SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of a tincture from the bark of *Cinnamomum verum* J. Presl (cinnamon tincture) for use in all animal species (FEFANA asbl)

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of a tincture from the bark of *Cinnamomum verum* J. Presl (cinnamon tincture) when used as a sensory additive in feed and water for drinking for all animal species. The product is a water/ethanol [redacted] solution, with a dry matter content of approximately 0.9%. The product contains on average 0.344% polyphenols (of which 0.001% are flavonoids) and 0.001% cinnamaldehyde. Methyleugenol was present at the limit of detection in one out of the five batches examined. The FEEDAP Panel concluded that cinnamon tincture is safe at the maximum proposed use level of 50 mg/kg complete feed for all animal species except horses. For horses, the maximum proposed use level of 60 mg/kg complete feed is considered safe. No safety concern would arise for the consumer from the use of cinnamon tincture up to the highest proposed use levels in feed. The additive under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser. The use of the cinnamon tincture as a flavour in animal feed is not expected to pose a risk for the environment. Since *C. verum* and cinnamon bark extracts are 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 for the tincture under application.

Keywords: sensory additives, flavouring compounds, *Cinnamomum verum* J. Presl, tincture, cinnamon tincture, *(E)*-cinnamaldehyde, methyleugenol, safrole

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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 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 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] In addition, during the course of the assessment, the application was split and the present opinion covers only one out of the initial 18 preparations under application: a tincture from the bark of *Cinnamomum verum* J. Presl[4] (cinnamon tincture) for all animal species.[5]

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 a tincture form the bark of *C. verum* J. Presl (cinnamon tincture), when used under the proposed conditions of use (see Section 3.2.3).

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

A tincture from *Cinnamomum zeylanicum* Bl., *C. verum* J. Presl (cinnamon tincture) 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). It has not been assessed as a feed additive in the EU.

There is no specific EU authorisation for any *C. verum* J. Presl 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

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 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, 1050 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] Accepted name: *Cinnamomum verum* J. Presl., synonym: *Cinnamomun zeylanicum* Blume.
[5] On 27 February 2019, EFSA was informed by the applicant about the transfer of contact point for this preparation to Manghebati SAS, zone de la Basse Haye – BP 42133 – 35221 Chateaubourg Cedex.
[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.

and approval 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'.

The European Medicines Agency (EMA) issued two summary reports for veterinary use on '*Cinnamomi ceylanici aetheroleum*' and '*Cinnamomi ceylanici cortex*', the stembark of *Cinnamonum verum* J. Presl (synonym: *C. zeylanicum* Blume) (EMA, 1998, 2000).

For *C. verum* J. Presl, cortex and cortices aetheroleum the European Medicines Agency (EMA) issued a monograph for human medicinal use and an assessment report (EMA, 2011a, b) and an addendum to the assessment report (EMA, 2021).

'Cinnamon' (Cinnamomi cortex) is described in a monograph of the European Pharmacopoeia 10.0 (PhEur, 2020). It is defined as the dried bark, freed from the outer cork and the underlying parenchyma, and shoots grown on cut stock of *C. verum* J. Presl.

The main identified individual component of cinnamon tincture is cinnamaldehyde, a compound identified with the EU Flavour Information System (FLAVIS) number [05.014], which has been assessed for use in feed and food by the FEEDAP Panel and the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), respectively. The genotoxicity of cinnamaldehyde [05.014][7] has been evaluated by EFSA (EFSA, 2009a,b) and is summarised in the EMA assessment report of cinnamon bark (EMA, 2011b). In its assessment of aryl-substituted primary alcohol, aldehyde, ester and acetal derivatives belonging to chemical group 22 (EFSA FEEDAP Panel, 2017a), the FEEDAP Panel concluded that cinnamaldehyde was safe at the maximum use level of 125 mg/kg complete feed for salmonids, veal calves and dogs, and at 25 mg/kg complete feed for the remaining target species. The additive was also considered safe for the consumer and the environment, whereas hazards for skin and eye contact and respiratory exposure were recognised for the majority of the compounds belonging to chemical group 22.

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[8] in support of the authorisation request for the use of cinnamon tincture 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.

The applicant submitted a written agreement to refer to 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 and to refer to the data submitted by FFAC for the assessment of other botanical preparations containing methyleugenol (publications) for the risk assessment of cinnamon tincture belonging to BDG 6.[9]

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 cinnamon tincture. The Executive Summary of the EURL report can be found in Annex A.[10]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of active substance (trade name of the product) is in line with the principles laid down in Regulation (EC) No 429/2008[11] and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 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, 2012a), Guidance on studies concerning the safety of use of the

[7] The configuration of the double bond in cinnamaldehyde [FL-no: 05.014] has not been specified. However, the substance is anticipated to contain more than 97% trans-cinnamaldehyde (EFSA, 2009a).
[8] FEED dossier reference: FAD-2010-0218.
[9] Technical dossier/Supplementary information/Letters dated 29/4/2021 and 26/10/2021.
[10] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0218-BDG06.doc_.pdf
[11] 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.

additive for users/workers (EFSA FEEDAP Panel, 2012b), 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 efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b) 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 Panel, 2021).[12]

3. Assessment

The additive under assessment, cinnamon tincture, is derived from the bark of *C. verum* J. Presl and is intended for use as a sensory additive (functional group: flavouring compounds) in feed for all animal species.

3.1. Origin and extraction

C. verum J. Presl (synonym: *C. zeylanicum*) is an evergreen tree belonging to the Lauraceae and is commonly referred as to Ceylon cinnamon tree or true cinnamon tree. It is native to Sri Lanka and is cultivated in Madagascar, India, Vietnam and Indonesia.

The tincture is produced from the bark of *C. verum* (from Madagascar, India, Indonesia, Vietnam) by extended extraction with a [redacted] mixture, [redacted] for 3 weeks under ambient conditions. After this period the tincture is recovered by pressing to separate solid and liquid phases and the extracted solution is then clarified by filtration.

3.2. Characterisation

3.2.1. Characterisation of the tincture

Cinnamon tincture is a brown liquid, with a characteristic cinnamon odour. It has an average density of 967 kg/m^3 (range: 965–969 kg/m^3) and a pH of 5.20 (5.06–5.33).[13] It is soluble in water.

Table 1 summarises the results of the proximate analysis of five batches of the additive. The solvent represents 99.08% of the additive leaving a dry matter (DM) content of 0.92%. The dry matter consists of ash (11.3% of the DM fraction, on average) and a plant-derived organic fraction (88.7% of the DM fraction), which includes proteins (1.53%), lipids (0.32%) and carbohydrates and fibre (86.8%).

Table 1: Proximate analysis of a tincture derived from the bark of *Cinnamomum verum* J. Presl based on the analysis of five batches (mean and range)

Constituent	Mean	Range
	% (w/w)	% (w/w)
Dry matter	0.92	0.78–1.02
Ash	0.10	0.10–0.11
Organic fraction	0.81	0.68–0.92
Proteins	0.014	0.013–0.015
Lipids	0.003	0.0028–0.0029
'Carbohydrates'(1)	0.80	0.66–0.99
Solvent	99.08	98.98–99.22

(1): 'Carbohydrates' (by difference) include secondary plant metabolites, such as phenolic compounds.

[12] https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf
[13] Technical dossier/Supplementary information February 2020/Annex_II_3_Results of analysis.
[14] Technical dossier/Supplementary information September 2021.

The constituent described as 'carbohydrate' in Table 1 represents the fraction of organic matter remaining after subtraction of the values for protein and lipids. It will contain a variety of plant-derived compounds including phenolic compounds, in addition to any carbohydrate present.

The fraction of secondary metabolites was characterised in the same batches of the tincture and the results are summarised in Table 2. The tincture was shown to contain polyphenols (0.344%) determined by spectrophotometry (at 760 nm) and expressed as gallic acid equivalents, several unidentified flavonoids (0.0008%), separately determined by high-performance thin-layer chromatography (HPTLC) and expressed as quercetin equivalents and cinnamaldehyde (0.0008%) determined by HPTLC at 254 nm using a reference standard.[15]

Table 2: Characterisation of the fraction of secondary metabolites of a tincture derived from the bark of *Cinnamomum verum* J. Presl based on the analysis of five batches (mean and range)

Constituent	Method	Mean	Range
		% (w/w)	% (w/w)
Total polyphenols	Folin–Ciocalteu	0.3440	0.2665–0.3948
Flavonoids	Spectrophotometry	0.0008	0–0.0018
(*E*)-Cinnamaldehyde	HPTLC	0.0008	0.0001–0.0012
Methyleugenol	GC-MS	–	ND[#]–0.00001

HPTLC: high-performance thin-layer chromatography.
[#]: ND: not detected, lower than 0.00001%, corresponding to 0.1 mg/kg.

The applicant performed a literature search to identify substances of concern in *C. verum* and its aqueous and hydroalcoholic extracts.[16] Among the compounds identified, methyleugenol, safrole, 1,8-cineole, camphor and coumarin are reported in the EFSA Compendium of botanicals as substances of concern for the essential oil obtained from the bark of *C. verum* (EFSA, 2012). No information on substances of concern in aqueous hydroalcoholic preparations was retrieved.

The applicant provided analytical data by gas chromatography–mass spectrometry (GC–MS) which showed that safrole and methyleugenol were below the corresponding limit of detection (LOD, 0.2 mg/kg for safrole and 0.1 mg/kg for methyleugenol), except in one batch where trace amounts of methyleugenol were present at the LOD.[14] Although not detected in the samples provided by the applicant, there is evidence from literature that safrole is commonly found in cinnamon bark (Tisserand and Young, 2014). There is no specification defining limit values for undesirable compounds in the tincture.

The identified secondary metabolites account on average for 37.6% of the dry matter content of the tincture and other plant constituents for about 62.4%.

The applicant controls contamination at the level of the raw material (bark). Specifications are set with suppliers covering heavy metals (cadmium < 1 mg/kg, mercury < 0.1 mg/kg and lead < 10 mg/kg) and arsenic (< 2 ppm), and microbial contamination.[17] Three certificates of analysis of the raw material (bark) showing compliance were provided.[18] Analysis of impurities in the tincture apparently is made on irregular basis and does not form part of the Hazard Analysis and Critical Control Points (HACCP) plan.

3.2.2. Stability

The shelf-life of the tincture is declared by the applicant to be at least 36 months when stored in tightly closed containers under standard conditions. No evidence was provided to support this claim.

3.2.3. Conditions of use

The additive is intended for use in feed and water for drinking for all animal species. The applicant proposes a maximum concentration of 50 mg tincture/kg complete feed or 50 mg/kg water for drinking for all animal species except for horses, for which the proposed use level is 60 mg/kg complete feed.

[15] Technical dossier/Supplementary information February 2020/ Section_II_Identity and Annex II_3.
[16] Technical dossier/Supplementary information February 2020/Annex II_6_Bibliographic data.
[17] Technical dossier/Supplementary information February 2020/Annex II_4_TDS_Cinnamon.
[18] Technical dossier/Supplementary information February 2020/Annex_II_5_COA_Cinnamon.

3.3. Safety

The safety assessment is based on the highest proposed use levels, which is 50 mg/kg complete feed for all animal species except for horses, for which the proposed use level is 60 mg/kg complete feed.

No studies to support the safety for target animals, consumers and users were performed with the additive under assessment.

The additive under assessment, cinnamon tincture, consists of 99.08% of a water/ethanol mixture. The concentration of plant derived compounds is about 0.92% of the tincture. The dry matter included ash, protein, lipids and carbohydrates, which are not of concern and are not further considered.

Among the secondary plant metabolites, phenolic compounds including flavonoids were quantified but not individually identified. They will be assessed based on considerations at the level of the assessment group (see Section 3.3.3). These compounds will be readily metabolised and excreted and are not expected to accumulate in animal tissues and products.

The additive contains (*E*)-cinnamaldehyde, a compound belonging to Chemical Group 22. Cinnamaldehyde [05.014] has been evaluated by EFSA for use as a flavour in food (EFSA, 2009a,b) and feed (EFSA FEEDAP Panel, 2017a) and is currently authorised for food[19] and feed[20] uses. It is rapidly absorbed and metabolised mainly into hippuric acid. After administration of cinnamaldehyde to rats, benzoic acid and cinnamic acid were found in urine samples. The genotoxicity of cinnamaldehyde has been evaluated by EFSA (EFSA, 2009a,b) and is summarised in the EMA assessment report (EMA, 2011b). EFSA concluded that 'Some concern could be raised by studies carried out with cinnamaldehyde [FL-no: 05.014], showing an ability to induce chromosomal damage *in vitro*, and by the positive result obtained for 2-methoxycinnamaldehyde [FL-no: 05.048] in an Ames test. For cinnamaldehyde the concern was not confirmed in *in vivo* studies. Thus, it is concluded that cinnamaldehyde does not have a genotoxic potential *in vivo*. In addition, the carcinogenicity studies with trans-cinnamaldehyde did not indicate a carcinogenic potential'.

Trace amounts (0.1 mg/kg) of methyleugenol, a compound with experimentally proven genotoxicity and carcinogenicity in rodents (as reviewed in EMA, 2005; IARC, 2018) were detected in one batch of the additive. Although not detected in the samples provided by the applicant, there is evidence from literature that safrole is commonly found in cinnamon bark. Information on the absorption, distribution, metabolism and excretion and on the toxicology of methyleugenol and safrole is summarised in the next sections.

3.3.1. Absorption, distribution, metabolism and excretion of methyleugenol and safrole

Methyleugenol is a highly lipophilic compound and as such readily and completely absorbed from the gastrointestinal tract. Phase I metabolism is catalysed by cytochromes P450 (CYP450) enzymes mainly in the liver. Demethylation of the 4-methoxygroup with formation of 4-allylphenol is followed by conjugation with glucuronic acid or sulfate and renal excretion. Oxidation of the allyl-side chain leads to methyleugenol-2',3'-epoxide, which is hydrolysed to the corresponding diol with subsequent glucuronidation and excretion. Both metabolic pathways represent detoxification of methyleugenol. The formation of genotoxic metabolites is initiated by oxidation of the side chain with formation of 1'-hydroxy-methyleugenol. Sulfate-conjugation of the hydroxyl group leads to 1'-sulfooxymethyleugenol, which is capable of disintegration to form a highly reactive carbonium ion, which can react covalently with DNA (as reviewed in EMA, 2005, IARC, 2018). The occurrence of DNA-adducts of methyleugenol in liver samples obtained at liver surgery of humans as a result of exposure to this compound via normal food has been demonstrated (Herrmann et al., 2013).

The same metabolic pathways have been described for safrole (European Commission, 2002; WHO, 2009) and other structurally related *p*-allylalkoxybenzenes.

[19] 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.

[20] 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

3.3.2. Toxicology of methyleugenol and safrole

Methyleugenol and safrole are compounds with experimentally proven genotoxicity and carcinogenicity in rodents (as reviewed in EMA, 2005; IARC, 2018; European Commission, 2002; WHO, 2009).

Methyleugenol

Methyleugenol was not mutagenic in the bacterial mutagenicity assay with *Salmonella* Typhimurium and *Escherichia coli* WP-*uvrA* in the presence and absence of S9-mix. However, positive results were obtained in a modified strain of *Salmonella* Typhimurium (TA100-hSULT1C2) expressing sulfotransferase (Honda et al., 2016), indicating that the formation of sulfate esters plays a key role in the genotoxicity of alkenylbenzenes. In Chinese hamster ovary (CHO) cells, sister chromatid exchange (SCE) was induced by methyleugenol exposure in the presence and absence of microsomal activation and chromosomal aberrations only in the presence of microsomal activation (NTP, 2000). The induction of malignant transformation by methyleugenol was demonstrated in Syrian hamster ovary cells (Kerkaert et al., 1996). DNA repair was induced by methyleugenol in primary hepatocytes from rats and mice (Howes et al., 1990; Chan and Caldwell, 1992; Burkey et al., 2000). The DNA damaging effects could be inhibited by addition of sulfotransferase inhibitors. DNA adducts were detected after i.p. injection of methyleugenol in the livers of female CD-1 mice and treatment of human HepG2 cells *in vitro* with methyleugenol (Zhou et al., 2007).

The carcinogenicity of methyleugenol was investigated in a 2-year National Toxicology Program (NTP) carcinogenicity study in rats and mice (NTP, 2000) using doses of 0, 37, 75, or 150 mg/kg body weight (bw) per day in both species and a higher dose of 300 mg/kg bw per day in rats. Rats of both sexes receiving methyleugenol had dose-related increased incidences of hepatocarcinoma and neuroendocrine tumours of the glandular stomach. Higher incidences of kidney neoplasms, malignant mesothelioma, mammary gland fibroadenoma and subcutaneous fibroma and fibrosarcoma were observed in male rats only. Increased incidence of hepatocarcinoma was seen in both sexes of mice although the incidence was not related to dose. Neuroendocrine tumours of the glandular stomach were also observed in male mice but only at the highest dose. The NTP concluded that there was clear evidence for the carcinogenicity of methyleugenol in rats and mice.

Suparmi et al. (2019) performed an evaluation of the available evidence using the benchmark dose (BMD) approach and found that the application of the appropriate dose-response modelling on the long-term chronic toxicity study (NTP, 2000) using hepatocellular carcinomas in male rats as a response, yielded a BMD lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) as 22.2 mg/kg bw per day applying model averaging, as recommended by the EFSA Scientific Committee (EFSA Scientific Committee, 2017).

Safrole

Safrole was included in the diet of female CD-1 mice at 0, 2.5 and 5.0 g/kg diet (corresponding to 58 and 117 mg/kg bw per day) for 12 months. At least 70% of the animals in the exposed groups developed hepatic tumours by 18 months, which were diagnosed as hepatoma types A (hepatocellular adenomas) or B (hepatocellular adenocarcinomas) or mixed types A and B. The animals which were fed with the control diet did not show any hepatic tumour (Miller et al., 1983).

van den Berg et al. (2011) performed an evaluation of the available evidence using the benchmark dose (BMD) approach and found that the application of the appropriate dose-response modelling on the long-term chronic toxicity study (Miller et al., 1983) using hepatocellular carcinomas as a response, yielded a BMD lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) of 1.9 mg safrole/kg bw per day.

3.3.3. Safety for the target species

In the absence of tolerance studies and/or toxicity data from repeated dose studies in laboratory animals performed with the additive under assessment or its individual components, the threshold of toxicological concern (TTC) is applied to derive maximum safe feed concentrations for the known individual components of the tincture (EFSA FEEDAP Panel, 2017b).

Cinnamaldehyde

At the maximum proposed use level of 50 mg tincture/kg in feed, the concentration of cinnamaldehyde (on average 0.0008%, up to 0.0011%, measured by HPTLC) would be 0.4 μg/kg on

average and up to 0.6 μg/kg feed. The corresponding figures for horses are 0.5 and 0.7 μg/kg feed. These concentrations are several orders of magnitude below the concentrations in feed which were considered safe by the FEEDAP Panel in its opinion on chemical group 22, i.e. 125 mg/kg complete feed for salmonids, veal calves and dogs, and at 25 mg/kg complete feed for the remaining target species (EFSA FEEDAP Panel, 2017a) and therefore no concern for the target species is expected.

Phenolic compounds including flavonoids

At the maximum proposed use level of 50 mg tincture/kg in feed, the concentration of the total phenolic fraction (on average 0.344%, up to 0.395%, measured by the Folin–Ciocalteu method) would be 0.17 mg/kg on average and up to 0.20 mg/kg feed. Since phenolic acids are assigned to Cramer Class I and the data indicate that they would not exceed the relevant threshold value (ranging from 0.3 mg/kg feed for poultry to 1.5 mg/kg feed for salmonids and dogs), no concern for the target species arises from the phenolic fraction. For horses, the concentration of the phenolic fraction would be 0.21 mg/kg on average and up to 0.24 mg/kg feed, which is also below the threshold value for TTC (1.2 mg/kg feed, EFSA FEEDAP Panel, 2017c) and is considered of no concern.

Concerning flavonoids (on average 0.0008%, up to 0.0018%), their concentration in feed would be 0.4 μg/kg on average and up to 0.9 μg/kg feed at the proposed use level of 50 mg/kg (or 0.5 and up to 1.1 μg/kg in feed for horses) and would be at least 3 orders of magnitude lower than the threshold value for Cramer Class III (ranging from 0.02 mg/kg feed for poultry to 0.08 mg/kg feed for salmonids and dogs). Therefore, the presence of flavonoids is not considered of concern for the target species.

Methyleugenol and safrole

Trace amounts of methyleugenol (LOD 0.1 mg/kg) were detected in one out of five batches of the additive. In the other four batches, methyleugenol was below the LOD (0.1 mg/kg) and safrole was below its LOD (0.2 mg/kg) in all the five batches.

At the maximum proposed use level of 50 mg tincture/kg in feed, a concentration of methyleugenol in the additive corresponding to the LOD would lead to a concentration of 5 ng/kg complete feed (6 ng/kg for horses at the use level of 60 mg tincture/kg complete feed). The intake of the target animals (as μg/kg bw per day) at the maximum proposed use level of 50 mg/kg complete feed would range between 0.03 and 0.45 ng methyleugenol/kg bw per day (0.14 ng methyleugenol/kg bw per day for horses).

If safrole is present at a concentration corresponding to the LOD, the corresponding figures for the concentration in complete feed would be 10 ng safrole/kg tincture (12 ng/kg for horses). The intake of the target animals (as μg/kg bw per day) would range between 0.05 and 0.90 ng safrole/kg bw per day (0.27 ng safrole/kg bw per day for horses).

Since methyleugenol and safrole share the same mode of action, they are allocated to the same assessment group (*p*-allylalkoxybenzenes) (EFSA Scientific Committee, 2019a) and an assessment of the combined exposure is performed (Appendix A).

When the estimated exposures for the different animal categories are compared to the corresponding $BMDL_{10}$ of 22.2 mg methyleugenol/kg bw per day (Suparmi et al., 2019), and of 1.9 mg safrole/kg bw per day (van den Berg et al., 2011) calculated from rodent carcinogenicity studies (NTP, 2000; Miller et al., 1993, see Section 3.3.2), a combined margin of exposure (MOET) of at least 2,029,000 is calculated (> 6,680,000 for horses) (Appendix A). The magnitude of this MOET is indicative of a low concern for the target species.

The applicant proposed a maximum use level in water for drinking of 50 mg/kg for all animal species except for horses. 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 which is considered safe when consumed via feed (EFSA FEEDAP Panel, 2010).

3.3.3.1. Conclusions on safety for the target species

The additive under assessment, cinnamon tincture, is safe up to the maximum proposed use level of 50 mg/kg complete feed for all animal species except for horses. For horses, the safe concentration in feed is 60 mg/kg complete feed.

The FEEDAP Panel considers that the use of the additive in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount which is considered safe when consumed via feed.

3.3.4. Safety for the consumer

The bark of *C. verum* and its preparations including extracts and tinctures are added to a wide range of food categories as spice or for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites values of 3.02 mg/kg bw per day for cinnamon and its preparations.

No data on residues in products of animal origin following the use of the tincture under assessment were made available. The phenolic compounds, including flavonoids, present in the additive at concentrations below the thresholds for Cramer Class I compounds or Cramer Class III compounds, respectively, will be readily metabolised and excreted and are not expected to accumulate in animal tissues and products. For cinnamaldehyde, the available data indicate that it is absorbed, metabolised and rapidly excreted and is not expected to accumulate in animal tissues and products. Also for methyleugenol, detected in trace amounts in one batch of the additive, and safrole, not detected but possibly occurring a concentration corresponding to the LOD, the available data indicate that it is absorbed, metabolised and rapidly excreted and is not expected to accumulate in animal tissues and products (see Section 3.3.1). Therefore, a relevant increase of the uptake of the individual constituents by humans consuming products of animal origin is not expected.

Considering the reported human exposure due to direct use of cinnamon bark and its preparations in food (Burdock, 2009), it is unlikely that consumption of products from animals given cinnamon tincture at the proposed maximum use level would increase human background exposure.

Consequently, no safety concern would be expected for the consumer from the use of cinnamon tincture up to the highest proposed 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 provided information according to Classification, Labelling and Packaging (CLP) Regulation (EC) 1272/2008[21] concerning the presence of ethanol in the tincture.[22]

The additive contains cinnamaldehyde, a compound for which hazards for skin and eye contact and respiratory exposure were recognised (EFSA FEEDAP Panel, 2017a).

The additive under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser.

3.3.6. Safety for the environment

C. verum J. Presl is not native to Europe. However, owing to the very low concentration of cinnamaldehyde and polyphenols, components which are present in many plants indigenous Europe, the use of the tincture derived from *C. verum* as a flavour in animal feed is not expected to pose a risk for the environment.

3.4. Efficacy

Cinnamon bark from *C. verum* J. Presl and its preparations are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by the Flavour and Extract Manufactures Association (FEMA) with the reference numbers 2289 (cinnamon), 2290 (cinnamon bark extract) and 2291 (cinnamon bark oil).

Since cinnamon bark from *C. verum* J. Presl and its preparations are 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 for the tincture under application.

4. Conclusions

Since cinnamon tincture from *C. verum* J. Presl may be produced from plants of different geographical origins and by various processes resulting in preparations with different composition and toxicological profiles, the following conclusions apply only to cinnamon tincture which contains $\leq$ 0.00001% methyleugenol and $<$ 0.00002% safrole and is produced from the bark of *C. verum*.

[21] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

[22] H319: causes serious eye irritation (relevant for dermal exposure).

The FEEDAP Panel concludes that the additive is safe at the maximum proposed use level of 50 mg/kg complete feed for all animal species except for horses. For horses, the maximum proposed use level of 60 mg/kg complete feed is considered safe. 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 which is considered safe when consumed via feed.

No safety concern would arise for the consumer from the use of cinnamon tincture in animal nutrition up to the highest proposed use levels in feeds.

The additive under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser.

The use of cinnamon tincture at the maximum proposed use level is not considered to be a risk for the environment.

Since cinnamon bark from *C. verum* J. Presl and its preparations are 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 for the tincture under application.

5. Recommendation

The specification should ensure that the methyleugenol and safrole concentrations should be as low as possible and should not exceed 0.1 mg/kg tincture and 0.2 mg/kg tincture, respectively.

6. Documentation provided to EFSA / Chronology

Date	Event
05/11/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 06 - Laurales, Magnoliales, 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. 7150727) 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
27/06/2013	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives- Scientific assessment remains suspended
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*
13/02/2020	Reception of supplementary information from the applicant (partial submission)
12/03/2021	The application was split and a new EFSA-Q-2021-00133 was assigned to the preparation included in the present assessment. Scientific assessment re-started for the preparation included in the present assessment
01/09/2021	Reception of supplementary information from the applicant (partial submission)
26/10/2021	Reception of supplementary information from the applicant (partial submission)
10/11/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment for the preparation included in the present assessment

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Abbreviations

CAS	Chemical Abstracts Service
CD	Commission Decision
CDG	chemically defined group
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
DM	dry matter
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
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–MS	gas chromatography–mass spectrometry
HACCP	hazard anaylsis and critical control points
HPTLC	high performance thin layer chromatography
LOD	limit of detection
LOQ	limit of quantification
MOE	Margin of exposure
MOET	Combined margin of exposure
NOAEL	no observed adverse effect level
TTC	threshold of toxicological concern
UF	uncertainty factor

Appendix A – Methyleugenol and safrole: maximum daily intake and combined margin of exposure for the different target species

The maximum daily intake of methyleugenol and safrole was calculated for chickens for fattening, the species with the highest ratio of feed intake/body weight, based on

- the default values for body weight and feed intake (EFSA FEEDAP Panel, 2017b)
- the maximum proposed use level of the additive in feed for the different target animal categories (50 mg/kg complete feed and 60 mg/kg for horses) and
- assuming that methyleugenol and safrole are both present at concentrations equal to the corresponding limit of detection (0.1 mg methyleugenol/kg tincture and 0.2 mg safrole/kg tincture).

According to the General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021),[12] 'for substances for which carcinogenicity studies in rodents are available, from which a $BMDL_{10}$ can be derived, the MOE approach (EFSA, 2005; EFSA Scientific Committee, 2012) can be applied. Similarly to human risk assessment, a combined (total) margin of exposure (MOET) with a magnitude of $\geq$ 10,000, when comparing estimated exposure to genotoxic and/or carcinogenic substances with a $BMDL_{10}$ from a rodent carcinogenicity study, would be indicative of a low concern for the target species (EFSA Scientific Committee, 2019a)'.

Since methyleugenol and safrole share the same structural features and the same mode of action, they are allocated to the same assessment group (p-allylalkoxybenzenes) and an assessment of the combined exposure is performed as described in the Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a).

The MOE for each component is calculated as the ratio of the reference point (the $BMDL_{10}$ of 22.2 mg methyleugenol/kg bw per day and of 1.9 mg safrole/kg bw per day, see Section 3.3.1) to the intake. The combined margin of exposure (MOET) is calculated for the assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

The assessment of the combined exposure to methyleugenol and safrole for chickens for fattening is reported in Table A.1.

Table A.1: Compositional data, intake values (calculated for chickens for fattening at 50 mg/kg complete feed), reference points and margin of exposure (MOE) for methyleugenol and safrole (if present in the additive at the corresponding limit of detection), and combined margin of exposure (MOET) for the assessment group *p*-allylalkoxybenzenes

Composition		Exposure		Hazard characterisation	Risk characterisation	
Assessment group	**Max conc. in the tincture**	**Max Feed conc.**	**Intake[(a)]**	**$BMDL_{10}$**	**MOE**	**MOET**
Constituent	**mg/kg**	**µg/kg**	**µg/kg bw per day**	**mg/kg bw per day**	–	–
Methyleugenol	0.1	0.005	0.0004	**22.2**	49,458,228	
Safrole	0.2	0.010	0.0009	**1.9**	2,116,456	
MOET						2,029,603

bw: body weight; $BMDL_{10}$: BMD lower confidence limit for a benchmark response of 10%.

(a): Intake calculations for the individual components are based on the use level of 50 mg/kg in feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point ($BMDL_{10}$) to the intake. The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

From the MOET for chickens for fattening, the MOET for *p*-allylalkoxybenzenes was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table A.2.

Table A.2: Combined exposure and combined margin of exposure (MOET) for the assessment group *p*-allylalkoxybenzenes calculated at the maximum proposed use level of the additive in feed for target animal category

Animal category	Daily feed intake	Body weight	Use level	Combined Intake	MOET
	kg DM/day	kg	mg/kg	ng/kg bw per day	–
Chicken for fattening	0.158	2	50	1.347	2,029,603
Laying hen	0.106	2	50	0.903	3,025,258
Turkey for fattening	0.176	3	50	1.000	2,717,604
Piglet	0.88	20	50	0.750	3,644,060
Pig for fattening	2.2	60	50	0.625	4,333,477
Sow lactating	5.28	175	50	0.514	5,344,622
Veal calf (milk replacer)	1.89	100	50	0.300	8,438,877
Cattle for fattening	8	400	50	0.341	8,016,933
Dairy cow	20	650	50	0.525	5,172,215
Sheep/goat	1.2	60	50	0.341	8,016,933
Horse	8	400	60	0.409	6,680,778
Rabbit	0.1	2	50	0.852	3,206,773
Salmon	0.0021	0.12	50	0.298	8,907,703
Dog	0.25	15	50	0.284	9,431,686
Cat	0.06	3	50	0.341	8,016,933
Ornamental fish	0.00054	0.012	50	0.077	32,067,732

bw: body weight.

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis eighteen compounds from botanically defined Group 06 (Laurales, Magnoliales, Piperales)

The *Botanically Defined Flavourings – Group 6 BDG 06* (*Laurales, Magnoliales, Piperales)* is an application comprising *eighteen flavouring compounds* (*) for which authorisation as feed additive is sought under the category/functional group 2(b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003. In the current application submitted according to Articles 4(1) and 10(2) of Regulation (EC) No 1831/2003, the authorisation for all species and categories is requested. *Mixtures of flavouring compounds* are intended to be incorporated only into *feedingstuffs* or drinking water. The Applicant suggested no minimum or maximum levels for the different *flavouring compounds*, but normal contents of flavouring compounds in *feedingstuffs* range up to from 0.1 to 100 mg/kg.

For the identification of volatile phytochemical markers in the *feed additive,* the Applicant submitted a qualitative multi-analyte gas-chromatography mass-spectrometry (GC-MS) method, using Retention Time Locking (RTL), which allows a close match of retention times on GC-MS. By making an adjustment to the inlet pressure, the retention times can be closely matched to those of a reference chromatogram. It is then possible to screen samples for the presence of target compounds using a mass spectral database of RTL spectra. The Applicant provided the typical chromatogram for the *BDG 06* of interest. In order to demonstrate the transferability of the proposed analytical method (relevant for the method verification), the Applicant tested two model premixtures of twenty chemically defined flavourings representing the whole spectrum of compounds in use as feed flavourings with respect to their volatility and polarity. All twenty substances were extracted either from a liquid premixture or a solid premixture, and subsequently analysed using the same GC/MS method. All twenty model substances were properly identified. Since the volatile phytochemical markers of *BDG 06* are within the volatility and polarity range of the model mixture tested, the Applicant concluded that the proposed analytical method is suitable to determine qualitatively the presence of the volatile phytochemical markers from *BDG 06* in the *mixture of flavouring compounds*.

For the qualitative identification of non-volatile phytochemical markers (*boldine*, *kavain* and *piperine*) in *mixture of flavouring compounds*, the Applicant submitted High-Performance Liquid Chromatography methods with UV detection (HPLC-UV), together with the ISO 11027 standard method for the determination of piperine.

Based on the satisfactory experimental evidence provided, the EURL recommends for official control for the qualitative identification in the *feed additive* of the individual (or mixture of) *flavouring compounds* of interest (*) the GC-MS-RTL and HPLC-UV methods submitted by the Applicant.