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SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of a tincture derived from the leaves of *Eucalyptus globulus* Labill. (eucalyptus tincture) for all animal species (FEFANA asbl)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of a tincture from the leaves of *Eucalyptus globulus* Labill. (eucalyptus tincture) when used as a sensory additive for all animal species. The product is a [redacted] solution, with a dry matter content of ~ 1.86%, which contains on average 0.454% phenolic acids and flavonoids (of which 0.280% was gallic acid), 0.0030% 1,8-cineole and 0.00012% methyleugenol. In the absence of analytical data on the occurrence of mono- or diformylated adducts of acylphloroglucinols with terpenes in the tincture and in the absence of toxicity data, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) could not conclude on the use of eucalyptus tincture for long-living and reproductive animals. For short-living animals (species for fattening), the additive was considered of no concern at 4 mg/kg complete feed for chickens for fattening, 5 mg/kg for turkeys for fattening, 6 mg/kg for piglets and rabbits for meat production, 7 mg/kg for pigs for fattening, 16 mg/kg for veal calves (milk replacer), 14 mg/kg for cattle for fattening, sheep/goats and horses for fattening, and 15 mg/kg for salmonids. These levels were extrapolated to physiologically related minor species. No safety concern would arise for the consumer from the use of eucalyptus tincture up to the levels in feed considered of no concern. Eucalyptus tincture should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser. The use of eucalyptus tincture as a flavour in animal feed was not expected to pose a risk for the environment. Since the leaves of *E. globulus* and their preparations were recognised to flavour food and their function in feed would be essentially the same, no demonstration of efficacy was considered necessary.

KEYWORDS

1,8-cineole, *Eucalyptus globulus* Labill., eucalyptus tincture, flavouring compounds, gallic acid, methyleugenol, sensory additives

CONTENTS

Abstract 1
1. Introduction 3
1.1. Background and Terms of Reference 3
1.2. Additional information 3
2. Data and methodologies 3
2.1. Data 3
2.2. Methodologies 4
3. Assessment 4
3.1. Origin and extraction 4
3.2. Uses other than feed flavouring 5
3.3. Characterisation 5
3.3.1. Characterisation of eucalyptus tincture 5
3.3.1.1. Substances of concern 6
3.3.1.2. Impurities 6
3.3.2. Stability 7
3.3.3. Conditions of use 7
3.4. Safety 7
3.4.1. Genotoxicity and carcinogenicity of methyleugenol 8
3.4.2. Safety for the target species 8
3.4.2.1. 1,8-Cineole 8
3.4.2.2. Total phenolic compounds 9
3.4.2.3. Methyleugenol 12
3.4.2.4. Use in water for drinking 13
3.4.2.5. Conclusions on safety for the target species 13
3.4.3. Safety for the consumer 13
3.4.4. Safety for the user 13
3.4.5. Safety for the environment 13
3.5. Efficacy 14
4. Conclusions 14
5. Recommendation 14
6. Documentation provided to EFSA/chronology 14
Abbreviations 15
Acknowledgements 16
Conflict of interest 16
Requestor 16
Question number 16
Copyright for non-EFSA content 16
Panel members 16
Legal notice 16
References 16

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. 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 7 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 additives (namely geranium oil, geranium rose oil, eucalyptus oil, eucalyptus tincture, clove oil, clove tincture, broom teatree oil, purple loosestrife tincture, tea tree oil, melaleuca cajuputi oil, niaouli oil, allspice oil, bay oil, pomegranate bark extract, bambusa tincture, citronella oil, lemongrass oil and vetiveria oil) belonging to botanically defined group (BDG) 07 – Geraniales, Myrtales, Poales when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). During the assessment, the applicant withdrew the application for six additives.[3] These additives were deleted from the register of feed additives.[4] During the course of the assessment, this application was split and the present opinion covers only one out of the remaining 12 additives under application: eucalyptus tincture from *Eucalyptus globulus* Labill.[5] for all animal species.

The remaining 11 additives belonging to botanically defined group (BDG) 07 – Geraniales, Myrtales, Poales under application are assessed in separate opinions.

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 21 December 2010.

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 the product eucalyptus tincture (*E. globulus*), when used under the proposed conditions of use (see Section 3.3.3).

1.2 | Additional information

A tincture from *E. globulus* Labill. (eucalyptus 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.

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[6] in support of the authorisation request for the use of eucalyptus tincture from *E. globulus* as a feed additive. The dossier was received on 26/3/2024 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2024-00192.[7]

The FEEDAP Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 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.

[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 13/3/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1050 Brussels, Belgium.
[3]Broom teatree oil, geranium oil, bay oil and vetiveria oil (27 February 2019); bambusa tincture and allspice oil (18 November 2022).
[4]Register of feed additives, Annex II, withdrawn by OJ L162, 10.5.2021, p. 5.
[5]Accepted name.
[6]FEED dossier reference: FAD-2010-0219.
[7]The original application EFSA-Q-2010-01282 was split on 26/3/2024 and a new EFSA-Q-2024-00192 was generated.

Some of the components of the tincture 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 additives belonging to BDG 07, including the current one under assessment.[8]

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 the additive. The evaluation report is related to the methods of analysis for each feed additive included in the group BDG 07 (Geraniales, Myrtales, Poales). During the assessment, upon request from EFSA, the EURL issued two amendments[9] of the original report. The additive under assessment, eucalyptus tincture, is included in the second amendment. In particular, the EURL recommended methods based on (i) spectrophotometry for the determination of *total polyphenols* and *flavonoids*, (ii) gas chromatography coupled with flame ionisation detection (GC-FID) for the determination of *1,8-cineole* (eucalyptol, the phytochemical marker) and (iii) high-performance thin-layer chromatography (HPTLC) for the determination of the phytochemical marker gallic acid in *eucalyptus tincture*.[10]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of eucalyptus tincture from *E. globulus* is in line with the principles laid down in Regulation (EC) No 429/2008[11] and the relevant guidance documents: Opinion of the Scientific Committee on harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic (EFSA, 2005), Statement on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed (EFSA Scientific Committee, 2012), 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 the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), 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 on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023), 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), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee, 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 Panel, 2021a).[12]

3 | ASSESSMENT

The additive under assessment, eucalyptus tincture, is derived from the leaves of *E. globulus* Labill. and 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

E. globulus Labill. is a fast-growing evergreen tree native to Australia belonging to the myrtle (Myrtaceae) family. The species is commonly known as the southern blue gum tree or simply the blue gum tree in reference to the glaucous colour of the adult leaves. There are four recognised sub-species arising from different geographical locations within Australia, each with a locally associated name (e.g. Tasmanian blue gum, Maidan's gum). *E. globulus* is now grown commercially in many countries.

The tincture is produced from the dried leaves by extended extraction for [redacted] under ambient conditions with a [redacted] and a plant to solvent ratio of [redacted]. The tincture is then recovered by pressing to separate solid and liquid phases and the extracted solution is then clarified by filtration.

[8]Technical dossier/Supplementary information February 2023/Letter dated 31/1/2023.

[9]Additives included in the first amendment: geranium rose oil, eucalyptus oil, lemongrass oil and clove oil; additives included in the second amendment: citronella oil, melaleuca cajuputi oil, tea tree oil, clove tincture and eucalyptus tincture.

[10]The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0219_en.

[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.

[12]https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf.

3.2 | Uses other than feed flavouring

While there is no specific EU authorisation for any *E. globulus* preparation when used to provide flavour in food, according to Regulation (EC) No 1334/2008[13] flavouring preparations produced from food, may be used without an evaluation and approval.

An essential oil produced from the leaves of *E. globulus* is used to flavour food. Apart from an herbal tea prepared from the leaf, there are no further food uses for *E. globulus* itself.

'Eucalyptus leaf (Eucalypti folium)' and 'Eucalyptus oil (Eucalypti aetheroleum)' from *E. globulus* Labill. are described in monographs of the European Pharmacopoeia 11.0 (PhEur, 2022a, 2022b) and of the European Medicines Agency (EMA, 2013a, 2013b, 2014a, 2014b, 2022) for medicinal uses. Eucalyptus oil has been also evaluated for veterinarian uses (EMA, 1998).

3.3 | Characterisation

3.3.1 | Characterisation of eucalyptus tincture

Eucalyptus tincture is a brown liquid, with a characteristic fresh, camphorated and mentholated odour which is characteristic of 1,8-cineole (synonym: eucalyptol). It has an average density of 956 kg/m^3 (range: 955–957 kg/m^3) and a pH of 5.22 (5.20–5.29).[14]

Table 1 summarises the results of proximate analysis of five batches of the additive.[15] The solvent represents about 98.1% of the additive leaving a dry matter (DM) content of about 1.9%. The DM consists of inorganic material measured as ash (5.1%) and a plant-derived organic fraction of 94.9%, which includes protein, lipids and 'carbohydrates', described as the fraction of organic matter remaining after subtraction of the values for protein and lipids. It contains a variety of plant-derived compounds including phenolic compounds, in addition to any carbohydrate present.

TABLE 1 Proximate analysis of a tincture derived from the leaves of *Eucalyptus globulus* Labill. based on the analysis of five batches.

Constituent	Mean % (w/w)	Range % (w/w)
Dry matter	1.86	1.77–1.98
Ash	0.09	0.07–0.11
Organic fraction	1.76	1.66–1.91
Proteins	0.50	0.24–0.30
Lipids	< 0.50	< 0.50
'Carbohydrates+fibre'[a]	0.76	0.33–1.00
Solvent	98.14	98.02–98.23

[a]'Carbohydrates + fibre' (by difference) include secondary plant metabolites, such as phenolic compounds.

The fraction of secondary metabolites was characterised in the same batches of the tincture and the results expressed as % (w/w)[16] are summarised in Table 2. The tincture was shown to contain total phenolic compounds (≤ 0.491%) determined by spectrophotometry (at 760 nm) and expressed as gallic acid equivalents (GAE).[17] Several unidentified phenolic acids and flavonoids were detected by HPTLC.[18] The concentration of gallic acid (≤ 0.303%) was determined by HPTLC and that of ellagic acid (≤ 0.018%) by high-performance liquid chromatography (HPLC). The concentration of flavonoids (≤ 0.032% expressed as quercetin equivalents) was determined by spectrophotometry at 415 nm (PhEur 10.0, p. 1627).[19] The concentration of 1,8-cineole [03.001] was determined by GC-FID in the same five batches of the tincture.[20]

[13]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.

[14]Technical dossier/Supplementary information October 2022/Annex_II_4_Results of analysis.

[15]Technical dossier/Supplementary information October 2022/Section II_ Identity and Annex_II_4_Results of analysis.

[16]For each batch, the values analysed in each individual batch and expressed as mg/mL were converted into % (w/w) considering the value of the density determined for each individual batch.

[17]Technical dossier/Supplementary information October 2022/Section_II_Identity and Annex II_4_Results of analysis.

[18]Technical dossier/Supplementary information October 2022/Annex II_8_Detailed report of gallic acid HPTLC.

[19]Technical dossier/Supplementary information May 2023.

[20]Technical dossier/Supplementary information October 2022/Annex II_7_Certificate of analysis of 1,8-cineole in Eucalyptus tincture.

TABLE 2 Characterisation of the fraction of secondary metabolites of a tincture derived from the leaves of *Eucalyptus globulus* Labill. based on the analysis of five batches (mean and range).

Constituent	Method	Mean % (w/w)	Range % (w/w)
Total phenolic compounds[a]	Folin–Ciocalteu	0.454	0.404–0.491
Gallic acid	HPTLC	0.280	0.262–0.303
Ellagic acid	HPLC	0.016	0.014–0.018
Flavonoids[b]	Spectrophotometry[c]	0.030	0.028-0.032
1,8-Cineole	GC-FID	0.0030	0.0023–0.0036
Methyleugenol	GC-FID	0.00012	0.00011–0.00012

Abbreviations: HPTLC, high-performance thin-layer chromatography; HPLC, high-performance liquid chromatography; GC-FID, gas chromatography-flame ionisation detector.

[a]Expressed as gallic acid equivalents (GAE).

[b]Expressed as quercetin equivalents.

[c]Determined by an internal method based on European Pharmacopoeia (PhEur, 2022c): chapter 2.8.14, Determination of tannins in herbal drugs.

The identified secondary metabolites account on average for 24.6% of the DM content of the tincture (range: 22.5%–25.3%).

According to existing monographs (PhEur, 2022a; PhEur Commentary, 2022; EMA, 2013a), the dried leaves from *E. globulus* Labill. are known to contain a fraction of phenolic compounds in addition to the occurrence of essential oil (1%–3%, main component 1,8-cineole) and of triterpenoids, such as derivatives of ursolic and oleanolic acids. The phenolic fraction consists of (i) mono- or diformylated adducts of acylphloroglucinols with terpenes (typical for the genus *Eucalyptus*), such as euglobals, macrocarpals and eucalypton (ii) hydrolysable tannins, especially of the ellagitannin type (iii) monoterpenoid galloyl-glucose-derivatives, such as eucaglobulin, cypellocarpin, globulusin, (iv) condensed tannins (proanthocyanidins), (v) flavonoids, especially quercetin derivatives (vi) phenolic acids (not abundant with the exception of ellagic acid) (e.g. PhEur Commentary, 2022; EMA, 2013a).

3.3.1.1 | *Substances of concern*

The applicant performed a literature search to identify substances of concern in *E. globulus* and its botanical preparations, essential oils and aqueous and hydroalcoholic extracts.[21] Among the compounds identified in the essential oil from the leaves of *E. globulus*, 1,8-cineole (up to 82.2%) is reported in the EFSA Compendium of botanicals as substance of concern (EFSA, 2012).[22] 1,8-Cineole is the main constituent of eucalyptus oil and is an authorised flavouring. The presence of methyleugenol (3.5%) in an essential oil from the leaves of *E. globulus* has been reported in one reference (Vieira et al., 2017). No information on the occurrence of substances of concern in aqueous alcoholic preparations was retrieved.

The applicant provided analytical data by GC-FID on the content of 1,8-cineole (23.0–35.6 mg/kg)[23] and methyleugenol (1.10–1.21 mg/kg)[24] in five batches of the additive (see Table 2). There is no specification defining limit values for undesirable compounds in the tincture.

Analytical data on the occurrence in the tincture of mono- or diformylated adducts of acylphloroglucinols with terpenes, which are considered of toxicological relevance, were not provided. In a worst-case scenario, it is assumed that the estimated maximum concentration of mono- or diformylated adducts of acylphloroglucinols with terpenes in the tincture would correspond to the highest analysed concentration for total phenolic compounds of 0.491% (w/w).

3.3.1.2 | *Impurities*

The applicant controls contamination at the level of the raw material, including knowledge of the cultivation conditions and pesticides applied. Specifications are set with suppliers covering cadmium, mercury, lead and arsenic, dioxins, mycotoxins, pesticides, and microbial contamination.[25] A certificate of analysis of the raw material (eucalyptus leaves) showing compliance with specifications was provided.[26] Analysis of impurities in the tincture is made on irregular basis and does not form part of the Hazard Analysis and Critical Control Points (HACCP) plan.

[21]Technical dossier/Supplementary information October 2022/Annex II_5_Bibliographic data.

[22]Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals.

[23]Technical dossier/Annex_II_7_Certificate of analysis 1,8-cineole in Eucalyptus tincture.

[24]Technical dossier/Supplementary information May 2023.

[25]Technical dossier/Section II.

[26]Technical dossier/Supplementary information October 2022/Annex_II_2_ Certificate of analysis for plant raw material.

3.3.2 | 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.

3.3.3 | Conditions of use

The additive is intended for use in feed and in water for drinking for all animal species. The applicant proposes a maximum concentration of 50 mg eucalyptus tincture/kg complete feed for all animal species, except for horses, for which the proposed use level is 200 mg/kg complete feed. No use level has been proposed by the applicant for the use in water for drinking.

3.4 | Safety

Eucalyptus leaves are toxic to most animals, except Koalas, which use the leaves as their only diet. The toxicity of eucalyptus leaves is due to a complex mixture of volatile and non-volatile compounds, among which 1,8-cineole and formylated phloroglucinols (Eschler et al., 2000) are the most toxic. The resistance of Koalas against the toxicity of eucalyptus leaves depends on a unique intestinal microflora and the ability to avoid the intake of eucalyptus leaves with high concentrations of toxic formylated phloroglucinol compounds (Moore & Foley, 2005; Littleford-Colquhoun et al., 2022).

The safety assessment is based on the highest proposed use levels in feed, which are 50 mg eucalyptus tincture/kg complete feed for all animal species, except for horses, for which the proposed use level is 200 mg/kg complete feed.

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

Eucalyptus tincture contains 1.9% (w/w) plant-derived material, which includes ash, protein, lipids and carbohydrates (other than secondary metabolites), which are not of concern, and are not further considered.

The main identified individual components of eucalyptus tincture are 1,8-cineole [03.001] (eucalyptol), a compound identified with the EU Flavour Information System (FLAVIS) number, and gallic acid [08.080]. 1,8-Cineole and gallic acid have 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 (EFSA CEF Panel, 2011a, 2011b; EFSA FEEDAP Panel, 2012b, 2012c). In its assessment of aliphatic and alicyclic ethers (chemical group 16), the FEEDAP Panel concluded that 1,8-cineole [03.001] was safe at the use level of 5 mg/kg complete feed for all animal species based on a no observed adverse effect level (NOAEL) of 562.5 mg/kg body weight (bw) per day from a 28-day study in mice (EFSA FEEDAP Panel, 2012b). Subsequently, the FEEDAP Panel reviewed the toxicological dataset on 1,8-cineole and identified a NOAEL of 100 mg/kg bw per day from a 50-day study in rat where effects on body weight were observed starting at 500 mg/kg bw per day (EFSA FEEDAP Panel, 2021b). For gallic acid [08.080], a compound belonging to chemical group 23, the FEEDAP Panel concluded that the additive was safe at the maximum proposed use level of 25 mg/kg for all animal species, applying a NOAEL of 119 mg/kg bw per day identified from a 90-day study in rat with gallic acid (Niho et al., 2001) based on effects on the liver and haematological changes[27] observed at higher doses (EFSA FEEDAP Panel, 2012c). 1,8-Cineole and gallic acid were considered safe for the consumer and the environment, whereas hazards for skin and eye contact and respiratory exposure were recognised for all compounds belonging to chemical groups 16 and 23. 1,8-Cineole is currently authorised for use in feed for all animal species with a recommended maximum content of 5 mg/kg complete feed.[28] Gallic acid is currently authorised for use in feed for all animal species (except fish) with a recommended maximum content of 25 mg/kg complete feed.[29]

Among the secondary plant metabolites present in the tincture, total phenolic compounds including flavonoids were quantified but not individually identified, with the exception of gallic acid and ellagic acid. Unidentified flavonoids will be evaluated at the level of the assessment group (see Section 3.4.2.2, flavonoids). These compounds are readily metabolised and excreted and are not expected to accumulate in animal tissues and products.

Besides phenolic acids and flavonoids (mainly quercetin derivatives according to PhEur Commentary, 2022), the phenolic fraction consists of mono- or diformylated adducts of acylphloroglucinols with terpenes, hydrolysable tannins, especially of the ellagitannin type, monoterpenoid galloyl-glucose-derivatives and condensed tannins (proanthocyanidins). As analytical data on the occurrence in the tincture of mono- or diformylated adducts of acylphloroglucinols with terpenes,

[27]Centrilobular liver cell hypertrophy, reflected in a significant increase in liver weight observed in animals of both sexes from 1.7%; a decrease in haemoglobin (Hb), haematocrit (Ht) and red blood count (RBC) observed at in males 0.6% and above; in female, decrease of mean corpuscular volume (MCV) in the 1.7% group, and decreases of RBC, Hb, Ht and MCH in the 5% group were observed.

[28]Commission Implementing Regulation (EU) 2017/57 of 14 December 2016 concerning the authorisation of 1,8-cineole, 3,4-dihydrocoumarin and 2-(2-methylprop-1-enyl)-4-methyltetrahydropyran as feed additives for all animal species. OJ L 13, 17.1.2017, p. 153–158.

[29]Commission Implementing Regulation (EU) 2017/63 of 14 December 2016 concerning the authorisation of benzyl alcohol, 4-isopropylbenzyl alcohol, benzaldehyde, 4-isopropylbenzaldehyde, salicylaldehyde, *p*-tolualdehyde, 2-methoxybenzaldehyde, benzoic acid, benzyl acetate, benzyl butyrate, benzyl formate, benzyl propionate, benzyl hexanoate, benzyl isobutyrate, benzyl isovalerate, hexyl salicylate, benzyl phenylacetate, methyl benzoate, ethyl benzoate, isopentyl benzoate, pentyl salicylate and isobutyl benzoate as feed additives for all animal a species and of veratraldehyde and gallic acid as feed additives for certain animal species. OJ L 13, 17.1.2017, p. 214–241.

which are considered of toxicological relevance, were not provided, their concentration in the tincture was estimated to be equal to the highest analysed concentration for total phenolic compounds (0.491%, w/w) (see Sections 3.3.1.1 and 3.4.2.2).

No (geno)toxicity data are available on mono- or diformylated adducts of acylphloroglucinols with terpenes and monoterpenoid galloyl-glucose derivatives.

Trace concentrations of methyleugenol (1.10–1.21 mg/kg) were detected in all batches of the additive. For the absorption, distribution, metabolism and excretion (ADME) and the toxicology of methyleugenol reference is made to the safety evaluation made by the FEEDAP Panel in the EFSA opinion on laurel oil (EFSA FEEDAP Panel, 2023).

3.4.1 | Genotoxicity and carcinogenicity of methyleugenol

Eucalyptus tincture contains trace amounts of methyleugenol, a compound with experimentally proven genotoxicity and carcinogenicity in rodents (as reviewed in IARC, 2018). The carcinogenicity of methyleugenol and other structurally related *p*-allylalkoxybenzenes has been reviewed by the FEEDAP Panel in the opinion on olibanum extract (EFSA FEEDAP Panel, 2022).

The FEEDAP Panel identified a reference point for neoplastic endpoints derived from a carcinogenicity study in rat with methyleugenol (NTP, 2000) by applying the benchmark dose (BMD) approach with model averaging. Dose–response modelling using hepatocellular carcinomas in male rats as a response yielded a BMD lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) of 22.2 mg/kg bw per day (Suparmi et al., 2019).

3.4.2 | Safety for the target species

Tolerance studies in the target species and/or toxicological studies in laboratory animals made with the tincture under application were not submitted.

In the absence of these data, the approach to the safety assessment of the mixture is based on its individual components (when individually identified and quantified) or groups of components. For 1,8-cineole and gallic acid, subchronic studies are available, from which a NOAEL can be derived (EFSA FEEDAP Panel, 2012c, 2021b). For methyleugenol rodent carcinogenicity studies are available from which a $BMDL_{10}$ can be derived (EFSA FEEDAP Panel, 2023b). For the group assessments of phenolic compounds and flavonoids, and for the mono- or diformylated adducts of acylphloroglucinols with terpenes, in the absence of data, the threshold of toxicological concern (TTC) was applied to derive maximum safe feed concentrations for the whole groups in the tincture (EFSA FEEDAP Panel, 2017b).

For the components, for which no concern for genotoxicity has been identified, the TTC values of Cramer structural Class I–III were assigned (EFSA FEEDAP Panel, 2017b).

For the components that have the potential to be genotoxic mutagens (EFSA FEEDAP Panel, 2021a; EFSA Scientific Committee, 2019c), i.e. mono- or diformylated adducts of acylphloroglucinols with terpenes, different TTC thresholds are applied for long-living and reproductive animals (including those animals reared for laying/breeding/reproduction) and for short-living animals. Short-living animals are 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:

- For long-living and reproductive animals, considering their long lifespan and the likelihood to develop cancer, the TTC value of 0.0025 μg/kg bw per day is applied. This value has been established for potential DNA-reactive mutagens and/or carcinogens in human risk assessment (EFSA Scientific Committee, 2019c) and is considered applicable in this context.
- For short-living animals (species for fattening), genotoxicity and carcinogenicity endpoints are not considered biologically relevant. Due to their short lifespan, cancer risk is not a relevant concern for short-living animals under farming conditions (animals for fattening). For those animals, the TTC value for non-genotoxic substances in Cramer Class III (1.5 μg/kg bw per day) is applied when comparing estimated exposures with the relevant thresholds established based on non-neoplastic endpoints.

3.4.2.1 | *1,8-Cineole*

The feed concentrations of 1,8-cineole calculated at the highest proposed use levels in complete feed and considering the highest analysed concentration of 1,8-cineole (0.0036%, w/w) are reported in Table 3. Applying an UF of 100 to the NOAEL of 100 mg/kg bw per day identified from a 50-day study in rat (EFSA FEEDAP Panel, 2021a), the safe concentrations of 1,8-cineole in complete feed for the target species were calculated according to the FEEDAP Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b). For cats an additional UF of 5 is applied, considering their unusually low capacity for glucuronidation of compounds (Court and Greenblatt, 1997; Lautz et al., 2021). The calculated safe concentrations of 1,8-cineole in feed for the target species are shown in Table 3.

TABLE 3 Highest feed concentration of **1,8-cineole** from eucalyptus tincture (*Eucalyptus globulus* Labill.) calculated at the highest proposed use levels in complete feed and maximum safe concentrations in feed for the target species calculated by using the NOAEL of 100 mg/kg bw per day.

Animal category	Daily feed intake (g DM/kg bw)	Proposed use level in feed (mg additive/kg complete feed)[a]	Highest feed concentration (mg 1,8-cineole/kg complete feed)[a]	Maximum safe concentration (mg 1,8-cineole/kg complete feed)[a]
Chickens for fattening	79	50	0.002	11
Laying hens	53	50	0.002	17
Turkeys for fattening	59	50	0.002	15
Piglets	44	50	0.002	20
Pigs for fattening	37	50	0.002	24
Sows lactating	30	50	0.002	31
Veal calves (milk replacer)	19	50	0.002	50
Cattle for fattening	20	50	0.002	44
Dairy cows	31	50	0.002	29
Sheep/goats	20	50	0.002	44
Horses	20	200	0.007	44
Rabbits	50	50	0.002	18
Salmonids	18	50	0.002	50
Dogs	17	50	0.002	53
Cats[b]	20	50	0.002	9
Ornamental fish	5	50	0.002	196

Abbreviations: bw, body weight; DM, dry matter.

[a]Complete feed containing 88% DM, milk replacer 94.5% DM.

[b]The uncertainty factor for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation.

The calculated safe concentrations of 1,8-cineole for the target species are several orders of magnitude higher than the concentrations in feed resulting from the use of eucalyptus tincture at the proposed use levels in feed. Therefore, the presence of 1,8-cineole in eucalyptus tincture is not considered of concern for the target species.

3.4.2.2 | *Total phenolic compounds*

Among the secondary metabolites, up to 0.491% are total phenolic compounds including gallic acid (0.303%), ellagic acid (0.018%) and flavonoids (0.032%).

Gallic acid

The feed concentrations of gallic acid calculated at the highest proposed use levels in complete feed and considering the highest analysed concentration of gallic acid (0.303%, w/w) are reported in Table 4. Applying an UF of 100 to the NOAEL of 119 mg/kg bw per day identified from a 90-day study in rat with gallic acid (EFSA FEEDAP Panel, 2012c), the safe concentrations of gallic acid in complete feed for the target species were calculated according to the FEEDAP Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b). As mentioned above, for cats an additional UF of 5 is applied, considering their unusually low capacity for glucuronidation of compounds (Court and Greenblatt, 1997; Lautz et al., 2021). The calculated safe concentrations of gallic acid in feed for the target species are shown in Table 4.

TABLE 4 Highest feed concentration of **gallic acid** from eucalyptus tincture (*Eucalyptus globulus* Labill.) calculated at the highest proposed use levels in complete feed and maximum safe concentrations in feed for the target species calculated by using the NOAEL of 119 mg/kg bw per day.

Animal category	Daily feed intake (g DM/kg bw)	Proposed use level in feed (mg additive/kg complete feed)[a]	Highest feed concentration (mg gallic acid/ kg complete feed)[a]	Maximum safe concentration (mg gallic acid/ kg complete feed)[a]
Chickens for fattening	79	50	0.152	13
Laying hens	53	50	0.152	20
Turkeys for fattening	59	50	0.152	18
Piglets	44	50	0.152	24
Pigs for fattening	37	50	0.152	29
Sows lactating	30	50	0.152	37
Veal calves (milk replacer)	19	50	0.152	60
Cattle for fattening	20	50	0.152	52
Dairy cows	31	50	0.152	34
Sheep/goats	20	50	0.152	52

(Continues)

TABLE 4 (Continued)

Animal category	Daily feed intake (g DM/kg bw)	Proposed use level in feed (mg additive/kg complete feed)[a]	Highest feed concentration (mg gallic acid/ kg complete feed)[a]	Maximum safe concentration (mg gallic acid/ kg complete feed)[a]
Horses	20	200	0.606	52
Rabbits	50	50	0.152	21
Salmonids	18	50	0.152	60
Dogs	17	50	0.152	63
Cats[b]	20	50	0.152	10
Ornamental fish	5	50	0.152	233

Abbreviations: DM, dry matter; bw, body weight.

[a]Complete feed containing 88% DM, milk replacer 94.5% DM.

[b]The uncertainty factor for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation.

The calculated safe concentrations of gallic acid for the target species are at least two orders of magnitude higher than the concentrations in feed resulting from the use of eucalyptus tincture at the proposed use levels in feed. Therefore, the presence of gallic acid in eucalyptus tincture is not considered of concern for the target species.

Ellagic acid and flavonoids

Ellagic acid represents ≤ 0.018% of eucalyptus tincture and unidentified **flavonoids** (quantified as quercetin equivalents) account together for ≤ 0.032% of the tincture. The feed concentrations of ellagic acid and of flavonoids calculated at the highest proposed use levels in complete feed were compared to maximum acceptable concentration in feed for Cramer Class III (EFSA FEEDAP Panel, 2017b). The results are shown in Table 5.

TABLE 5 Highest feed concentration of ellagic acid and **flavonoids** from eucalyptus tincture (*Eucalyptus globulus* Labill.) calculated at the highest proposed use levels in complete feed and maximum safe concentrations in feed for the target species calculated by applying the threshold of toxicological concern for Cramer Class III compounds.

Animal category	Daily feed intake (g DM/kg bw)	Proposed use level in feed (mg additive/kg complete feed)[a]	Highest feed concentration ellagic acid	Highest feed concentration flavonoids	Maximum safe concentration
			(mg/kg complete feed)[a]		
Chickens for fattening	79	50	0.009	0.016	0.02
Laying hens	53	50	0.009	0.016	0.02
Turkeys for fattening	59	50	0.009	0.016	0.02
Piglets	44	50	0.009	0.016	0.03
Pigs for fattening	37	50	0.009	0.016	0.04
Sows lactating	30	50	0.009	0.016	0.05
Veal calves (milk replacer)	19	50	0.009	0.016	0.08
Cattle for fattening	20	50	0.009	0.016	0.07
Dairy cows	31	50	0.009	0.016	0.04
Sheep/goats	20	50	0.009	0.016	0.07
Horses	20	200	0.036	0.064	0.07
Rabbits	50	50	0.009	0.016	0.03
Salmonids	18	50	0.009	0.016	0.08
Dogs	17	50	0.009	0.016	0.08
Cats	20	50	0.009	0.016	0.07
Ornamental fish	5	50	0.009	0.016	0.29

Abbreviations: DM, dry matter; bw, body weight.

[a]Complete feed containing 88% DM, milk replacer 94.5% DM.

The results shown in Table 5 indicate that the feed concentration of ellagic acid and flavonoids would be below the maximum acceptable feed concentration for Cramer Class III (EFSA FEEDAP Panel, 2017b). Therefore, no concern for the target species arises from ellagic acid and flavonoids in eucalyptus tincture.

Other phenolic compounds

The occurrence of other phenolic derivatives, such as mono- or diformylated adducts of acylphloroglucinols with terpenes, hydrolysable tannins, especially of the ellagitannin type, monoterpenoid galloyl-glucose-derivatives and condensed tannins (proanthocyanidins) has been described in literature for the leaves of *E. globulus* (see Section 3.3.1). Analytical data on the occurrence of these phenolic compounds and especially of the mono- or diformylated adducts of acylphloroglucinols with terpenes, which are considered of toxicological relevance, were not provided for the additive. In the absence of data, the present assessment is based on the assumption that the highest analysed concentration for total phenolic compounds of 0.491% (w/w) is the estimated maximum value for mono- or diformylated adducts of acylphloroglucinols with terpenes in the additive.

The highest feed concentration and the highest estimated intake of mono- or diformylated adducts of acylphloroglucinols with terpenes for long-living and reproductive animals and for target species for fattening are reported in Table 6.

TABLE 6 Highest feed concentration and estimated intake of mono- or diformylated adducts of acylphloroglucinols with terpenes calculated for the target animals at the maximum proposed use level of the additive in feed.

Target species	Daily feed intake g DM/kg bw	Use level in feed mg additive/kg complete feed	Highest feed concentration[a] mg/kg complete feed	Intake[a] µg/kg bw per day
Long-living and reproductive animals				
Laying hens	53	50	0.246	14.8
Sows lactating	30	50	0.246	8.4
Dairy cows	31	50	0.246	8.6
Sheep/goats	20	50	0.246	5.6
Horses	20	200	0.982	22.3
Rabbits	50	50	0.246	13.9
Dogs	17	50	0.246	4.6
Cats	20	50	0.246	5.6
Ornamental fish	5	50	0.246	1.3
Short-living animals (species for fattening)				
Chickens for fattening	79	50	0.246	22.0
Turkeys for fattening	59	50	0.246	16.4
Piglets	44	50	0.246	12.3
Pigs for fattening	37	50	0.246	10.2
Veal calves (milk replacer)	19	50	0.246	5.3
Cattle for fattening	20	50	0.246	5.6
Sheep/goats for meat production	20	50	0.246	5.6
Horses for meat production	20	200	0.982	22.3
Rabbits for meat production	50	50	0.246	13.9
Salmonids	18	50	0.246	4.9

Abbreviations: DM, dry matter; bw, body weight.

[a]The highest feed concentration and the intake values of mono- or diformylated adducts of acylphloroglucinols with terpenes are calculated assuming that they are present in the tincture at the highest analysed concentration for total phenolic compounds of 0.491% (w/w).

Long-living and reproductive animals

For long-living and reproductive animals, the estimated highest intake of mono- or diformylated adducts of acylphloroglucinols with terpenes ranges from 1.3 µg/kg bw per day in ornamental fish to 22.3 µg/kg bw per day in horses. These intake levels are several orders of magnitude higher than the TTC value of 0.0025 µg/kg bw per day established for potential DNA reactive mutagens and/or carcinogens in human risk assessment (EFSA Scientific Committee, 2019c).

For long-living animals, the TTC value is exceeded, and generation of further data would be required. In the absence of analytical data on the occurrence of mono- or diformylated adducts of acylphloroglucinols with terpenes in the tincture and in the absence of toxicity data, no conclusion can be drawn on the use of eucalyptus tincture for long-living and reproductive animals.

Short-living animals (species for fattening)

For short-living animals, the TTC based on non-cancer endpoints has been applied. For these species, the estimated highest intake of mono- or diformylated adducts of acylphloroglucinols with terpenes ranges from 4.9 µg/kg bw per day

in salmonids to 22.3 μg/kg bw per day in horses for meat production. These intake levels are 3- to 15-fold higher than the TTC value for Cramer class III compounds (1.5 μg/kg bw per day).

Therefore, a reduction of the use levels of eucalyptus tincture in feed would be needed to ensure that the maximum acceptable concentrations in feed established based on the application of the TTC for Cramer class III compounds are not exceeded. This would correspond to the following concentrations in complete feed: 4 mg/kg for chickens for fattening, 5 mg/kg for turkeys for fattening, 6 mg/kg for piglets and rabbits for meat production, 7 mg/kg for pigs for fattening, 16 mg/kg for veal calves, 14 mg/kg for cattle for fattening, sheep/goats and horses for meat production and 15 mg/kg for salmonids. These levels are extrapolated to physiologically related minor species.

3.4.2.3 | *Methyleugenol*

Trace concentrations of methyleugenol (≤ 0.00012%) were detected in all five batches of the additive.

Methyleugenol belongs to the group of *p*-allylalkoxybenzenes and is a genotoxic carcinogen. For this kind of compounds, different reference points and a different magnitude of the margin of exposure (MOE) would be applied for long-living and reproductive animals (including those animals reared for laying/breeding/reproduction) and for short-living animals (animal for fattening).

In the current assessment, considering the very low concentrations of methyleugenol in feed resulting from the use of eucalyptus tincture at the proposed use levels, the FEEDAP Panel did not consider it necessary to distinguish between long-living and reproductive animals and short-living animals.

For all animals, an MOE with a magnitude > 10,000 when comparing estimated exposure to genotoxic and/or carcinogenic substances with a $BMDL_{10}$ from a rodent carcinogenicity study is considered indicative of low concern. The FEEDAP Panel identified the $BMDL_{10}$ of 22.2 mg/kg bw per day derived from rodent carcinogenicity studies with methyleugenol (NTP, 2000; Suparmi et al., 2019), as the reference point for the entire group of *p*-allylalkoxybenzenes (EFSA FEEDAP Panel, 2022). In the current assessment this reference point is applied to assess the exposure of all animal species to methyleugenol.

At the maximum proposed use level of 50 mg tincture/kg complete feed, the highest concentration of methyleugenol in the additive (≤ 0.00012%) would lead to a concentration of 0.06 μg/kg complete feed (0.24 μg/kg for horses at the use level of 200 mg tincture/kg complete feed).

The methyleugenol intake calculated for the target species at the maximum proposed use level in feed (200 mg/kg complete feed for horses and 50 mg/kg complete feed for the other species) and the margin of exposure (MOE) are reported in Table 7.

TABLE 7 Intake of methyleugenol and margin of exposure (MOE) calculated for the target animals at the maximum proposed use level of the additive in feed.

Target species	Daily feed intake g DM/kg bw	Body weight Kg	Use level in feed mg/kg	Methyleugenol intake[a]	MOE[b]
				μg/kg bw per day	
Chickens for fattening	79	2	50	0.0054	4,087,457
Laying hens	53	2	50	0.0036	6,092,624
Turkeys for fattening	59	3	50	0.0040	5,504,132
Piglets	44	20	50	0.0030	7,338,843
Pigs for fattening	37	60	50	0.0025	8,806,612
Sows lactating	30	175	50	0.0021	10,702,479
Veal calves (milk replacer)	19	100	50	0.0012	18,347,107
Cattle for fattening	20	400	50	0.0014	16,145,455
Dairy cows	31	650	50	0.0021	10,494,545
Sheep/goats	20	60	50	0.0014	16,145,455
Horses	20	400	200	0.0055	4,036,364
Rabbits	50	2	50	0.0034	6,458,182
Salmonids	18	0.12	50	0.0012	18,451,948
Dogs	17	15	50	0.0011	19,374,545
Cats	20	3	50	0.0014	16,145,455
Ornamental fish	5	0.012	50	0.0003	71,757,576

Abbreviations: DM, dry matter; bw, body weight.

[a]The values of methyleugenol in feed is calculated considering the highest analysed value in the additive (0.00012% w/w).

[b]The MOE for methyleugenol is calculated as the ratio of the reference point ($BMDL_{10}$) to the intake.

When the estimated exposures for the different animal categories are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day (Suparmi et al., 2019) calculated from rodent carcinogenicity studies with methyleugenol (NTP, 2000, see Section 3.3.2),

a MOE of at least 4,036,364 is calculated. The magnitude of this MOE indicates that the presence of methyleugenol in eucalyptus tincture is very unlikely to be of safety concern for the target species.

3.4.2.4 | *Use in water for drinking*

The FEEDAP Panel considers that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered of no concern when consumed via feed alone.

3.4.2.5 | *Conclusions on safety for the target species*

In the absence of analytical data on the occurrence of mono- or diformylated adducts of acylphloroglucinols with terpenes in the tincture and in the absence of toxicity data, no conclusion can be drawn on the use of eucalyptus tincture for long-living and reproductive animals.

The additive under assessment, eucalyptus tincture, is considered of no concern for short-living animals (species for fattening) at the following concentrations in complete feed: 5 mg/kg for turkeys for fattening, 4 mg/kg for chickens for fattening and other poultry for fattening, 7 mg/kg for pigs for fattening, 6 mg/kg for piglets and other Suidae for meat production, 16 mg/kg for veal calves (milk replacer), 14 mg/kg for sheep/goats for meat production, cattle for fattening and other ruminants for fattening and camelids at the same physiological stage, horses and other Equidae for meat production, 6 mg/kg for rabbits for meat production, and 15 mg/kg for salmonids and minor fin fish.

The FEEDAP Panel considers that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered of no concern when consumed via feed alone.

3.4.3 | Safety for the consumer

Preparations of eucalyptus leaves including tinctures and fluid extracts are added to food for flavouring purposes according to Fenaroli's handbook of flavour ingredients (Burdock, 2009), without intake figures being cited.

No data on residues in products of animal origin were made available for any of the constituents of the tincture. When considering the ADME of the individual components, for 1,8-cineole and gallic acid, the available data indicate that they are absorbed, metabolised and rapidly excreted and are not expected to accumulate in animal tissues and products (EFSA FEEDAP Panel, 2012a, 2012b). Similarly, the phenolic compounds will either not be absorbed (condensed tannins with a high degree of polymerisation), or poorly absorbed and rapidly metabolised (quercetin, the main flavonoid) or be readily metabolised and excreted and are not expected to accumulate in animal tissues and products (phenolic acids). Equally methyleugenol, occurring in the additive at trace concentrations, is not expected to accumulate in animal tissues and products (see Section 3.3.1).

Considering the above and the known human exposure due to the use of preparations of eucalyptus leaves to flavour food (Burdock, 2009), it is unlikely that consumption of products from animals given eucalyptus tincture at the proposed maximum use level would significantly increase human background exposure.

No safety concern would be expected for the consumer from the use of eucalyptus tincture up to the highest safe use level in feed.

3.4.4 | 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[30] concerning the presence of ethanol in the tincture.[31]

The additive contains 1,8-cineole and gallic acid, two compounds for which hazards for skin and eye contact and respiratory exposure were recognised (EFSA FEEDAP Panel, 2012a, 2012b).

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

When handling the tincture, exposure of unprotected users to methyleugenol may occur. Therefore, to reduce the risk, the exposure of the users should be minimised.

3.4.5 | Safety for the environment

Although *E. globulus* is not native to Europe, the blue gum is among the most extensively planted eucalypts because of its uses and its adaptability to a range of climatic conditions. It is particularly suited to areas with a Mediterranean climate and so is found widely distributed in southern parts of Europe. In addition, the most abundant components in the tincture,

[30]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.

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

i.e. 1,8-cineole and phenolic compounds including gallic acid and flavonoids, are naturally occurring in European plants. Therefore, the use of the tincture under the proposed conditions of use in animal feed is not expected to pose a risk to the environment.

3.5 | Efficacy

The leaves of *E. globulus* and their preparations are used to flavour food according to Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009).

Since eucalyptus leaves and their 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.

4 | CONCLUSIONS

Eucalyptus tincture from *Eucalyptus globulus* Labill. may be produced from plants of different origins and by various processes resulting in preparations with different composition and toxicological profiles. Thus, the following conclusions apply only to eucalyptus tincture which contains ≤ 1.2 mg/kg (0.00012%) methyleugenol and is produced by ethanol/water extraction from the leaves of the plant.

In the absence of analytical data on the occurrence of mono- or diformylated adducts of acylphloroglucinols with terpenes in the tincture and in the absence of toxicity data, no conclusion can be drawn on the use of eucalyptus tincture for long-living and reproductive animals.

For short-living animals (species for fattening), the FEEDAP Panel concludes that the additive is considered of no concern at the following concentrations in complete feed: 5 mg/kg for turkeys for fattening, 4 mg/kg for chickens for fattening and other poultry for fattening, 7 mg/kg for pigs for fattening, 6 mg/kg for piglets and other Suidae for meat production, 16 mg/kg for veal calves (milk replacer), 14 mg/kg for sheep/goats for meat production, cattle for fattening and other ruminants for fattening and camelids at the same physiological stage, horses and other Equidae for meat production, 6 mg/kg for rabbits for meat production, and 15 mg/kg for salmonids and minor fin fish. The FEEDAP Panel considers that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered of no concern when consumed via feed alone.

No safety concern would arise for the consumer from the use of eucalyptus tincture in animal nutrition up to the levels in feeds considered of no concern.

The additive under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser. When handling the additive, exposure of unprotected users to methyleugenol may occur. Therefore, to reduce the risk, the exposure of the users should be minimised.

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

Since the leaves of *E. globulus* and their 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 assessment.

5 | RECOMMENDATION

The specification should ensure that the concentration of methyleugenol should be as low as possible and should not exceed 1.2 mg/kg eucalyptus tincture, corresponding to 0.00012%.

6 | DOCUMENTATION PROVIDED TO EFSA/CHRONOLOGY

Date	Event
28/10/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 07 – Geraniales, Myrtales, Poales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)
09/11/2010	Reception mandate from the European Commission
22/03/2011	Comments received from Member States
08/04/2011	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*
08/01/2013	Reception of supplementary information from the applicant

Date	Event
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
20/01/2014	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
17/06/2016	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". Discussion on the ongoing work regarding the pilot dossiers BDG08 and BDG 09
27/04/2017	Trilateral meeting organised by the European Commission with EFSA and the applicant FEFANA on the assessment of botanical flavourings: characterisation, substances of toxicological concern present in the botanical extracts, feedback on the pilot dossiers
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: broom teatree oil, geranium oil, bay oil and vetiveria oil
12/12/2019	EFSA informed the applicant that the evaluation process restarted
02/03/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization, safety for the target species, safety for the consumer, safety for the user, safety for the environment*
30/09/2022	Reception of supplementary information from the applicant (partial dataset on eucalyptus tincture) - Scientific assessment remains suspended
18/11/2022	Partial withdrawal by applicant (EC was informed) for the following additives: bambusa tincture and allspice oil
17/02/2023	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: characterization
11/05/2023	Reception of supplementary information from the applicant (partial dataset on clove tincture) - Scientific assessment remains suspended
06/06/2023	Reception of an amendment of the Evaluation report of the European Union Reference Laboratory for Feed Additives related to geranium rose oil, eucalyptus oil, lemongrass oil and clove oil
01/03/2024	Reception of an amendment of the Evaluation report of the European Union Reference Laboratory for Feed Additives related to citronella oil, melaleuca oil, tea tree oil, eucalyptus tincture, clove tincture
26/03/2024	The application was split and a new EFSA-Q-2024-00192 was assigned to the additive included in the present assessment. Scientific assessment re-started
18/14/2024	Opinion adopted by the FEEDAP Panel on eucalyptus tincture (EFSA-Q-2024-00192). End of the Scientific assessment for the additive included in the present assessment. The assessment of other additives in BGD 07 is still ongoing

ABBREVIATIONS

ADME absorption, distribution, metabolism and excretion
BDG botanically defined group
BMD benchmark dose
$BMDL_{10}$ benchmark dose (BMD) lower confidence limit for a benchmark response of 10%
bw body weight
CAS Chemical Abstracts Service
CEF EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CLP Classification, Labelling and Packaging
DM dry matter
EEIG European economic interest grouping
EMA European Medicines Agency
EURL European Union Reference Laboratory
FEMA Flavour and Extract Manufactures Association
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)
FLAVIS The EU Flavour Information System
JECFA Joint FAO/WHO Expert Committee of Food Additives
GAE gallic acid equivalent
GC-FID gas chromatography-flame ionisation detector
HACCP Hazard Analysis and Critical Control Points
HPLC high-performance liquid chromatography
HPTLC high-performance thin-layer chromatography
LOD limit of detection
MOE margin of exposure

NOAEL no observed adverse effect level
PhEur European Pharmacopoiea
SC EFSA Scientific Committee
TTC threshold of toxicological concern
WHO World Health Organization

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CONFLICT 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.

REQUESTOR
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