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Safety and efficacy of a feed additive consisting of a tincture derived from the leaves of Ocimum basilicum L. (basil tincture) for use in all animal species (FEFANA asbl)

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SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of a tincture derived from the leaves of *Ocimum basilicum* L. (basil tincture) for use in all animal species (FEFANA asbl)

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

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 *Ocimum basilicum* L. (basil tincture) when used as a sensory additive in feed and in water for drinking for all animal species. The product is a [redacted] solution, with a dry matter content of approximately 1.79%. Basil tincture contains on average 0.187% (w/w) total polyphenols (of which 0.0247% are flavonoids). Methyleugenol (0.0006%) and estragole (0.00005%) were detected in the additive. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the use of basil tincture is very unlikely to induce adverse effects in long-living and reproductive animals and is of no safety concern for species for fattening at the following concentrations in complete feed: 65 mg/kg for chickens for fattening, 97 mg/kg for laying hens, 87 mg/kg for turkeys for fattening, 140 mg/kg for pigs for fattening, 116 mg/kg for piglets, 170 mg/kg for sows, 291 mg/kg for veal calves, 256 mg/kg for cattle for fattening, sheep/goats and horses, 166 mg/kg for dairy cows, 102 mg/kg for rabbits, 292 mg/kg for salmonids, 307 mg/kg for dogs, 256 mg/kg for cats and 500 mg/kg for ornamental fish. These conclusions were extrapolated to other physiologically related species. For any other species, the additive is very unlikely to induce adverse effects at 65 mg/kg complete feed. No safety concerns were identified for the consumer and the environment from the use of the additive in animal feed. Regarding user safety, basil tincture should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser. When handling the additive, exposure of unprotected users to methyleugenol and estragole may occur. Therefore, to reduce the risk, the exposure of the users should be minimised. Since the leaves of *O. basilicum* are recognised to flavour food and their function in feed would be essentially the same as that in food, no further demonstration of efficacy was necessary.

KEYWORDS

1,8-cineole, basil tincture, camphor, estragole, flavouring compounds, methyleugenol, *Ocimum basilicum* L., sensory additives, thujones

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1 | INTRODUCTION

1.1 | Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. 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 41 additives (king of bitter extract, thyme leaved gratiola tincture, devils claw extract, devils claw tincture, lavender oil, lavender tincture, spike lavender oil, melissa oil, balm leaves extract, mentha arvensis/corn mint oil, pennyroyal oil, spearmint oil, peppermint oil, peppermint tincture, basil oil, basil tincture, olive extract, marjoram oil, oregano oil, oregano tincture, patchouli oil, rosemary oil, rosemary oleoresin, rosemary extract, rosemary tincture, Spanish sage oil, sage oil, sage tincture, clary sage oil, savory summer oil, savory summer tincture, Pau darco tincture, thymus origanum oil, thyme oil, thyme oleoresin, thyme extract, thyme tincture, lilac chastetree extract, lilac chastetree tincture, Spanish marjoram oil and wild thyme tincture) belonging to botanically defined group (BDG) 01 – Lamiales, 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 12 additives.[3] These additives were deleted from the register of feed additives.[4] In addition, during the course of the assessment, the application was split, and the present opinion covers only one out of the remaining 29 additives under application: basil tincture from *Ocimum basilicum* L.[5] for use in all animal species.

The remaining 28 additives belonging to botanically defined group (BDG) 01 – Lamiales, 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 1 June 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 the feed additive consisting of basil tincture from the leaves of *O. basilicum*, when used under the proposed conditions of use (see **Section 3.3.3**).

1.2 | Additional information

Basil tincture from *O. basilicum* L. 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 basil tincture from *O. basilicum* as a feed additive. The dossier was received on 26 May 2025 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2025-00324.[7]

[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]Thyme leaves gratiola tincture, spike lavender oil, melissa oil, pennyroyal oil, basil oil and savoury summer oil (27 February 2019); Spanish majoram oil (28 September 2023); lilac chastetree extract and savoury summer tincture (8 July 2024); devils claw extract (wb), balm leaves extract (sb), olive extract (sb) (16 December 2024).

[4]Register of feed additives, Annex II, withdrawn by OJ L162, 10.5.2021, p. 5.

[5]Accepted name: *Ocimum basilicum* L.

[6]Dossier reference: FAD-2010-0137.

[7]The original application EFSA-Q-2010-01307 was split on 26/5/2025 and a new EFSA-Q-2025-00324 was generated.

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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.

Several components of the tincture under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings (CDGs). The applicant submitted a written agreement to reuse the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 01, 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. During the assessment, upon request of EFSA, the EURL issued two partial reports.[9] The additive under assessment is included in the second partial report. In particular, for the characterisation of basil tincture the EURL recommended methods based on (i) spectrophotometry for the determination of total polyphenols and total flavonoids; (ii) gas chromatography coupled with flame ionisation detection (GC-FID) for the determination of 1,8-cineole, camphor, estragole and methyleugenol; (iii) gas chromatography coupled with mass spectrometry detection (GC–MS) for the determination of eugenol; (iv) high-performance liquid chromatography (HPLC) for the quantification of the phytochemical marker rosmarinic acid in basil tincture; and (v) high-performance thin layer chromatography (HPTLC) profile for the unequivocal identification of basil tincture.[10]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of basil tincture from *O. basilicum* 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 naturally occurring substances of potential concern for human and animal health,[12] 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 safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2024), Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023a), 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).[13]

3 | ASSESSMENT

The additive under assessment, basil tincture, is obtained from the dried leaves of *O. basilicum* L. and is intended for use as a sensory additive (functional group: flavouring compounds) in feed and in water for drinking for all animal species.

3.1 | Origin and extraction

O. basilicum L. (basil) is an annual herb belonging to the family Lamiaceae. It is native to the tropical regions of the Indian sub-continent, most of south-east Asia and parts of Australia but, because of its commercial value, it is now found throughout the world, grown in the open or under glass. The generic term 'basil' is generally taken to refer to the variety of *O. basilicum* described as sweet basil or Genovese basil, but multiple other cultivars of the species have been developed with different sensory characteristics (e.g. lemon basil, thai basil). In addition, the term basil is also applied to other *Ocimum* species, notably clove basil (*Ocimum grantissimum* L.), holy basil (*Ocimum tenuiflorum* L.) and camphor basil (*Ocimum*

[8]Technical dossier/Supplementary information August 2024/Letter dated 27/8/2024.

[9]Additives included in the first partial report: Spanish sage oil, peppermint oil, thymus origanum oil, patchouli oil, clary sage oil, lavender oil and sage oil; additives included in the second partial report: cornmint oil, spearmint oil, thyme oil, rosemary oil, marjoram oil, rosemary tincture, basil tincture, lavender tincture, peppermint tincture, sage tincture and wild thyme tincture.

[10]Evaluation report received on 18/06/2025 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/reports-and-technical-documentation/fad-2010-0137_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]Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals.

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

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killimandschericum Gurke). Depending on the variety, plants may reach 30–100 cm in height with pairs of leaves produced on a square stem. Under commercial production, plants are cut above the second pair of leaves for a single harvest of dried leaves or above the fourth set to allow regrowth. With regrowth, plants may be harvested on 2–3 occasions.

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

3.2 | Uses other than feed flavouring

While there is no specific EU authorisation for any *O. basilicum* preparation when used to provide flavour in food, according to Regulation (EC) No 1334/2008[14] flavouring preparations produced from food, may be used without an evaluation 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'.

3.3 | Characterisation

3.3.1 | Characterisation of basil tincture

Basil tincture is a brown liquid, with a characteristic odour. It has an average density of [redacted] kg/m^3 ([redacted] kg/m^3) and a pH of [redacted] ([redacted]).[15] No Chemical Abstract Service (CAS) number or European Community (EC) number is specifically associated to basil tincture.[16] The Council of Europe (CoE) number 308 is associated to *O. basilicum* L.

Table 1 summarises the results of the proximate analysis of five batches of the additive.[17] The solvent represents on average 98.21% of the additive leaving a dry matter (DM) content of 1.79%. The DM consists of inorganic material measured as ash (25.7%) and a plant-derived organic fraction of 74.3%, which includes proteins, lipids and reducing sugars (after hydrolysis). The unidentified organic fraction remaining after subtracting the values for proteins, lipids and reducing sugars, contains a variety of non-volatile secondary metabolites including phenolic compounds (see Table 2).

TABLE 1 Proximate analysis of basil tincture based on the analysis of five batches.

Constituent	Mean % (w/w)	Range % (w/w)
Dry matter	1.79	1.79–1.80
Ash	0.46	0.45–0.48
Organic fraction	1.33	1.31–1.34
Proteins	< 0.08	< 0.08
Lipids	0.012	0.008–0.016
Reducing sugars[1]	< 0.3	< 0.3
'Unidentified'[2]	0.94	0.92-0.96
Solvent	98.21	98.20–98.22

[1]After hydrolysis, expressed as glucose equivalents.

[2]'Unidentified' (by difference) calculated subtracting ash, proteins, lipids and sugar from the dry matter. It includes non-volatile secondary plant metabolites.

The fraction of secondary metabolites was characterised in the same five batches of basil tincture and the results are summarised in Table 2. The tincture was shown to contain polyphenols (0.187% on average, w/w) determined by spectrophotometry (at 760 nm) and expressed as gallic acid equivalents (GAE).[18] The FEEDAP Panel notes that the polyphenols were determined by the Folin–Ciocalteu method, which is a widely used method for the determination of total content of polyphenols in plant extracts, but which does not give the true concentrations of polyphenols of different types. This may

[14]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.

[15]Technical dossier/Supplementary information April 2024/Annex_II_4_Results of analysis.

[16]The following entries were found at https://echa.europa.eu home: 'Ocimum basilicum, ext. (Extractives and their physically modified derivatives such as tinctures, concretes, absolutes, essential oils, oleoresins, terpenes, terpene-free fractions, distillates, residues, etc., obtained from Ocimum basilicum, Labiatae)', CAS number 84775–71-3, EC number 283-900-8.

[17]Technical dossier/Supplementary information April 2024/Section II_Identity and Annex_II_4_Results of analysis.

[18]Technical dossier/Supplementary information April 2024/Section_II_Identity and Annex_II_4_Results of analysis.

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lead to an overestimation of their concentrations in comparison to results obtained by HPLC depending on the substances of interest and the standard used (Everette et al., 2010; Samara et al., 2022). The concentration of flavonoids (0.024% on average, w/w, expressed as quercetin equivalents) was determined by spectrophotometry (at 415 nm). Five unidentified polyphenols (phenolic acids) were detected by HPTLC and expressed as GAE.[19] Hydroxycinnamic acid derivatives were quantified by HPLC with ultraviolet (UV) detection[20] and volatile compounds by GC-FID[21] in the same five batches of the tincture. Safrole and coumarin were below the limit of detection (LOD, 1.0 mg/kg, corresponding to less than 0.0001%) in all batches.

TABLE 2 Concentrations of the fraction of secondary metabolites in basil tincture based on the analysis of five batches (mean and range).

				Mean	Range
Constituent	**CAS no**	**FLAVIS no**	**Method**	**% (w/w)**	**% (w/w)**
Total polyphenols[1]	–		Folin–Ciocalteu[2]	0.187	0.178–0.198
Flavonoids	–		Spectrophotometry[3]	0.0247	0.0228-0.0257
Hydroxycinnamic acid derivatives					
Rosmarinic acid	20283-92-5	–	HPLC-UV (330 nm)	0.0107	0.0104–0.0110
Caffeic acid	331-39-5	–	HPLC-UV (320 nm)	0.0074	0.0070–0.0076
p-Coumaric acid	501-98-4	–	HPLC-UV (310 nm)	0.0010	0.0008–0.0011
Volatile compounds					
Camphor[4]	76-22-2	–	GC-FID	0.0045	0.0043–0.0047
1,8-Cineole	470-82-6	03.001	GC-FID	0.0017	0.0016–0.0019
Eugenol	97-53-0	04.003	GC-FID	0.0022	0.0021–0.0024
Geraniol	106-24-1	02.012	GC-FID	0.0002	0.0002–0.0002
Linalool	78-70-6	02.013	GC-FID	0.0026	0.0022–0.0031
α-Terpineol	98-55-5	02.013	GC-FID	0.0002	0.0002–0.0002
4-Terpinenol	562-74-3	02.072	GC-FID	0.0002	0.0002–0.0003
Thymol	89-83-8	04.006	GC-FID	0.0001	0.0001–0.0001
Thujones[5]	–	–	GC-FID	0.00007	0.00007–0.00008
Methyleugenol	93-15-2	–	GC-FID	0.0006	0.0005–0.0006
Estragole	140-67-0	–	GC-FID	0.00005	0.00005–0.00005

Abbreviations: CAS no, Chemical Abstracts Service number; EU, European Union; FLAVIS no, EU Flavour Information System number; GC-FID, gas chromatography coupled with flame ionisation detection; HPLC: high-performance liquid chromatography; UV, ultraviolet.

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

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

[3]Determined by an internal method based on European Pharmacopoeia (PhEur, 2022b). Determination of flavonoids in motherwort.

[4]Present in the additive as a mixture of enantiomers (*d,l*-camphor), the ratio between *d*- and *l*-stereoisomers not given.

[5]Present in the additive as a mixture of α-thujone (CAS no 546-80-5) and β-thujone (CAS no 471-15-8), the ratio between α- and β-thujones not given.

The EFSA Compendium of botanicals[22] reports as substances of potential concern for human and animal health the occurrence of 1,8-cineole (3.74%–16.7%), camphor (1.2%), α-selinene (1.67%), β-selinene (1.04%), eugenol (1.4%–10.3%), estragole (2.4%–16.5%) and methyleugenol (0.5%–1.6%) in the essential oil from the aerial parts of *O. basilicum*.

The applicant made a literature search on the chemical composition of *O. basilicum* and its botanical preparations and the possible presence of substances of concern,[23] the results of which confirmed the information in the EFSA Compendium. In addition, other publications were identified which reported the presence of thujones (0.001%–14.84%), safrole (0.1%) and coumarin (0.12%–0.37%) in essential oils and other preparations from the leaves of *O. basilicum* (e.g. Awad et al., 2022; Elsherbiny et al., 2017; López et al., 2005; Vârban et al., 2022). The result of the literature search also indicated that apart from the compounds identified in Table 2, hydroxycinnamic acids, such as caffeoyl esters with quinic acid (chlorogenic acid) and tartaric acid (caftaric acid and chicoric acid), and ferulic acid are found in extracts from the leaves of *O. basilicum*. Additionally, phenolic acids such as vanillic acid, gallic acid, 4-hydroxybenzoic acid, 3-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, and their derivatives and other polyphenols (ellagic acid, catechol and epicatechin) have been

[19]Technical dossier/Supplementary information April 2024/ Annex II_7_Detailed report of basil tincture HPTLC analysis.

[20]Technical dossier/Supplementary information April 2024/Annex II_8_Certificate of analysis of rosmarinic acid in basil tincture.

[21]Technical dossier/Supplementary information April 2024/ Annex II_6_Certificate of analysis of 1,8-cineole, camphor, methyleugenol, estragole, safrole, thujones and coumarin in basil tincture.

[22]Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals, accessed on 15 May 2025.

[23]Technical dossier/Supplementary information April 2024/Annex II_5_Bibliographic data concerning chemical composition of basil and its extracts.

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identified. In the flavonoid fraction, apigenin-*O*-glucoside, luteolin, rutin, kaempferol, quercetin and quercitrin are predominant (e.g. Güez et al., 2017; Ibrahim et al., 2020; Tamfu et al., 2021).

3.3.1.1 | *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, mycotoxins, pesticides, dioxins and polychlorinated biphenyls (PCBs) and microbial contamination.[24] An example of a quality control certificate of the raw material was provided. The applicant stated that analysis of impurities in the tincture is made on an irregular basis and does not form part of the Hazard Analysis and Critical Control Points (HACCP) plan. However, no data were provided on the presence of these impurities in the additive under assessment.

3.3.2 | Shelf-life

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 500 mg basil tincture/kg complete feed for all animal species. No use level has been proposed for the use of the additive in water for drinking.

3.4 | Safety

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

Basil tincture contains plant-derived proteins, lipids and sugars (see Table 1), which are not of concern and are not further considered.

Among the secondary plant metabolites, total polyphenols were quantified, but only rosmarinic acid, caffeic acid and *p*-coumaric acid were individually identified and quantified. Hydroxycinnamic acid derivatives (rosmarinic acid, caffeic acid and *p*-coumaric acid) are ubiquitous in food and feeds of plant origin. They are readily metabolised and excreted and are not expected to accumulate in animal tissues and products. These compounds are not of concern at concentrations resulting from the use of the additive at the maximum proposed use level in feed and are not further considered in the assessment.

Several individual components identified in the tincture have been already assessed as chemically defined flavourings for use in feed and food by the FEEDAP Panel, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) superseded in 2008 by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). The flavouring compounds currently authorised for food[25] and/or feed[26] use, together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000,[27] and the corresponding EFSA opinion are listed in Table 3.

TABLE 3 Flavouring compounds identified among the components of basil tincture, which were already assessed by EFSA as chemically defined flavourings, grouped according to the chemical group (CG) as defined in Commission Regulation (EC) No 1565/2000, with indication of the EU Flavour Information System (FLAVIS) number and the corresponding EFSA opinion.

CG	Chemical group	Product (EU register name)	FLAVIS no.	EFSA* opinion, year
03	α,β-Unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters	Geraniol	02.012	2016a
06	Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers	Linalool	02.013	2012a
		α-Terpineol	02.014	
		4-Terpinenol	02.072	

(Continues)

[24]Technical dossier/Supplementary information April 2024/ Annex II_1_Basil leaves description from plant supplier and certificate of analysis of raw material.

[25]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.

[26]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.

[27]Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 1 80, 19.7.2000, p. 8.

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TABLE 3 (Continued)

CG	Chemical group	Product (EU register name)	FLAVIS no.	EFSA* opinion, year
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	Camphor *d*-Camphor[1]	– 07.215	2008, AFC 2016b, 2023b
16	Aliphatic and alicyclic ethers	1,8-Cineole	03.001	2012b, 2021b
18	Allylhydroxybenzenes	Eugenol	04.003	2011
25	Phenol derivatives containing ring-alkyl, ring-alkoxy and side-chains with an oxygenated functional group	Thymol	04.006	2012c

[1]Present in the additive as a mixture of enantiomers (*d,l*-camphor), the ratio between *d*- and *l*-stereoisomers not given. JECFA and EFSA evaluated the enantiomer *d*-camphor (name in the register: *(1R)*-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one [07.215]) for use in food (EFSA, 2008) and in feed (EFSA FEEDAP Panel, 2016b).

*FEEDAP opinion unless otherwise indicated.

Trace concentrations of methyleugenol (up to 0.0006%) and estragole (up to 0.00005%), two compounds with experimentally proven genotoxic and carcinogenic activities, were detected in all batches of the additive. For the absorption, distribution, metabolism and excretion (ADME) and the toxicology of these compounds, reference is made to the safety evaluation made by the FEEDAP Panel in the opinions on laurel oil and star anise oil (EFSA FEEDAP Panel, 2023c, 2023d).

3.4.1 | Safety for the target species

Tolerance studies in the target species and/or toxicological studies in laboratory animals 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 the safety assessment of its individual components (when individually identified and quantified) or groups of components.

Camphor is assessed based on the results of the tolerance studies carried out in the target species with the mixture of flavourings 'Herbal' (EFSA FEEDAP Panel, 2023b).

For geraniol [02.012], linalool [02.013], terpineol [02.230], 1,8-cineole [03.001], eugenol [04.003] and thymol [04.006], subchronic oral toxicity studies are available, from which no observed adverse effect levels (NOAELs) were identified (EFSA FEEDAP Panel, 2011, 2012a, 2012c, 2016a, 2021b). For thujones,[28] the FEEDAP Panel applied a benchmark dose lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) derived from the long-term chronic toxicity study in mice and rats using clonic seizures as the critical effect (EFSA FEEDAP Panel, 2022a).

For the group assessment of phenolic compounds including flavonoids, in the absence of data, the threshold of toxicological concern (TTC) is applied to derive maximum safe concentrations in feed for the whole groups in the tincture (EFSA FEEDAP Panel, 2017b).

For *p*-allylalkoxybenzenes, rodent carcinogenicity studies with methyleugenol are available from which a benchmark dose lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) can be derived (EFSA FEEDAP Panel, 2023c).

3.4.1.1 | *Camphor*

Camphor (as a mixture of isomers) has not been evaluated for use as a flavouring. However, *d*-camphor [07.215] was assessed in tolerance studies with a mixture of flavourings referred to as 'Herbal' in chickens for fattening, piglets, cattle for fattening and salmons. The tolerance studies showed that *d*-camphor was safe for the target species at 5 mg/kg complete feed (EFSA FEEDAP Panel, 2023b). The FEEDAP Panel considers that the conclusions reached for *d*-camphor can be extrapolated to the mixture of isomers of camphor by applying read-across.

At the maximum proposed use level of 500 mg basil tincture/kg complete feed and considering the highest analysed concentration of camphor[29] (0.0047%) in the tincture, the corresponding level of camphor in complete feed would be 0.024 mg/kg. This concentration is well below the concentration of 5 mg *d*-camphor/kg complete feed considered safe for all animal species when tested in the mixture 'Herbal' (EFSA FEEDAP Panel, 2023b). Therefore, no concern for the target species is expected from the presence of camphor in basil tincture.

[28]Present in the additive as a mixture of α-thujone and β-thujone, the ratio between α- and β-thujones not given.

[29]Present in the additive as a mixture of enantiomers (*d,l*-camphor), the ratio between *d*- and *l*-stereoisomers not given.

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3.4.1.2 | *Volatile components other than camphor*

The volatile compounds present in the tincture were allocated to five assessment groups, corresponding to the chemical groups (CGs) 3, 6, 16, 18 and 25, as defined in Annex I of Regulation (EC) No 1565/2000. The allocation of the components to the assessment groups is shown in Table 4.

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification using Toxtree (version 3.1.0, May 2018[30]). For some components in the assessment group, toxicological data were available to identify no observed adverse effect levels (NOAEL). Structural and metabolic similarity among the components in the assessment groups were assessed to explore the application of read-across, allowing extrapolation from a known NOAEL of a component of an assessment group to the other components of the same group with no available NOAEL or, if sufficient evidence were available for members of an assessment group, to derive a group NOAEL.

Sub-chronic studies, from which from which NOAEL values (or a $BMDL_{10}$ value for thujones) could be identified, were available for the following volatile components of the tincture: 345 mg/kg body weight (bw) per day for geraniol [02.012] (EFSA FEEDAP Panel, 2016a), 117 mg/kg bw per day for linalool [02.013], 250 mg/kg bw per day for terpineol [02.230][31] (EFSA FEEDAP Panel, 2012a), 100 mg/kg bw per day for 1,8-cineole [03.001] (EFSA FEEDAP Panel, 2021b), 300 mg/kg bw per day for eugenol [04.003] (EFSA FEEDAP Panel, 2011), 36 mg/kg bw per day for thymol [04.006] (EFSA FEEDAP Panel, 2012c) and 11 mg/kg bw per day for thujones (EFSA FEEDAP Panel, 2022a).

In CG 6, a NOAEL of 250 mg/kg bw per day was identified for terpineol [02.230], divided by a factor of 2 to take account of the nature of the study (EFSA FEEDAP Panel, 2012a). The resulting value of 125 mg/kg bw per day was used as the reference point for terpinyl derivatives, α-terpineol [02.014] and 4-terpinenol [02.072].

For each component in the assessment group, exposure in target animals (expressed as mg/kg bw per day) was estimated considering the maximum proposed use level of 500 mg basil tincture/kg complete feed, the percentage of the component in the tincture, and the default values for body weight and feed intake according to the guidance on the safety of feed additives for target species (EFSA FEEDAP Panel, 2017b). For each component the highest analysed concentration in the additive is used.

For risk characterisation, the margin of exposure (MOE) was calculated for each component as the ratio between the reference point and the exposure. For each assessment group, the combined (total) margin of exposure (MOET) was calculated as the reciprocal of the sum of the reciprocals of the MOE for the individual substances (EFSA Scientific Committee, 2019a). A MOE(T) > 100 allowed for interspecies differences and intra-individual variability.

The approach to the safety assessment of basil tincture is shown in Table 4 for chickens for fattening, the species with the highest ratio of feed intake/body weight and which represents the worst-case scenario among the target species.

TABLE 4 Compositional data, intake values (calculated for chickens for fattening at 500 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of basil tincture classified according to assessment groups, and combined margin of exposure (MOET) for each assessment group.

Tincture composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS-No	Highest concentration in the tincture	Highest concentration in feed	Daily intake[1]	Cramer class[2]	NOAEL[3]	MOE[4]	MOET[5]
Constituent	–	%	mg/kg	mg/kg bw per day	–	mg/kg bw per day	–	–
CG 4								
Geraniol	02.012	0.0002	0.0010	0.0009	(I)	**345**	3,940,125	
CG 6								
Linalool	02.013	0.0031	0.0011	0.00010	(I)	**117**	1,297,801	
α-Terpineol	02.014	0.0002	0.0014	0.00012	(I)	125[6]	1,031,333	
4-Terpinenol	02.072	0.0003	0.0153	0.00137	(I)	125[6]	85,224	
MOET CG 6								**74,217**
CG 16								
1,8-Cineole	03.001	0.0019	0.0093	0.00083	(II)	**100**	120,008	
CG 18								
Eugenol	04.003	0.0024	0.0118	0.00106	(I)	**300**	283,034	

(Continues)

[30]Toxtree includes both the original Cramer rule base with the 33 structural rules (Cramer et al., 1978) and an extended rule base with five additional rules which were introduced to overcome misclassification (in Class I or Class II) of several substances with low NOAELs. https://toxtree.sourceforge.net/.

[31]Terpineol is a mixture of four structural isomers: α-terpineol [02.014], β-terpineol, γ-terpineol and 4-terpinenol [02.072]. α-Terpineol [02.014], is defined as a mixture of *(R)*-(+)-α-terpineol and *(S)*-(−)-α-terpineol.

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TABLE 4 (Continued)

Tincture composition			Exposure		Hazard characterisation		Risk characterisation
CG 25							
Thymol	04.006	0.0001	0.0004	0.00003	(I)	36	1,084,962
Thujones	–	0.00008	0.0004	0.00003	(III)	**11**	318,256

Abbreviations: bw, body weight; MOE, margin of exposure; MOET, combined (total) MOE; NOAEL, no observed adverse effect level.

[1]Rounded intake values are shown in the Table.

[2]When a NOAEL value is available or read-across is applied, the allocation to the Cramer class is put into parentheses.

[3]Values **in bold** refer to those components for which the NOAEL value was available, values *in italics* are the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class, other values (plain text) are NOAELs extrapolated by using read-across.

[4]The MOE for each component is calculated as the ratio of the reference point (no observed adverse effect level, NOAEL) to the intake (non-rounded values are use in the calculations).

[5]The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE for the individual substances. MOET values are reported **in bold**.

[6]A factor of 2 was applied to the NOAEL of 250 mg/kg bw per day for terpineol because of the nature of the study.

As shown in Table 4, for all assessment groups the MOE(T) calculated for chickens for fattening at the maximum proposed use level of 500 mg basil tincture/kg complete feed was substantially > 100. Therefore, no safety concern was identified for the volatile components of basil tincture when used as a feed additive for chickens for fattening at the maximum proposed use levels (500 mg/kg complete feed). As chickens for fattening is the species with the highest ratio of feed intake/body weight and represents the worst-case scenario, the same conclusion can be extended to all animal species.

3.4.1.3 | *Flavonoids*

At the maximum proposed use level of 500 mg basil tincture/kg complete feed, the highest concentration of flavonoids (0.026% of the tincture) would be up to 0.129 mg/kg complete feed. Since the individual compounds were not identified, they are assigned to Cramer Class III. A comparison between the highest concentration of flavonoids in feed resulting from the use of the additive at the maximum proposed use level of 500 mg/kg and the maximum acceptable concentrations in feed for Cramer Class III compounds (EFSA FEEDAP Panel, 2017b) is shown in Table 5.

TABLE 5 Highest concentration of flavonoids from basil tincture in complete feed, calculated at the maximum proposed use level of 500 mg/kg complete feed, and maximum safe concentrations of flavonoids and of the additive 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)	Highest concentration of flavonoids[1] (mg/kg complete feed)[2]	Maximum safe concentration of flavonoids (mg/kg complete feed)[2]	Maximum safe concentration of basil tincture (mg/kg complete feed)[2]
Chickens for fattening	79	0.129	0.02	65
Laying hens	53	0.129	0.02	97
Turkeys for fattening	59	0.129	0.02	87
Piglets	44	0.129	0.03	116
Pigs for fattening	37	0.129	0.04	140
Sows lactating	30	0.129	0.04	170
Veal calves (milk replacer)	19	0.129	0.08	291
Cattle for fattening	20	0.129	0.07	256
Dairy cows	31	0.129	0.04	166
Sheep/goats	20	0.129	0.07	256
Horses	20	0.129	0.07	256
Rabbits	50	0.129	0.03	102
Salmonids	18	0.129	0.08	292
Dogs	17	0.129	0.08	307
Cats	20	0.129	0.07	256
Ornamental fish	5	0.129	0.29	–[3]

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

[1]Calculated at the proposed use level of basil tincture in complete feed (500 mg/kg) and considering the highest analysed concentration of flavonoids in the additive (0.026%, w/w).

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

[3]The proposed use level of 500 mg/kg complete feed is considered safe.

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The results shown in Table 5 indicate that the concentration of flavonoids in feed would be above the maximum acceptable concentration for Cramer Class III compounds in feed for all animal categories except ornamental fish. For ornamental fish, the maximum proposed use level of 500 mg/kg complete feed is safe when considering the presence of flavonoids. For the other animal categories, the calculated maximum safe concentration of basil tincture in feed for the target species (when considering only the flavonoid fraction) are shown in Table 5.

3.4.1.4 | *Polyphenols other than flavonoids, rosmarinic acid, caffeic acid and* p*-coumaric acid*

Among the secondary metabolites, up to 0.198% of the tincture are polyphenols measured by the Folin–Ciocalteu method and expressed as GAE. After subtraction of flavonoids, rosmarinic acid, caffeic acid and *p*-coumaric acid, the fraction of polyphenols is 0.154% of the tincture. At the maximum proposed use level of 500 mg basil tincture/kg complete feed, this would equate to 0.77 mg/kg complete feed. Although the individual components of this fraction were not identified, the results of the literature search provided by the applicant (see Section 3.3.1) indicated that substances belonging to Cramer class III are not expected in the polyphenolic fraction. Unidentified flavonoids have been separately assessed by applying the TTC for Cramer Class III compounds (see Section 3.4.1.3). Therefore, the remaining polyphenols belonging to this fraction are assigned to Cramer Class I and assessed as a group. A comparison between the highest concentration of polyphenols in feed resulting from the use of the additive at the maximum proposed use level of 500 mg/kg and the maximum acceptable concentrations in feed for Cramer Class I compounds (EFSA FEEDAP Panel, 2017b) is shown in Table 6.

TABLE 6 Highest concentration of polyphenols (after subtraction of flavonoids, rosmarinic acid, caffeic acid and *p*-coumaric acid from the value determined by Folin–Ciocalteu method) from basil tincture in complete feed calculated at the maximum proposed use level of 500 mg/kg complete feed, and maximum safe concentrations in feed of polyphenols and of the additive for the target species calculated by applying the threshold of toxicological concern for Cramer Class I compounds.

Animal category	Daily feed intake (g DM/kg bw)	Highest concentration of polyphenols[1] (mg/kg complete feed)[2]	Maximum safe concentration of polyphenols (mg/kg complete feed)[1]	Maximum safe concentration of basil tincture (mg/kg complete feed)[1]
Chickens for fattening	79	0.77	0.3	217
Laying hens	53	0.77	0.5	323
Turkeys for fattening	59	0.77	0.5	292
Piglets	44	0.77	0.6	390
Pigs for fattening	37	0.77	0.7	468
Sows lactating	30	0.77	0.9	–[3]
Veal calves (milk replacer)	19	0.77	1.5	–
Cattle for fattening	20	0.77	1.3	–
Dairy cows	31	0.77	0.9	–
Sheep/goats	20	0.77	1.3	–
Horses	20	0.77	1.3	–
Rabbits	50	0.77	0.5	343
Salmonids	18	0.77	1.5	–
Dogs	17	0.77	1.6	–
Cats	20	0.77	1.3	–
Ornamental fish	5	0.77	5.9	–

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

[1]Calculated at the proposed use level of basil tincture in complete feed (500 mg/kg) and considering the highest estimated analysed value of polyphenols (after subtraction of flavonoids, rosmarinic acid, caffeic acid and *p*-coumaric acid) in the additive (0.154%, w/w).

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

[3]The proposed use level of 500 mg/kg complete feed is considered safe.

The results shown in Table 6 indicate that the concentration of polyphenols in feed (after subtraction of flavonoids, rosmarinic acid, caffeic acid and *p*-coumaric acid) would be above the maximum acceptable concentration for Cramer Class I compounds in feed for poultry, piglets, pigs for fattening and rabbits. For these species, the calculated safe concentrations of basil tincture (when considering only polyphenols) in feed for the target species are shown in Table 6. For sows, veal calves, cattle for fattening, dairy cows, sheep/goats, horses, salmonids, dogs, cats and ornamental fish the maximum proposed use level of 500 mg/kg complete feed is safe (when considering only polyphenols).

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3.4.1.5 | *Methyleugenol and estragole*

Trace concentrations of methyleugenol (≤ 0.0006%) and estragole (≤ 0.00005%) were detected in all five batches of the additive.

Methyleugenol and estragole belong to the group of *p*-allylalkoxybenzenes and are genotoxic and carcinogenic. 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, 2021a), different reference points and a different magnitude of the MOE are applied for long-living and reproductive animals (including those animals reared for laying/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, a 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 carcinogenicity studies in rodents (rats) with methyleugenol (NTP, 2000; Suparmi et al., 2019), as the reference point for the entire group of *p*-allylalkoxybenzenes (EFSA FEEDAP Panel, 2022b).

For short-living animals, genotoxicity and carcinogenicity endpoints are not considered biologically relevant, therefore a lower magnitude of the MOE (> 100) when comparing estimated exposure with a reference point based on non-neoplastic endpoints is considered adequate (EFSA FEEDAP Panel, 2021a). The FEEDAP Panel identified a NOAEL of 10 mg/kg bw per day for non-neoplastic lesions (changes in organ weight[32] and function, including effects on liver[33] and the glandular stomach[34]) from a 90-day study in mice with methyleugenol (EFSA FEEDAP Panel, 2023b; NTP, 2000).

The highest daily combined intake of methyleugenol and estragole was calculated for the different animal categories considering the maximum proposed use level of 500 mg basil tincture/kg complete feed and the highest analysed concentrations of methyleugenol and estragole in the additive. The intake values are reported in Table 7, together with the corresponding group MOE for the combined intake. This is calculated considering the relevant reference point for long-living and reproductive animals or for short-living animals (species for fattening).

TABLE 7 Individual intake of methyleugenol and estragole, their combined intake and group margin of exposure (MOE) calculated for the target animal categories at the maximum proposed use level of 500 mg/kg of basil tincture in complete feed.

Animal category	Daily feed intake g DM/kg bw	Use level in feed[1] mg/kg	Methyleugenol intake[2] μg/kg bw per day	Estragole intake[2]	Combined intake[2]	MOE[3,4] –
Long-living and reproductive animals						
Laying hens	53	500	0.1807	0.015	0.1957	113,417
Piglets	44	500	0.1500	0.013	0.1625	136,615
Sows lactating	30	500	0.1029	0.009	0.1114	199,231
Dairy cows	31	500	0.1049	0.009	0.1136	195,360
Sheep/goats	20	500	0.0682	0.006	0.0739	300,554
Horses	20	500	0.0682	0.006	0.0739	300,554
Rabbits	50	500	0.1705	0.014	0.1847	120,222
Dogs	17	500	0.0568	0.005	0.0616	360,665
Cats	20	500	0.0682	0.006	0.0739	300,554
Ornamental fish	5	500	0.0153	0.0013	0.0166	1,335,795
Short-living animals (species for fattening)						
Chickens for fattening	79	500	0.2693	0.022	0.2918	34,275
Turkeys for fattening	59	500	0.2000	0.017	0.2167	46,154
Pigs for fattening	37	500	0.1250	0.010	0.1354	73,846
Veal calves (milk replacer)	19	500	0.0600	0.005	0.0650	153,846
Cattle for fattening	20	500	0.0682	0.006	0.0739	135,385
Sheep/goats for fattening	20	500	0.0682	0.006	0.0739	135,385

[32]Increases in absolute liver weights of rats (at doses of 100 mg/kg of higher in males and at doses of 300 mg/kg of higher in females) and mice (at 30, 100 and 300 mg/kg in males and at 300 mg/kg in females) and the increase in testis weight of rats administered 1000 mg/kg.

[33]Cytologic alteration, cytomegaly, Kupffer cell pigmentation, bile duct hyperplasia and foci of cellular alteration.

[34]Incidences of atrophy and chronic inflammation of the mucosa of the glandular stomach were significantly increased in rats administered 300 or 1000 mg/kg; the incidences of lesions of the glandular stomach were increased in one or more groups administered 30 mg/kg or greater.

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TABLE 7 (Continued)

Animal category	Daily feed intake g DM/kg bw	Use level in feed[1] mg/kg	Methyleugenol intake[2] µg/kg bw per day	Estragole intake[2]	Combined intake[2]	MOE[3,4] –
Horses for fattening	20	500	0.0682	0.006	0.0739	135,385
Rabbits for fattening	50	500	0.1705	0.014	0.1847	54,154
Salmonids	18	500	0.0597	0.005	0.0646	154,725

Abbreviations: bw, body weight; DM, dry matter; MOE, margin of exposure.

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

[2]The intake value of methyleugenol and estragole is calculated at the highest analysed concentrations of 0.0006% and 0.00005%, respectively.

[3]The group MOE for long-living and reproductive animals is calculated as the ratio of the reference point ($BMDL_{10}$ of 22.2 mg/kg bw per day) to the combined intake.

[4]The group MOE for short living animals (species for fattening) is calculated as the ratio of the reference point (NOAEL of 10 mg/kg bw per day) to the combined intake.

When the estimated combined exposures of long-living and reproductive animals are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day, a MOE > 10,000 is obtained for long-living and reproductive animals (Table 7). In accordance with the EFSA Statement on the Margin of Exposure (EFSA Scientific Committee, 2012), the use of an additive in feed is considered of low concern for long-living and reproductive animals if the MOE is > 10,000. Consequently, the FEEDAP Panel considers it very unlikely that the use of that feed additive will induce adverse effects during their lifetime.

For short-living animals (species for fattening), the magnitude of the MOE is > 100 (Table 7) and is of no safety concern, when comparing the exposure to the reference point of 10 mg methyleugenol/kg bw per day for non-neoplastic endpoints.

Overall evaluation

When considering the assessment of the individual components or groups of components of basil tincture, the lowest concentrations of the additive considered very unlikely to induce adverse effects in long-living and reproductive animals and of no safety concern for short-living animals (species for fattening) were derived for the assessment group flavonoids (see Table 5). These levels are extrapolated to physiologically related species. For the other species not considered, the calculated maximum level of 65 mg additive/kg complete feed is applied.

No specific proposals have been made by the applicant for the use of basil tincture 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 very unlikely to induce adverse effects in long-living and reproductive animals and of no safety concern for short-living animals (species for fattening) when consumed via feed alone.

3.4.1.6 | *Conclusions on safety for the target species*

The FEEDAP Panel considers that the use of basil tincture is very unlikely to induce adverse effects in long-living and reproductive animals and is of no safety concern for short-living animals (species for fattening) up to the maximum concentrations in complete feed summarised in Table 8.

TABLE 8 Concentrations of basil tincture in complete feed (mg/kg) considered very unlikely to induce adverse effects in long-living and reproductive animals and of no safety concern for short-living animals (species for fattening).

Animal categories	Concentration (mg/kg complete feed)[1]
Turkeys for fattening	87
Chickens for fattening and minor poultry for fattening	65
Laying hens and other laying/reproductive birds including animals reared for laying/reproduction and ornamental birds	97
Piglets and piglets of minor porcine species including animals reared for reproduction	116
Pigs for fattening and other porcine species for meat production	140
Sows and other porcine species for reproduction	170
Veal calves (milk replacer)	291
Sheep/goats	256
Cattle for fattening, other ruminants for fattening or reared for milk production/reproduction and camelids at the same physiological stage	256
Dairy cows and other ruminants and camelids for milk production or reproduction	166
Horses and other equids	256
Rabbits and other leporids	102
Salmonids and minor fin fish	292

(Continues)

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TABLE 8 (Continued)

Animal categories	Concentration (mg/kg complete feed)[1]
Dogs	307
Cats	256
Ornamental fish	500
Other species	65

[1]Complete feed containing 88% dry matter, milk replacer 94.5% dry matter.

The FEEDAP Panel considers that the use of basil tincture in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered very unlikely to induce adverse effects in long-living and reproductive animals and of no safety concern for short-living animals (species for fattening) when consumed via feed alone.

3.4.2 | Safety for the consumer

O. basilicum L. (basil) and its preparations are added to a wide range of food categories for flavouring purposes. Although individual consumption figures are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites intake values of 1.54 mg/kg bw per day for basil and 0.00073 mg/kg bw per day for basil oil. Fenaroli also reports use levels in food and beverages in the range of 3 mg/kg up to 3216 mg/kg for basil and in the range of 2 mg/kg up to 294 mg/kg for basil oil.

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, the data available for 1,8-cineole, camphor, eugenol and thujones indicate that they are absorbed, metabolised and rapidly excreted and are not expected to accumulate in animal tissues and products (EFSA FEEDAP Panel, 2011, 2012a, 2016b, 2021b). Similarly, the phenolic compounds including flavonoids, will be readily metabolised and excreted and are not expected to accumulate in animal tissues and products.

The results of in vitro kinetic studies in the target species combined with physiologically based kinetic (PBK) modelling indicated that the carry-over of methyleugenol and estragole and their corresponding 1′-hydroxy metabolites to tissues and products (e.g. eggs, milk) is limited due to rapid elimination and a decline in concentrations in edible tissues (Noorlander et al., 2024). Since methyleugenol and estragole occur in the additive at trace concentrations, residues in food products of animal origin are unlikely.

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

No safety concern would be expected for the consumer from the use of basil tincture up to the maximum proposed use level in feed.

3.4.3 | 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[35] concerning the presence of ethanol in the tincture.[36]

The additive contains camphor, 1,8-cineole, and eugenol compounds for which hazards for skin and eye contact and respiratory exposure were recognised (EFSA FEEDAP Panel, 2012b, 2016b). 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 and estragole may occur. Therefore, to reduce the risk, the exposure of the users should be minimised.

3.4.4 | Safety for the environment

O. basilicum is a species native to and widely distributed in Europe, where it is cultivated for commercial, culinary and ornamental purposes.

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

[35]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, pp. 1–1355.*

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

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3.5 | Efficacy

Basil (*O. basilicum* L.) and its oil are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference numbers 2118 and 2119, respectively.

Since basil 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.

4 | CONCLUSIONS

Basil tincture from *O. basilicum* L. 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 basil tincture which contains ≤ 6 mg/kg (0.0006%) methyleugenol and ≤ 0.5 mg/kg (0.00005%) estragole and is produced from the leaves of *O. basilicum*.

The concentrations in complete feed of basil tincture, which are considered very unlikely to induce adverse effects in long-living and reproductive animals and of no safety concern for short-living animals (species for fattening) are summarised as follows:

Animal categories	Concentration (mg/kg complete feed)[1]
Turkeys for fattening	87
Chickens for fattening and minor poultry for fattening	65
Laying hens and other laying/reproductive birds including animals reared for laying/reproduction and ornamental birds	97
Piglets and piglets of minor porcine species including animals reared for reproduction	116
Pigs for fattening and other porcine species for meat production	140
Sows and other porcine species for reproduction	170
Veal calves (milk replacer)	291
Sheep/goats	256
Cattle for fattening, other ruminants for fattening or reared for milk production/reproduction and camelids at the same physiological stage	256
Dairy cows and other ruminants and camelids for milk production or reproduction	166
Horses and other equids	256
Rabbits and other leporids	102
Salmonids and minor fin fish	292
Dogs	307
Cats	256
Ornamental fish	500
Other species	65

[1]Complete feed containing 88% dry matter, milk replacer 94.5% dry matter.

The FEEDAP Panel considers that the use of the basil tincture in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered very unlikely to induce adverse effects in long-living and reproductive animals and of no safety concern for short-living animals (species for fattening) when consumed via feed alone.

No safety concerns were identified for the consumer and the environment from the use of the additive in animal feed.

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

Since the leaves of *O. basilicum* 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.

5 | RECOMMENDATION

The specifications should ensure that basil tincture contains ≤ 0.0006% methyleugenol and ≤ 0.00005% estragole.

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6 | DOCUMENTATION PROVIDED TO EFSA/CHRONOLOGY

Date	Event
23/11/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 01 – Lamiales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)
03/01/2011	Reception mandate from the European Commission
06/01/2011	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*
08/01/2013	Reception of supplementary information from the applicant – Scientific assessment remains suspended
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
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
27/02/2019	Partial withdrawal of application for the following additives: thyme leaves gratiola tincture, spike lavender oil, melissa oil, pennyroyal oil, basil oil and savory summer oil
30/06/2021	EFSA informed the applicant that the evaluation process restarted
08/07/2021	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*
28/09/2023	Partial withdrawal of the application for the following additive: Spanish marjoram oil
04/04/2024	Reception of supplementary information from the applicant (partial dataset: basil tincture) – Scientific assessment remains suspended
08/07/2024	Partial withdrawal of the application for the following additives: lilac chastetree extract and savory summer tincture
17/07/2024	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*
26/08/2024	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives. Partial report related to seven additives: Spanish sage oil, peppermint oil, thymus origanum oil, patchouli oil, clary sage oil, lavender oil and sage oil
27/08/2024	Reception of supplementary information from the applicant (letter of agreement)
16/12/2024	Partial withdrawal of the application for the following additives: devils claw extract (wb), balm leaves extract (sb), olive extract (sb)
14/01/2025	Reception of supplementary information from the applicant (partial dataset: basil tincture, lavender tincture, peppermint tincture, sage tincture and wild thyme tincture) – Scientific assessment remains suspended
26/05/2025	The application was split and a new EFSA-Q-2025-00324 was assigned to the additive included in the present assessment
18/06/2025	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives. Partial report related to 11 additives: cornmint oil, spearmint oil, thyme oil, rosemary oil, marjoram oil, rosemary tincture, basil tincture, lavender tincture, peppermint tincture, sage tincture and wild thyme tincture.
23/06/2025	Scientific assessment re-started for the additive included in the present assessment
24/06/2025	Opinion adopted by the FEEDAP Panel on basil tincture (EFSA-Q-2025-00324). End of the Scientific assessment for the additive included in the present assessment. The assessment of other additives in BGD 01 is still ongoing

ABBREVIATIONS

ADME	absorption, distribution, metabolism and excretion
AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
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
CoE	Council of Europe
DM	dry matter
EEIG	European economic interest grouping
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavour and Extract Manufactures Association

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FFAC	Feed Flavourings authorisation Consortium of FEFANA (EU Association of Specialty Feed Ingredients and their Mixtures)
FLAVIS	The EU Flavour Information System
GAE	gallic acid equivalent
GC-FID	gas chromatography-flame ionisation detection
HACCP	Hazard Analysis and Critical Control Points
HPLC	high-performance liquid chromatography
HPTLC	high-performance thin layer chromatography
JECFA	Joint FAO/WHO Expert Committee of Food Additives
LOD	limit of detection
MOE	margin of exposure
MOE(T)	combined (total) margin of exposure
NOAEL	no observed adverse effect level
PBK	physiologically based kinetics
PCB	polychlorinated biphenyl
PhEur	European Pharmacopoeia
SC	EFSA Scientific Committee
TTC	threshold of toxicological concern
UV	ultraviolet detection
WHO	World Health Organization

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REQUESTOR

European Commission

QUESTION NUMBER

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