SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 5 May 2021

doi: 10.2903/j.efsa.2021.6623

Safety and efficacy of a feed additive consisting of an essential oil from the fruits of *Litsea cubeba* (Lour.) Pers. (litsea berry oil) for use in all animal species (FEFANA asbl)

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of an essential oil from the fruits of *Litsea cubeba* (Lour.) Pers. (litsea berry oil), when used as a sensory additive (flavouring) in feed and water for drinking for all animal species. The FEEDAP Panel concluded that litsea berry oil is safe up to the maximum proposed use level of 125 mg/kg complete feed for ornamental fish. For the other species, the calculated safe concentration in complete feed are 11 mg/kg for chicken for fattening, 16 mg/kg for laying hen, 14 mg/kg for turkey for fattening, 19 mg/kg for piglet, 23 mg/kg for pig for fattening, 28 mg/kg for lactating sow, 48 mg/kg for veal calf (milk replacer), 43 mg/kg for cattle for fattening, sheep, goat and horse, 28 mg/kg for dairy cow, 17 mg/kg for rabbit, 47 mg/kg for salmon, 50 mg/kg for dog and 8.5 mg/kg for cat. The FEEDAP Panel also concluded that the use of litsea berry oil at the maximum proposed use level in water for drinking of 1 mg/kg is safe for all animal species. Simultaneous use in feed and water for drinking may lead to the maximum safe dose being exceeded. No concerns for consumer safety were identified following the use of the additive up to the highest safe use level in feed for the target animals. The essential oil under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser. The use of the additive in animal feed under the proposed conditions was not expected to pose a risk for the environment. Litsea berry oil is recognised to flavour food. Since its function in feed would be essentially the same as that in food, no further demonstration of efficacy was considered necessary.

Keywords: sensory additives, flavouring compounds, *Litsea cubeba* (Lour.) Pers., litsea berry oil, geranial, neral, component-based approach

Requestor: European Commission

Question number: EFSA-Q-2021-00132

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgements: The Panel wishes to acknowledge the contribution of Jaume Galobart and Jordi Tarres-Call to this opinion.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Fašmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brantom P, Chesson A, Westendorf J, Manini P, Pizzo F and Dusemund B, 2021. Scientific Opinion on the safety and efficacy of a feed additive consisting of an essential oil from the fruits of *Litsea cubeba* (Lour.) Pers. (litsea berry oil) for use in all animal species (FEFANA asbl). EFSA Journal 2021;19(6):6623, 22 pp. https://doi.org/10.2903/j.efsa.2021.6623

ISSN: 1831-4732

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

Table of contents

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 seven years after the entry into force of this Regulation.

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

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

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

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

1.2. Additional information

Litsea berry oil from *Litsea cubeba* (Lour.) Pers. 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.

Many of the individual components of litsea berry oil have been already assessed as chemically defined flavourings for use in feed and food by the FEEDAP Panel, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) and the EFSA Panel on Food Additives and Flavourings (FAF). The list of flavouring compounds together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000[5] and the corresponding EFSA opinion is given in Table 1.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[2] On 13/03/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1050 Brussels, Belgium.

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

[4] Accepted name: *Litsea cubeba* (Lour.) Pers., synonym *Litsea cubeba* Lour., *Litsea cubeba* var. *cubeba*.

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

Table 1: Flavouring compounds already assessed by EFSA as chemically defined flavourings, grouped according to the chemical group (CG) as defined in Commission Regulation (EC) No 1565/2000, with indication of the EU Flavour Information System (FLAVIS) number and the corresponding EFSA opinion. They are currently authorised for food[6] and feed[7] uses unless otherwise indicated

CG	Chemical group	Product – EU register name (common 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 with esters containing α, β-unsaturated alcohol and acetal containing α, β-unsaturated alcohols or aldehydes	Geraniol	02.012	2016a
		(*Z*)-Nerol	02.058	
		Neral	05.170	
		trans-3,7-Dimethylocta-2,6-dienal (geranial)	05.188	
		Geranic acid	08.081	
		Geranyl acetate	09.011	
04	Non-conjugated and accumulated unsaturated straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters	Citronellal	05.021	2016b
05	Saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols	6-Methyhept-5-en-2-one	07.015	2015a
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	
		α-Terpinyl acetate[(a)]	09.015	JECFA
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	Sabinene hydrate[(a)]	02.085	JECFA
		Borneol	02.016	2016c
		d,l-Bornyl acetate	09.017	
16	Aliphatic and alicyclic ethers	1,8-Cineole	03.001	2012b, 2021
23	Benzyl alcohols/aldehydes/acids/esters/acetals	Methyl salicylate	09.749	2012c
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	Limonene[(a),(b)]	01.001	2008, AFC
		1-Isopropyl-4-methylbenzene (p-cymene)	01.002	2015b
		Terpinolene	01.005	
		α-Phellandrene	01.006	
		α-Terpinene	01.019	
		γ-Terpinene	01.020	
		Pin-2(10)-ene (β-pinene)	01.003	2016d
		Pin-2(3)-ene (α-pinene)	01.004	
		β-Caryophyllene	01.007	
		Myrcene	01.008	
		Camphene	01.009	
		3,7-Dimethyl-1,3,6-octatriene (β-ocimene)[(c)]	01.018	

[6] 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.

[7] 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

CG	Chemical group	Product – EU register name (common name)	FLAVIS no	EFSA opinion,* Year
		δ-Cadinene[(a),(d)]	01.021	2011, CEF
		β-Bisabolene[(a)]	01.028	
		δ-Germacrene[(a),(d)]	01.042	
		3,7,10-Humulatriene[(a),(d)]	01.043	
		4(10)-Thujene (sabinene)[(a)]	01.059	2015a, CEF
		cis-β-Ocimene[(a)]	01.064	
		β-Farnesene[(a)]	01.041	
32	Epoxides	β-Caryophyllene epoxide[(a)]	16.043	2014, CEF

*: FEEDAP opinion unless otherwise indicated.
(a): Evaluated for use in food. According to Regulation (EC) 1565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.
(b): JECFA and EFSA evaluated d-limonene [01.045] (EFSA, 2008). d-Limonene [01.045] and l-limonene [01.046] were also evaluated for use in feed (EFSA FEEDAP Panel, 2015b).
(c): β-Ocimene [01.018], as a mixture of (E)- and (Z)-isomers, containing 50-70% (E)-isomer and 17-17% (Z)-isomer, was evaluated.
(d): Evaluated applying the 'Procedure' described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[8] in support of the authorisation request for the use of litsea berry oil as a feed additive.

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.

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings. The applicant submitted a written agreement to refer to the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 6.[9]

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the 18 compounds from botanically defined flavourings Group (BDG 06) – Laurales, Magnoiales, Piperales. The Executive Summary of the EURL report can be found in Annex A.[10]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of litsea berry oil is in line with the principles laid down in Regulation (EC) No 429/2008[11] and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 2009), Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012d), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012e), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 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 document on harmonised methodologies for

[8] FEED dossier reference: FAD-2010-0218.
[9] Technical dossier/Supplementary information/Letter dated 29/4/2021.
[10] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2010-0218?search&form-return
[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.

human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b) and Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee, 2019c).

3. Assessment

The additive under assessment, litsea berry oil, is an essential oil obtained by steam distillation from the fruits of *Litsea cubeba* (Lour.) Pers. It is intended for use as a sensory additive (functional group: flavouring compounds) in feed and water for drinking for all animal species.

3.1. Origin and extraction

L. cubeba, sometimes referred to as May Chang or the mountain pepper, is a small tree or shrub belonging to the family Lauraceae. It is native to mountainous regions of China and parts of SE Asia but is now cultivated commercially in China, Japan and Indonesia, largely for the production of the essential oil from its fruits. The berry-like fruits are consumed as a food and virtually all parts of the plant find use in traditional medicine (Kamle et al., 2019).

The essential oil is extracted from the fruit by steam distillation and then separated from the aqueous phase by decantation.

3.2. Characterisation

3.2.1. Characteriation of litsea berry oil

The essential oil under assessment is a pale yellow to amber clear mobile liquid with a characteristic fresh aroma (citral). In six batches of the additive (all originating from China), the density (20°C) ranged between 881 and 885 kg/m^3 (specification: 872–895 kg/m^3), the refractive index (20°C) between 1.488 and 1.490 (specification: 1.483–1.485) and the specific optical rotation (at 20°C, four batches) between 9 and 11.[12] Litsea berry oil is identified with the single Chemical Abstracts Service (CAS) number 68855-99-2, the European Inventory of Existing Chemical Substances (EINECS) number 290-018-7,[13] the Flavor Extract Manufacturers Association (FEMA) number 3846 and the Council of Europe (CoE) number 491.

The product specifications are based on the standards developed by the International Organisation for Standardization (ISO) 3214:2000 for litsea berry oil,[14] which were adapted to reflect the concentrations of the main volatile components, analysed by gas chromatography with flame ionisation detection (GC-FID) and expressed as % of gas chromatographic peak area (% GC area). These components are geranial (36–45%) neral (25–35%, selected as phytochemical marker), limonene (9–15%), linalool (0.4–3%) and geraniol (1–4%). Analysis of six batches of the additive analysed by GC-FID showed compliance with these specifications.[15] When analysed by gas chromatography–mass spectrometry (GC–MS) these five compounds account for about 82.3% on average (range 74.8–85.2%) of the % GC area[16] (Table 2).

Table 2: Major constituents of the essential oil from the fruits of *Litsea cubeba* (Lour.) Pers. as defined based on ISO standard (3214:2000): specifications and batch to batch variation based on the analysis of six batches. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent			% GC area		
EU register name	**CAS no**	**FLAVIS no**	**Specification**	**Mean[(a)]**	**Range**
Geranial	141-27-5	05.188	36–45	36.35	30.3–39.4
Neral	106-26-3	05.170	25–35	29.77	26.4–31.6

[12] Technical dossier/Supplementary information March 2020/Annex_II_ SIn_Reply_litsea_berry_oil_COA_chromatograms.
[13] Further registry numbers were found at https://echa.europa.eu/home: 'Litsea cubeba oil': CAS 68855-99-2; EINECS 614-741-8 'Litsea cubeba, ext.': CAS 90063-59-5; EINICS 290-018-7.
[14] Technical dossier/Supplementary information March 2020/Annex III_SIn_Reply_litsea_berry_oil_ISO_3214_2000.
[15] Technical dossier/Supplementary information March 2020/SIn reply_litsea_berry oil/GC-FID analysis: geranial (38.8–41.2%), neral (29.5–31.4%), d-limonene (11.7–12.8%), linalool (1.1–2.5%), geraniol (0.9–1.6%).
[16] The concentration of geranial determined by GC-MS was underestimated and below the proposed specification in two batches of the additive.

Constituent			% GC area		
EU register name	**CAS no**	**FLAVIS no**	**Specification**	**Mean[(a)]**	**Range**
Limonene	138-86-3	01.001	9–15	12.98	11.7–14.2
Linalool	78-79-6	02.013	0.4–3	1.92	1.03–4.12
Geraniol	106-24-1	02.012	1–4	1.32	0.95–2.16
Total				82.3	74.8–85.2

CAS no.: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.
(a): Mean calculated on six batches.

The applicant provided the full characterisation of the six batches obtained by GC-MS.[17] In total, up to 110 peaks were detected in the chromatogram, 50 of which were identified and accounted on average for 99.8% (99.4–100%) of the % GC area. Besides the five compounds indicated in the product specifications, 21 other compounds were detected at individual levels > 0.1% and are listed in Table 3. These 26 compounds together account on average for 99.1% (98.3–99.6%) of the % GC area. The remaining 24 compounds (ranging between 0.01% and 0.09%) and accounting for 0.71% are listed in the footnote.[18]

Table 3: Other constituents of the essential oil from the fruits of *Litsea cubeba* (Lour.) Pers. accounting for > 0.1% of the composition (based on the analysis of six batches) not included in the specification. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent			% GC area	
EU register name	**CAS no**	**FLAVIS no**	**Mean[(a)]**	**Range**
(*E*)-Isocitral[(b)] (isogeranial)	72203-98-6	–	2.22	1.44–3.37
α-pinene (pin-2(3)-ene)	80-56-8	01.004	1.65	1.40–1.99
Myrcene	123-35-3	01.008	1.55	0.89–2.23
Sabinene (4(10)-thujene)	3387-41-5	01.059	1.53	0.87–1.98
6-Methylhept-5-en-2-one	110-93-0	07.015	1.48	0.49–3.18
β-Caryophyllene	87-44-5	01.007	1.46	0.95–2.37
Citronellal	106-23-0	05.021	1.25	0.57–2.18
1,8-Cineole	470-82-6	03.001	1.25	0.97–2.02
β-Pinene (pin-2(10)-ene)	127-91-3	01.003	1.16	0.97–1.41
(*Z*)-Isocitral[(b)] (isoneral)	72203-97-5	–	0.97	0.75–1.35
Nerol	106-25-2	02.058	0.75	0.44–1.21
trans-1-Methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol	7212-40-0	–	0.56	0.02–1.64
α-Terpineol	98-55-5	02.014	0.52	0.35–0.83
Camphene	79-92-5	01.009	0.36	0.27–0.47
exo-Isocitral[(b)]	55050-40-3	–	0.26	0.12–0.62
4-Terpinenol	562-74-3	02.072	0.24	0.13–0.29
Bicyclogermacrene	67650-90-2	–	0.15	0.06–0.22
Borneol	507-70-0	02.016	0.15	0.11–0.19
Geranic acid	459-80-3	08.081	0.12	0.08–0.17
3,7,10-Humulatriene	6753-98-6	01.043	0.11	0.06–0.19
α-Copaene	3856-25-5	–	0.10	0.05–0.14

[17] Technical dossier/Supplementary information April 2020/Annex_II_ SIn_Reply_litsea_berry_oil_COA_chromatograms.
[18] Additional constituents: constituents (n = 12) between < 0.1% and ≥ 0.05%: terpinolene, β-elemene, geranyl acetate, rose furan epoxide, β-bisabolene, bornyl acetate, γ-terpinene, methyl salicylate, δ-terpineol, cis-3,7-dimethyl-1,3,6-octatriene (Z β-ocimene), α-phellandrene, β-caryophyllene epoxide; constituents (n = 12) between < 0.05 and > 0.01%: α-thujene, cis-sabinene hydrate, α-terpinyl acetate, spathulenol, α-terpinene, β-farnesene, 1-isopropyl-4-methylbenzene, cis-limonene epoxide, trans-limonene epoxide, δ-cadinene, trans-3,7-dimethyl-1,3,6-octatriene (trans β-ocimene) and germacra-1(10),4(14),5-triene.

Constituent			% GC area	
EU register name	**CAS no**	**FLAVIS no**	**Mean[(a)]**	**Range**
Total			17.85	13.74–24.24

EU: European Union; CAS no. Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.
(a): Mean calculated on six batches.
(b): Structurally related to citral.

The applicant performed a literature search regarding substances of concern and chemical composition of the plant species *L. cubeba* and its preparations.[19] No substances of concern were identified. The presence of safrole (1.12%) was reported in an essential oil from the fruit of *L. cubeba* (Wang and Liu, 2010), but was not detected in the additive under assessment (LOD: 0.01%).

3.2.2. Impurities

The applicant makes reference to the 'periodic testing' of some representative flavourings premixtures for heavy metals (mercury, cadmium and lead), arsenic, fluoride, dioxins and polychlorinated biphenyls (PCBs), organo-chloride pesticides, organo-phosphorous pesticides, aflatoxins B1, B2, G1, G2 and ochratoxin A. However, no data have been provided. Since litsea berry oil is produced by steam distillation, the likelihood of any measurable carry-over of heavy metals is low except for mercury.

3.2.3. Shelf-life

The typical shelf-life of the additive is stated to be at least 12 months, when stored in tightly closed containers under standard conditions (in a cool, dry place protected from light).[20]

3.2.4. Conditions of use

Litsea berry oil is intended to be added to feed and water for drinking for all animal species without a withdrawal time. The maximum proposed use level in complete feed for the different target species are reported in Table 4. The proposed use level in water for drinking is 1 mg/kg.[21]

Table 4: Conditions of use for the essential oil from the fruit of *Litsea cubeba* (Lour.) Pers.: maximum proposed use levels in complete feed for the different target species

Animal category	Maximum use level (mg/kg complete feed)
Chicken for fattening	12
Laying hens	18
Turkey for fattening	16
Piglet	21.5
Pig for fattening	26
Sow lactating	31.5
Veal calf (milk replacer)	51
Cattle for fattening	48
Dairy cow	31
Sheep/goat	48
Horse	48
Rabbit	19
Salmon	55
Dog	57.5
Cat	48
Ornamental fish	125

[19] Technical dossier/Supplementary information April 2020/Literature search_litsea_berry_oil.
[20] Technical dossier/Section II.
[21] Technical dossier/Supplementary information July 2020.

3.3. Safety

The assessment of safety of litsea berry oil is based on the maximum use levels proposed by the applicant.

Many of the components of litsea berry oil, accounting for about 84% of the % GC peak areas (97% when considering limonene), have been previously assessed and considered safe for use as flavourings, and are currently authorised for food[6] and feed[7] uses. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see section 1.2).

Three compounds, δ-cadinene [01.021], germacra-1(10),4(14),5-triene [01.042] and 3,7,10-humulatriene [01.043], have been evaluated in FGE25.Rev2 (EFSA CEF Panel, 2011) by applying the procedure described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). For these compounds, for which there is no concern for genotoxicity, EFSA requested additional subchronic toxicity data (EFSA CEF Panel, 2011, 2015b). In the absence of such toxicological data, the EFSA CEF Panel was unable to complete its assessment. As a result, these compounds are not authorised for use as flavours in food. In the absence of toxicity data, the FEEDAP Panel applies the threshold of toxicological concern (TTC) approach or read-across from structurally related substances.

Several volatile components accounting for < 0.5% of the % GC area have not been previously assessed for use as flavourings. The FEEDAP Panel notes that they are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 3, 6, 8 and 31 and a similar metabolic and toxicological profile is expected. These lipophilic compounds are expected to be rapidly absorbed from the gastrointestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2012a, 2016a,c,d). In particular, (*Z*)-isocitral, (*E*)-isocitral and exo-isocitral are considered sufficiently structurally related to compounds already evaluated in CG 3 for use in food and/or feed as flavourings to consider that there is no concern for genotoxicity.

The following sections focus on those compounds not previously assessed or not structurally related to flavourings previously assessed, based on the evidence provided by the applicant in the form of literature searches and Quantitative Structure-Activity Relationship (QSAR) analysis. The safety of 6-methylhept-5-en-2-one [07.015] has also been reviewed, as the FEEDAP Panel identified a toxicological study submitted for the evaluation of CG 5 (BASF AG, 2002, as reported in OECD SIDS, 2003), which was not considered at the time of the previous assessment (EFSA FEEDAP Panel, 2015a).

3.3.1. Genotoxicity

For fully defined mixtures, the EFSA Scientific Committee (EFSA SC) recommends applying a component-based approach, i.e. assessing all components individually for their genotoxic potential (EFSA Scientific Committee, 2019b).

The genotoxicity of (+)-limonene epoxide, investigated in the Ames test and the SOS Chromotest, gave negative results (Basler et al., 1989 as referenced in EFSA CEF Panel, 2014). When V79 Chinese hamster cells were incubated with (+)-limonene epoxide, no increase in sister chromatid exchange was observed (von der Hude et al., 1991, as referenced in EFSA CEF Panel, 2014).

The genotoxic potential for seven substances (spathulenol, bicyclogermacrene, α-copaene, α-thujene germacra-1(10),4(14),5-triene, 3,7,10-humulatriene and rose furan epoxide) was predicted using the QSAR Toolbox.[22] No structural alerts were found with the exception of rose furan epoxide due to the presence of the epoxide. In this case, predictions of Ames mutagenicity were made by 'read-across' analyses of data available for similar substances to the target compounds (i.e. analogues obtained by categorisation). Categories were defined using general mechanistic and endpoint profilers[23] as well as empirical profilers.[24] Ames test (with and without S9) read across predictions

[22] Technical dossier/Supplementary information April 2020/Annex_VI_Sin reply_litsea_berry_oil_QSAR.

[23] Mechanistic and endpoint profilers: 'US-EPA New chemical Categories'; 'DNA binding by OASIS'; 'NA binding by OECD'; 'Protein binding by OASIS'; 'Protein binding by OECD'; 'Aquatic toxicity classification by ECOSAR'; 'in vitro mutagenicity (Ames test) alert'; 'in vivo mutagenicity (Micronucleus) alert'.

[24] Empirical profilers: 'organic functional groups', 'organic functional groups (nested)', 'organic functional groups (US EPA)' and 'organic functional groups, Norbert Haider'.

were found consistently negative for all categories of analogues.[25] On this basis, the alert raised for rose furan epoxide was discounted.

The Panel noted that rose furan epoxide is structurally related to rose furan, being the epoxide of rose furan (synonym: 3-methyl-2(3-methylbut-2-enyl)furan [13.148]). This additive was recently evaluated by the EFSA Panel on Food Additives and Flavourings (EFSA FAF Panel), which concluded that the genotoxicity concern for rose furan was ruled out and therefore there is 'no safety concern at the estimated levels of intake as flavouring substances based on the maximised survey-derived intake (MSDI) approach' (EFSA FAF Panel, 2021a,b).

The applicant also provided a structured database literature search,[19] which identified two references to the genotoxicity of two essential oils extracted from the berries of the Chinese *L. cubeba* (Luo et al., 2005; Gogoi et al., 2018). Only one of the two studies tested an oil showing similar composition as the oil under assessment (Luo et al., 2005).

In particular, Luo et al. (2005) tested a litsea berry oil extracted by distillation from the fresh fruits obtained in Guangxi, (China). The major ingredients were citral (66.80%), consisting predominantly of geranial (35.22%) and neral (31.58%), limonene (11.04%), 6-methyl-5-hep-ten-2-one (4.31%), β-linalool (2.90%), myrcene (1.66%), α-pinene (1.01%) and β-pinene (0.95%), followed by α-terpineol (0.81%), sabinene (0.49%), and β-caryophyllene (% not reported). The composition is similar to the essential oil under assessment. The essential oil was tested for the induction of gene mutations in bacteria and chromosome damage in ICR (Institute of Cancer Research) mice. The Ames test was performed in *Salmonella* Typhimurium strains TA98, TA100, TA1535 and TA1537 both in the absence and presence of metabolic activation applying the pre-incubation method. Four concentrations were tested ranging from 4 to 500 μg/plate. At concentrations above 500 μg/plate bacterial growth was restrained. No significant changes in the number of revertant colonies were observed with any strain and in any test conditions. The FEEDAP Panel notes that the sensitivity of *in vitro* genotoxicity tests for complex mixtures is limited (EFSA Scientific Committee, 2019b).

A chromosomal aberration test was carried out in ICR mice spermatocytes. Mice (five animals/group) were given litsea berry oil by oral gavage once a day for 5 consecutive days at either 0, 460 mg/kg (1/8 LD_{50}), 925 (1/4 LD_{50}) or 1,850 mg/kg body weight (bw) per day (1/2 LD_{50}). On day 12 of the study mice were euthanised. At least 100 spermatocytes per animal were analysed for the presence of structural and numerical chromosome aberrations. The frequencies of chromosomal aberrations in all the litsea berry oil dose groups were comparable to the concurrent solvent group.

In addition, a mouse bone marrow micronucleus test was performed in ICR male mice. Five animals per dose group received litsea berry oil by oral gavage twice at 30-h intervals at concentrations of 0, 185, 740 and 1,850 mg/kg bw per day, representing 1/20, 1/5 and 1/2 LD_{50}, respectively. Mice were euthanised 24 h after the second oral administration. Two thousand polychromatic erythrocytes per animal were scored for the frequency of micronuclei (MN) and the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) was calculated as measure of target tissue toxicity. Comparable frequencies of MN were observed in treated and control groups. No toxicity was observed in the bone marrow up to the top dose tested, corresponding to 1/2 LD_{50} (Luo et al., 2005).

In summary, there is no evidence for genotoxicity of litsea berry oil from investigation of its single components or the complex mixture.

3.3.2. Repeated-dose toxicity studies

A 90-day study (compliant with Organisation for Economic Co-operation and Development (OECD) TG 408) with 6-methylhept-5-en-2-one [07.015] was submitted for the evaluation of CG 5 (BASF AG, 2002, as reported in OECD SIDS, 2003) and is now considered for the present assessment. Four groups of Wistar rats (10/sex per dietary intake level) received 6-methylhept-5-en-2-one by gavage at doses of 0, 50, 200 and 1,000 mg/kg bw per day. The administration of 6-methylhept-5-en-2-one by gavage at all doses resulted in substance-related effects in all dosed groups. The target organs were kidney, liver and testes. At the highest dose tested, biomarkers of kidney and liver toxicity were altered, as well as the kidney weight (relative and absolute) and the number and the morphology of spermatozoa. Under the conditions of this study, the no observed adverse effect level (NOAEL) was 50 mg/kg bw per day for females due to an increase of 21% in platelet counts at 200 mg/kg bw per day

[25] Analogues: *trans*-β-propylstyrene-7,8-oxide, *trans*-β-ethylstyrene-7,8-oxide, cis-stilbene oxide, *p*-phenylstyrene oxide, 2-methyl-2-phenyloxirane (epoxides); benzofuran, 2,5-dimethylfuran, 2-ethylfuran, methylfuran, dibenzofuran (furans). Negative results were found for the closest related epoxide analogues: *trans*-β-propylstyrene-7,8-oxide, *trans*-β-ethylstyrene-7,8-oxide, *cis*-stilbene oxide.

(low observed adverse effect level (LOAEL)); and ≤ 50 mg/kg bw per day for males due to an increase of 12 and 14% in relative and absolute kidney weights. The kidney effects in all dose groups in the males were induced by accumulation of α-2-microglubulin (α2μ), which is known to be a rat specific phenomenon without a toxicological correlation in other species including humans. Since the effects on the kidney observed in male rats were not considered relevant, a NOAEL of 50 mg/kg bw per day was derived from this study.

The applicant provided three studies investigating acute and subchronic toxicity of Chinese litsea berry oil. An LD_{50} of 4,000 mg/kg was determined in an acute toxicity study performed with an essential oil whose composition is similar to that the additive under assessment (Luo et al., 2005). No adverse effects were reported in two 28-day studies in rats (Tubtim and Wasiksiri, 2007; Budin et al., 2012), however in both studies the composition of the test item was not reported, and it is not possible to assess the relevance of the results for the additive under assessment.

3.3.3. Safety for the target species

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

In the absence of toxicological data with the additive under assessment, the approach to the safety assessment of a mixture whose individual components are known is based on the safety assessment of each individual component (component-based approach). This approach requires that the mixture is sufficiently characterised. The individual components can be grouped into assessment groups, based on structural and metabolic similarity. The combined toxicity can be predicted using the dose addition assumption within an assessment group, taking into account the relative toxic potency of each component.

As the additive under assessment is sufficiently characterised (> 99.4%), the FEEDAP Panel applied a component-based approach to assess the safety for target species of the essential oil.

Based on considerations related to structural and metabolic similarities, the components were allocated to nine assessment groups, corresponding to the chemical groups (CGs) 3, 4, 5, 6, 8, 16, 23, 31 and 32, as defined in Annex I of Regulation (EC) No 1565/2000. For chemical group 31 ('aliphatic and aromatic hydrocarbons'), sub-assessment groups as defined in Flavouring Group Evaluation 25 (FGE.25) and FGE.78 are applied (EFSA CEF Panel, 2015a,b). The allocation of the components to the (sub-)assessment groups is shown in Table 5.

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

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification. For some components in the assessment group, toxicological data were available to derive NOAEL values. Structural and metabolic similarity among the components in the assessment groups were evaluated 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 group with no available NOAEL or, if sufficient evidence were available for members of a (sub-)assessment group, to derive a (sub-)assessment group NOAEL.

Toxicological data for subchronic studies, from which NOAEL values could be derived, were available for citral [05.020] in CG 3 (EFSA FEEDAP Panel, 2016a), citronellol [02.011] in CG 4 (EFSA FEEDAP Panel, 2016b), 6-methylhept-5-en-2-one [07.015] in CG 5 (see Section 3.3.2), terpineol [02.230] and linalool [02.013] in CG 6 (EFSA FEEDAP Panel, 2012a), 1,8-cineole in CG 16 (EFSA FEEDAP Panel, 2012b, 2021), methyl salicylate [09.749] in CG 23 (EFSA FEEDAP Panel, 2012c), myrcene [01.008], d-limonene [01.045], p-cymene [01.002] and β-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015b, 2016d), and β-caryophyllene epoxide in CG 32 (EFSA CEF Panel, 2014).

Considering the structural and metabolic similarities, read-across was applied using the NOAEL of 345 mg/kg bw per day for citral [05.020] to extrapolate to geranial [05.188], geraniol [02.012], geranic acid [08.081], geranyl acetate [09.011], neral [05.170] and nerol [02.058] in CG 3, and to the structurally related compounds (*Z*)-isocitral, (*E*)-isocitral and exo-isocitral. Since these aldehydes share the same C-skeleton with citral but are free of the α,β-unsaturated bond, they are expected to be less reactive than citral.

For the subgroup of terpinyl derivatives in CG 6, i.e. α-terpineol [02.072], terpinen-4-ol [02.072] and α-terpinyl acetate [09.015], the reference point was selected based on the NOAEL of 250 mg/kg bw per day available for terpineol [02.230] and d-limonene [01.045].

The NOAELs for the representative compounds of CG 31, myrcene [01.008], d-limonene [01.045] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within sub-assessment group II (*cis*-β-ocimene [01.064], trans-β-ocimene and β-farnesene [01.041]), group III (limonene [01.001], terpinolene [01.005], β-bisabolene [01.028], γ-terpinene [01.020], α-phellandrene [01.006] and α-terpinene [01.019]) and group V (α-pinene [01.004], sabinene [01.059], β-pinene [01.003], camphene [01.009], α-copaene, α-thujene and δ-cadinene [01.021]) (EFSA CEF Panel, 2015a, b). The same NOAEL value for sabinene [01.059] is applied to and *cis*-sabinene hydrate in CG 8.

For the remaining compounds, *trans*-1-methyl-4-(1-methyl vinyl)cyclohex-2-en-1-ol, (-terpineol and spathulenol in CG 6, borneol [02.016] and bornyl acetate [09.017] in CG 8, bicyclogermacrene, 3,7,10-humulatriene [01.043] and germacra-1(10),4(14),5-triene [01.042] in CG 31, rose furan epoxide, *cis*-limonene epoxide and *trans*-limonene epoxide in CG 32, toxicity studies and NOAEL values performed with the compounds under assessment were not available and read-across was not possible. Therefore, the TTC approach was applied (EFSA FEEDAP Panel, 2017b).

As the result of the hazard characterisation, a reference point was identified for each component in the assessment group based on the toxicity data available (NOAEL from *in vivo* toxicity study or read across) or from the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class (i.e. 3, 0.91 and 0.15 mg/kg bw per day, respectively, for Cramer Class I, II and III compounds). Reference points selected for each compound are shown in Table 5.

For risk characterisation, the margin of exposure (MOE) was calculated for each component as the ratio between the reference point and the exposure. For each assessment group, the combined (total) margin of exposure (MOET) was calculated as the reciprocal of the sum of the reciprocals of the MOE of the individual substances (EFSA Scientific Committee, 2019a). A MOET > 100 allowed for interspecies- and intra-individual variability (as in the default 10 × 10 uncertainty factor). The compounds resulting individually in an MOE > 50,000 were not further considered in the assessment group as their contribution to the MOE(T) is negligible. They are listed in the footnote.[26]

The approach to the safety assessment of litsea berry oil for the target species is summarised in Table 5. The calculations were done for chicken for fattening, the species with the highest ratio of feed intake/body weight and represent the worst-case scenario at the use level of 12 mg/kg complete feed.

Table 5: Compositional data, intake values, reference points and margin of exposure (MOE) for the individual components of litsea berry oil classified according to assessment groups

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS No**	**Max conc. in the oil**	**Max feed conc.**	**Intake[(a)]**	**Cramer class**	**NOAEL[(b)]**	**MOE**	**MOET**
Constituent	–	%	mg/kg	mg/kg bw per day	–	mg/kg bw per day	–	–
CG 3								
Geranial	05.188	39.40	4.728	0.4244	I	345	813	
Neral	05.170	31.60	3.792	0.3404	I	345	1,013	
(*E*)-Isocitral	–	3.37	0.404	0.0363	I	345	9,503	
Geraniol	02.012	2.16	0.259	0.0233	I	345	14,827	
(*Z*)-Isocitral	–	1.35	0.162	0.0145	I	345	23,722	
Nerol	02.058	1.21	0.145	0.0130	I	345	26,467	
MOET CG 3								405
CG 4								
Citronellal	05.021	2.18	0.262	0.0235	I	50	2,129	
CG 5								

[26] Compounds included in the assessment groups but not reported in the table: exo-citral, geranic acid and geranyl acetate (CG 3); 4-terpineol and α-terpinyl acetate (CG 6); *cis*-sabinene hydrate (CG 8); methyl salicylate (CG 23); β-farnesene and *trans*-β-ocimene (CG 31, II); terpinolene, β-elemene, β-bisabolene, γ-terpinene, α-phellandrene and α-terpinene (CG 31, III); *p*-cymene (CG 31,IVe); α-copaene, α-thujene and δ-cadinene (CG 31, V); β-caryophyllene epoxide (CG 32).

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS No**	**Max conc. in the oil**	**Max feed conc.**	**Intake[(a)]**	**Cramer class**	**NOAEL[(b)]**	**MOE**	**MOET**
6-Methylhept-5-en-2-one	07.015	3.18	0.382	0.0343	II	**50**	1,460	
CG 6								
Linalool	02.013	4.12	0.494	0.0444	I	**117**	2,636	
trans-1-methyl-4-(1-methyl vinyl)cyclohex-2-en-1-ol	–	1.64	0.197	0.0177	I	*3*	170	
α-Terpineol	02.014	0.83	0.100	0.0090	I	250	27,893	
δ-Terpineol	–	0.05	0.006	0.0005	III	*0.15*	278	
Spathulenol	–	0.06	0.007	0.0006	I	*3*	4,973	
MOET CG 6								100
CG 8								
Borneol	02.016	0.19	0.022	0.0020	I	*3*	1,497	
Bornyl acetate	09.017	0.13	0.015	0.0013	I	*3*	2,228	
MOET CG 8								895
CG 16								
1,8-Cineole	03.001	2.02	0.242	0.0218	II	100	4,595	
CG 31, II (Acyclic alkanes)								
Myrcene	01.008	2.23	0.268	0.0240	I	**44**	1,832	
β-*cis*-Ocimene	01.064	0.09	0.011	0.0010	I	44	45,892	
MOET CG 31, II								1,715
CG 31, III (Cyclohexene hydrocarbons)								
Limonene	01.001	14.20	1.704	0.1346	I	250	1,625	
CG 31, V (Bi-, tricyclic, non aromatic hydrocarbons)								
β-Caryophyllene	01.007	2.37	0.284	0.284	I	**222**	8,695	
α-Pinene	01.004	1.99	0.239	0.239	I	222	10,356	
Sabinene	01.059	1.98	0.238	0.238	I	222	10,408	
β-Pinene	01.003	1.41	0.169	0.169	I	222	14,615	
Camphene	01.009	0.47	0.056	0.056	I	222	44,033	
Bicyclogermacrene	–	0.22	0.027	0.027	I	*3*	1,249	
MOET CG 31, V								834
CG 31, VI								
3,7,10-Humulatriene	01.043	0.19	0.023	0.0021	I	*3*	1,450	
Germacra-1(10),4(14),5-triene	01.042	0.01	0.001	0.0001	I	*3*	25,316	
MOET CG 31, VI								1,372
CG 32 (epoxides)								
Rose furan epoxide		0.15	0.018	0.0016	III	*0.15*	91	
Cis-Limonene epoxide	–	0.03	0.003	0.0003	I	*3*	10,711	
Trans-limonene epoxide		0.02	0.003	0.0002	I	*3*	13,261	
MOET CG 32								90

(a): Intake calculations for the individual components are based on the use level of 12 mg/kg in feed for chicken for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point (NOAEL) to the intake. The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

(b): 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.

As shown in Table 5, for all the assessment groups, the MOET was ≥ 90. From the lowest MOET of 90 for chicken for fattening, the MOET was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 6.

Table 6: Combined margin of exposure (MOET) for the assessment group "Epoxides" (CG 32) calculated for the different target animal categories at the proposed use level and maximum safe use levels in feed calculated to ensure a MOET ≥ 100 (500 for cats)

Animal category	Body weight (kg)	Feed intake (g DM/day)	Proposed use level (mg/kg feed)	Lowest MOET	Maximum safe use level (mg/kg feed)[(a)]
Chicken for fattening	2	158	12	90	11
Laying hen	2	106	18	89	16
Turkey for fattening	3	176	16	90	14
Piglet	20	880	21.5	90	19
Pig for fattening	60	2,200	26	89	23
Sow lactating	175	5,280	31.5	90	28
Veal calf (milk replacer)	100	1,890	51	88	48
Cattle for fattening	400	8,000	48	89	43
Dairy cow	650	20,000	31	89	28
Sheep/goat	60	1,200	48	89	43
Horse	400	8,000	48	89	43
Rabbit	2	100	19	90	17
Salmon	0.12	2.1	55	86	47
Dog	15	250	57.5	87	50
Cat	3	60	48	89	8.5[(b)]
Ornamental fish	0.012	0.054	125	137	–

DM: dry matter.
(a): Complete feed containing 88% DM, milk replacer 94.5% DM.
(b): The MOET for cats is increased to 500 because of the reduced capacity of glucuronidation.

At the proposed use levels, the MOET was below the value of 100 for all species except ornamental fish. The maximum safe use levels in feed were calculated in order to ensure a MOET ≥ 100 for the different target species and > 500 for cats, considering their unusually low capacity for glucuronidation (Court and Greenblatt, 1997; Lautz et al., 2021). The calculated maximum safe levels in feed are shown in Table 6.

The applicant proposed a maximum use level in water for drinking of 1 mg/kg, which would ensure a lower exposure and is considered safe for all animal species. Simultaneous use in feed and water for drinking may lead to the maximum safe dose being exceeded.

Conclusions on safety for the target species

The FEEDAP Panel concludes that litsea berry oil is safe up to the maximum proposed use levels of 125 mg/kg complete feed for ornamental fish. For the other species, the calculated safe concentration in complete feed are 11 mg/kg for chicken for fattening, 16 mg/kg for laying hen, 14 mg/kg for turkey for fattening, 19 mg/kg for piglet, 23 mg/kg for pig for fattening, 28 mg/kg for lactating sow, 48 mg/kg for veal calf (milk replacer), 43 mg/kg for cattle for fattening, sheep, goat and horse, 28 mg/kg for dairy cow, 17 mg/kg for rabbit, 47 mg/kg for salmon, 50 mg/kg for dog and 8.5 mg/kg for cat.

The FEEDAP Panel also concludes that the use of litsea berry oil at the maximum proposed use level in water for drinking of 1 mg/kg is safe for all animal species.

Simultaneous use in feed and water for drinking may lead to the maximum safe dose being exceeded.

3.3.4. Safety for the consumer

Litsea berry oil is 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) reports use levels in food and beverages ranging from 2 mg/kg in meat products to 500 mg/kg in gelatines and puddings.

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

No data on residues in products of animal origin were made available for any of the constituents of the essential oil. However, the Panel recognises that the constituents of litsea berry oil are expected to be extensively metabolised and excreted in the target species (see Section 3.3.1). Therefore, a relevant increase of the uptake of the individual constituents by humans consuming products of animal origin is not expected.

The applicant applied the Feed Additives Consumer Exposure (FACE) and Food Additives Intake Model (FAIM) models to estimate the intake via the feed additive (assuming occurrence levels of litsea berry oil in animal meat and other animal products are anticipated to be equivalent to that ingested daily by the animal) and as a food additive. Estimated consumer daily exposure resulting from the use of litsea berry oil as a feed flavouring (maximum ranging from ca. 0.03 to 0.16 mg/kg per day considering a worse-case scenario) was considerably lower than estimated human daily exposure due to the use of litsea berry oil in food (ca. 0.6 to 1.8 mg/kg per day).[27]

It is recognised that the occurrence levels of litsea berry oil in food (taken from the blue books of the Council of Europe) used in the FAIM tool may not reflect real use levels. Nevertheless, as human exposure resulting from the use of litsea berry oil as a feed additive might also be considerably overestimated, the conclusion that it is unlikely that consumption of products from animals given the essential oil at the proposed maximum use level would significantly increase human background exposure is considered valid.

Consequently, no safety concern would be expected for the consumer from the use of litsea berry oil up to the highest safe use level in feed for the target animals.

3.3.5. Safety for user

No specific data were provided by the applicant regarding the safety of the additive for users.

The applicant produced a safety data sheet[28] for litsea berry oil, where hazards for users have been identified.

A review of essential oils (Tisserand and Young, 2014) described litsea berry oil (May Chang) as a dermal sensitiser with evidence of effects in both animals (Local Lymph Node Assay) and human dermatitis patients (Rudzki et al. 1976, as referenced in Tisserand and Young, 2014).

Lalko and Api (2006) investigated dermal sensitisation potential of litsea berry oil provided by commercial supplier (further details not provided but stated by the applicant to have a similar composition as that of litsea berry oil) using a local lymph node assay at concentrations of 2.5, 5, 10, 25, 50% w/v in 1:3 ethanol:diethyl phthalate. The estimated concentration required to elicit a positive effect was calculated to be 8.4% and thus, the authors concluded that the oil was a weak sensitiser.

It is stated by Tisserand and Young (2014) that undiluted oil is moderately irritating to rabbits and strongly irritating to the skin of pigs and mice. This irritant potential is also supported by evidence from the ECHA submission on essential oil of *Litsea*.

In an acute inhalation toxicity test, male and female Sprague Dawley (SD) rats were exposed to 2,500 ppm test oil and none of the rats died. When the test was continued at a concentration of 5,000 ppm with three SD rats of each sex one female died during the exposure period. Inhalation lethal concentration 50 (LC_{50}) value was approximately 12,500 ppm. According to the globally harmonised classification system, the hazard of the oil was class 5, which is the lowest toxic class (Luo et al., 2005).

Litsea berry oil is irritant to skin and thus presumed also irritant to eyes. The oil is also a dermal sensitiser. Although there is no evidence for significant respiratory toxicity it may also be assumed to be a respiratory sensitiser.

[27] Technical dossier/Supplementary information April 2020/SIn reply, Annex_V_ SIn_reply_litsea_berry_oil_safety_assessment and Annex_VII SIn_reply_litsea_berry_oil_FAIM_occurrence_data.

[28] Technical dossier/Supplementary Information April 2020/Annex_VIII_SIn reply_litsea berry_oil_MSDS. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1), serious eye damage/eye irritation (H319, category 2).

3.3.6. Safety for the environment

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

The major components (geranial, neral, limonene, linalool and geraniol) and additional 13 components (α-pinene, myrcene, 6-methylhept-5-en-2-one, β-caryophyllene, citronellal, 1,8-cineole, β-pinene, nerol, α-terpineol, camphene, 4-terpinenol, borneol and geranic acid) accounting together for 95% of the composition of the oil have been evaluated by EFSA as sensory additives for animal feed, they were considered to be safe for the environment at use individual levels higher than those resulting from the use of the essential oil in feed.

The remaining identified constituents of the essential oil are mainly aliphatic mono- or sesquiterpenes partially substituted with functional groups. They are structurally related to the substances evaluated by EFSA as CG 3 ((*Z*)-isocitral, (*E*)-isocitral and exo-isocitral) and CG 31 for use in animal feed (EFSA FEEDAP Panel, 2015a,b, 2016a–d) for which EFSA concluded that they were 'extensively metabolised by the target species (see Section 3.3) and excreted as innocuous metabolites or carbon dioxide'. Therefore, no risk for the safety for the environment is foreseen. Average feed levels of constituents of the essential oil are much lower than the use levels for substances belonging to CG 3 and 31.

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

3.4. Efficacy

Litsea berry oil is listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference number 3826.

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

4. Conclusions

Litsea berry oil is safe up to the maximum proposed use level of 125 mg/kg complete feed for ornamental fish. For the other species, the calculated safe concentration in complete feed are 11 mg/kg for chicken for fattening, 16 mg/kg for laying hen, 14 mg/kg for turkey for fattening, 19 mg/kg for piglet, 23 mg/kg for pig for fattening, 28 mg/kg for lactating sow, 48 mg/kg for veal calf (milk replacer), 43 mg/kg for cattle for fattening, sheep, goat and horse, 28 mg/kg for dairy cow, 17 mg/kg for rabbit, 47 mg/kg for salmon, 50 mg/kg for dog and 8.5 mg/kg for cat. The use of litsea berry oil at the maximum proposed use level in water for drinking of 1 mg/kg is safe for all animal species. Simultaneous use in feed and water for drinking may lead to the maximum safe dose being exceeded.

No concerns for consumer safety were identified following the use of the additive up to the highest safe use level in feed for the target animals.

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

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

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

5. Documentation as provided to EFSA/Chronology

Date	Event
05/11/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 06 – Laurales, Magnoliales, Piperales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG) and registered with Question number EFSA-Q-2010-01296
11/11/2010	Reception mandate from the European Commission
01/01/2011	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: EURL*

Date	Event
05/04/2011	Comments received from Member States
26/02/2013	EFSA informed the applicant (EFSA ref. 7150727) that, in view of the workload, the evaluation of applications on feed flavourings would be re-organised by giving priority to the assessment of the chemically defined feed flavourings, as agreed with the European Commission
27/06/2013	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives - Scientific assessment remains suspended
24/06/2015	Technical hearing during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products": data requirement for the risk assessment of botanicals
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
18/12/2018	EFSA informed the applicant that the scientific assessment restarted
07/02/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization, safety for the target species, safety for the consumer, safety for the user, safety for the environment*
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: cassia bark extract (sb), cinnamon bark oleoresin, laurel leaves extract/oleoresin, mace oil, nutmeg oleoresin, boldo extract (wb), boldo tincture and kawakawa tincture
29/04/2020	Reception of supplementary information from the applicant (partial submission)
01/07/2020	Reception of supplementary information from the applicant (partial submission)
12/03/2021	The application was split and a new EFSA-Q-2021-00132 was assigned to the preparation included in the present assessment. Scientific assessment re-started for the preparation included in the present assessment
05/05/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment for the preparation included in the present assessment

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Abbreviations

BDG	botanically defined group
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CDG	chemically defined group
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
CoE	Council of Europe
DM	dry matter
EEIG	European economic interest grouping
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FAF	EFSA Panel on Food Additives and Flavourings
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavor Extract Manufacturers Association
FFAC	Feed Flavourings authorisation Consortium of (FEFANA) the EU Association of Specialty Feed Ingredients and their Mixtures
FGE	Flavouring Group Evaluation
FLAVIS	the EU Flavour Information System
FL-No	FLAVIS number
GC	gas chromatography
GC-FID	gas chromatography with flame ionisation detector
GC–MS	gas chromatography–mass spectrometry
HPLC-UV	high-performance liquid chromatography methods with uv detection
ISO	International standard organization
LC_{50}	lethal concentration 50
LOAEL	low observed adverse effect level
LOD	limit of detection
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MOE	margin of exposure
MOET	combined margin of exposure (total)
MSDI	maximised survey-derived intake
NOAEL	no observed adverse effect level
OECD	Organization for Economic Co-operation and Development
PCB	polychlorinated biphenyl
QSAR	Quantitative Structure–Activity Relationship
RTL	retention time locking
SC	EFSA Scientific Committee
SD	Sprague Dawley
TTC	threshold of toxicological concern
UF	uncertainty factor
WHO	World Health Organization

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

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

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

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

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