damage/eye irritation (H319, category 2).
additive is considered as safe at 28.5 mg/kg complete feed. The FEEDAP Panel considers the use in water for drinking as safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

No concerns for consumers were identified following the use of the additive at the use level considered safe in feed for the target animals.

When handling the essential oil, exposure of unprotected users to styrene cannot be excluded. Therefore, to reduce the risk, the exposure of the users should be minimised.

The use of the additive under the proposed conditions in animal feed is not expected to pose a risk for the environment.

Cassia leaf oil is recognised to flavour food. Since its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

5. Recommendations

The specification should ensure that the concentration of styrene in the additive should be as low as possible and should not exceed 0.1%.

Data on the generation and levels of styrene in feed and food (from, e.g. cassia preparations, cinnamaldehyde and cinnamic acid) are needed and should be considered in the context of genotoxicity and mechanistic data, comparative toxicokinetics and analysis of species differences to complete a full safety assessment of styrene.

6. Documentation provided to EFSA/Chronology

| Date       | Event                                                                 |
|------------|----------------------------------------------------------------------|
| 28/10/2010 | Dossier received by EFSA. Botanically defined flavourings from Botanical Group 06 - Laurales, Magnoliæales, Piperales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG) |
| 11/11/2010 | Reception mandate from the European Commission                         |
| 03/01/2011 | Application validated by EFSA – Start of the scientific assessment     |
| 01/04/2011 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: analytical methods |
| 05/04/2011 | Comments received from Member States                                  |
| 20/04/2012 | Reception of supplementary information from the applicant              |
| 26/02/2013 | EFSA informed the applicant (EFSA ref. 7,150,727) that, in view of the workload, the evaluation of applications on feed flavourings would be re-organised by giving priority to the assessment of the chemically defined feed flavourings, as agreed with the European Commission |
| 02/08/2013 | Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives |
| 24/06/2015 | Technical hearing during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products": data requirement for the risk assessment of botanicals |
| 18/12/2018 | EFSA informed the applicant that the evaluation process restarted       |
| 07/02/2019 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: characterisation, safety for target species, safety for the consumer, safety for the user and environment |
| 30/01/2020 | Reception of supplementary information from the applicant (partial submission: cassia oil) |
| 16/02/2022 | The application was split and a new EFSA-Q-2022-00104 was assigned to the preparation included in the present assessment. Scientific assessment re-started |
| 24/06/2022 | Reception of an amendment of the Evaluation report of the European Union Reference Laboratory for Feed Additives related to ylang ylang oil, camphor white oil and cinnamon tincture |
| 31/08/2022 | Reception of a second amendment of the Evaluation report of the European Union Reference Laboratory for Feed Additives related to nutmeg oil, laurel leaves oil, pepper oil black, cinnamon oil, cassia oil and pepper oleoresin black |
| 27/09/2022 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment |
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### Abbreviations

- **ADME**: Absorption, distribution, metabolism and excretion
- **AFC**: EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
- **bw**: body weight
- **CAS**: Chemical Abstracts Service
- **CEF**: EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
- **CG**: chemical group
- **CoE**: Council of Europe
- **ECHA**: European Chemicals Agency
- **EINECS**: European Inventory of Existing Chemical Substances
- **EMA**: European Medicines Agency
- **EURO**: European Union Reference Laboratory
- **FAO**: Food and Agriculture Organization
- **FCM**: food contact material
- **FEEDAP**: EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
| Acronym | Full Form |
|---------|-----------|
| FEMA    | Flavor Extract Manufacturers Association |
| FFAC    | Feed Flavourings authorisation Consortium of FEFANA (EU Association of Specialty Feed Ingredients and their Mixtures) |
| FGE     | food group evaluation |
| FLAVIS  | The EU Flavour Information System |
| FL-no   | FLAVIS number |
| GC-FID  | gas chromatography-flame ionisation detection |
| GC-MS   | gas chromatography-mass spectrometry |
| IARC    | International Agency for Research on Cancer |
| ISO     | International Organization for Standardization |
| JECFA   | The Joint FAO/WHO Expert Committee on Food Additives |
| MOE     | margin of exposure |
| MOET    | combined margin of exposure |
| NOAEL   | no observed adverse effect level |
| NTP     | National Toxicology Program |
| OECD    | Organisation for Economic Co-operation and Development |
| PCBs    | polychlorinated biphenyls |
| QSAR    | quantitative structure-activity relationship |
| SC      | EFSA Scientific Committee |
| SCF     | Scientific Committee on Food |
| TTC     | threshold of toxicological concern |
| WHO     | World Health Organization |
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for eighteen compounds from botanically defined Group 06 (Laurales, Magnoniales, Piperales) – second amendment of the EURL report

In the period between the publication of the original EURL evaluation report [1] and the current date, eight flavouring compounds (cassia bark extract, cinnamon bark oleoresin, laurel leaves extract/oleoresin, boldo extract, boldo tincture, mace oil, nutmeg oleoresin and kawakawa tincture) were withdrawn from the grouped application FAD-2010-0218 Botanically defined flavourings from Group 06 - Laurales, Magnoliales, Piperales [2].

Upon request of DG SANTE, the EURL evaluated the new methods of analysis provided by the Applicant for three feed additives from the group, namely: ylang ylang oil, camphor white oil and cinnamon tincture and recently issued a partial amendment of the original EURL report [3].

Following an additional request from EFSA [4], the EURL evaluated in the frame of this second amendment the new supplementary information provided by the Applicant related to the methods of analysis proposed for other six feed additives so-called: nutmeg oil, laurel leaves oil, pepper oil black, cinnamon oil, cassia oil and pepper oleoresin black which belong to the same grouped application.

Hereafter is the amended report on the evaluation of the new methods of analysis submitted by the Applicant and proposed for official control of the following feed additives: nutmeg oil, laurel leaves oil, pepper oil black, cinnamon oil, cassia oil and pepper oleoresin black. The updated recommendations of this amendment replace the ones stated for these six feed additives in the original report issued by the EURL [1].

For nutmeg oil, laurel leaves oil, pepper oil black, cinnamon oil and cassia oil the Applicant proposed the quantification of their respective phytochemical markers, by gas chromatography coupled with flame ionisation detection (GC-FID), based on different available ISO standard methods.

Furthermore, the Applicant provided the analytical procedure with the specific operating conditions for the GC and applied it to the mentioned feed additives for the quantification of their respective phytochemical markers. According to the analytical procedure, 1 μl of the oil is injected into the GC using split ratio 100:1. The eluted compounds are detected by FID and the quantification is performed using the normalisation approach for the estimation of the area percentage of individual components (including also the phytochemical marker) in the obtained chromatograms.

(...)

Cassia oil

According to the Applicant cassia oil is an essential oil obtained by distillation from leaves, stalks and twigs of the plant species 'Cinnamomum aromaticum' Nees (synonym: Cinnamomum cassia Nees ex Blume) with a content of (E)-cinnamaldehyde (phytochemical marker) ranging from 70 to 89% (expressed as the relative individual peak area in the chromatogram) [15].

For the quantification of (E)-cinnamaldehyde in cassia oil the Applicant proposed a gas chromatography mass spectrometry (GC-MS) method according to the generic ISO 11024:1998 standard for ‘Essential oils: General guidance on chromatographic profiles’ [16]. Furthermore, the Applicant presented typical chromatograms of cassia oil using this GC-MS method thus demonstrating a good separation of the marker [17].

The Applicant provided the specific operating conditions in the method procedure [16] where, 1 μl of the diluted oil (1:10) is injected into the GC using split ratio 100:1. The quantification is performed using the normalisation approach for the estimation of the area percentage of individual components (including also the phytochemical marker) in the chromatogram [16].

Moreover, in the frame of the characterisation of the feed additive, the Applicant provided data for the quantification of (E)-cinnamaldehyde in eight different batches of cassia oil by using the mentioned GC-MS method. The reported content of the phytochemical marker (E)-cinnamaldehyde was ranging from 80.3 to 89.0% [15].

Furthermore, the Applicant also analysed the phytochemical marker (E)-cinnamaldehyde in cassia oil using a gas chromatography coupled with flame ionisation detection (GC-FID) method in line with the standard method ISO 3216:1997 for 'Oil of cassia, Chinese type [Cinnamomum aromaticum Nees, syn. Cinnamomum cassia Nees ex Blume]' [18]. The mentioned GC-FID method led to a (E)-cinnamaldehyde contents in cassia oil ranging from 76.9 to 87.4% [18] which are within the range
specified in the ISO 3216 standard [21], thus proving its applicability for the quantification of the phytochemical marker (E)-cinnamaldehyde in cassia oil [15].

Therefore, based on the evidences provided by the Applicant the EURL recommends for official control the GC-MS and the GC-FID methods mentioned above for the quantification of (E)-cinnamaldehyde (phytochemical marker) in cassia oil.

(...)