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Safety and efficacy of a feed additive consisting of an essential oil from the flowers of *Cananga odorata* (Lam.) Hook.f. & Thomson (ylang ylang 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 flowers of *Cananga odorata* (Lam.) Hook.f. & Thomson (ylang ylang oil), when used as a sensory additive in feed and water for drinking for all animal species. The FEEDAP Panel concluded that the essential oil under assessment is safe up to the maximum proposed use levels in complete feed of 1 mg/kg for chickens for fattening, 1.5 mg/kg for laying hens, turkeys for fattening and rabbits, 2 mg/kg for piglets, 2.5 mg/kg for pigs for fattening, 3 mg/kg for sows, 4.5 mg/kg for cattle for fattening, sheep, goats and horses, 5 mg/kg for veal calves (milk replacer), fish, dogs and ornamental fish. For cats, the calculated safe concentration in complete feed is 1 mg/kg feed. The FEEDAP Panel considered that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed. No concerns for consumer safety were identified following the use of the additive up to the maximum proposed use level in feed. 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 of use was not expected to pose a risk for the environment. Ylang ylang 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.

Keywords: sensory additives, flavouring compounds, essential oil, *Cananga odorata* (Lam.), Ylang ylang oil, safety, component-based approach, β-caryophyllene, estragole

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Amendment note/erratum note: In Section 3.2.1 the specifications for β-caryophyllene have been modified as wronlgy reported in the text. An editorial correction was carried out that does not materially affect the contents or outcome of this scientific output. To avoid confusion, the original version of the output has been removed from the EFSA Journal, but is available on request.

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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 7 years after the entry into force of this Regulation.

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation/re-evaluation of 18 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. During the course of the assessment, this application was split, and the present opinion covers only one out of the 18 initial preparations under application: an essential oil from the flower of *Cananga odorata* (Lam.) Hook.f. & Thomson (ylang ylang 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 flowers of *C. odorata* (ylang ylang oil), when used under the proposed conditions of use (see Section 3.2.3).

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

1.2. Additional information

Ylang ylang oil from *C. odorata* (Lam) Hook f.& Thomson 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 ylang ylang oil have been already assessed as chemically defined flavourings for use in feed and food by the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 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

[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.

flavouring compounds currently authorised for food[4] and feed[5] uses together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000[6] and the corresponding EFSA opinion is given in Table 1.

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

CG	Chemical Group	Product (EU register name)	FLAVIS No	EFSA opinion*, Year
01	Straight-chain primary aliphatic alcohols/ aldehydes/ acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes	Nonanal	05.025	2013
		Butyl acetate	09.004	
		Hexyl acetate	09.006	
02	Branched-chain primary aliphatic alcohols/ aldehydes/ acids, acetals and esters with esters containing branched-chain alcohols and acetals containing branched-chain aldehydes	Isopentyl acetate	09.024	2012a
		2-Methylbutyl acetate	09.266	
03	a, β-Unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters	Geraniol	02.012	2016a
		Geranyl acetate	09.011	
		Prenyl acetate	09.692	
04	Non-conjugated and accumulated unsaturated straight-chain and branched-chain aliphatic primary alcohols, aldehydes, acids, acetals and esters	3-Methyl-but-3-enyl acetate[(a)]	09.655	2010a, CEF
		(3*E*)-Hexenyl acetate[(a)]	09.928	2008a, AFC
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	2012b
		α-Terpineol	02.014	
		(*E*)-Nerolidol[(b)]	02.072	
		(-)-α-Elemol[(a)]	02.149	2015a, CEF
13	Furanones and tetrahydrofurfuryl derivatives	Linalool oxide[(c)]	13.140	2012c
15	Phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters	Phenethyl acetate	09.031	2012d
16	Aliphatic and alicyclic ethers	1,8-Cineole	03.001	2012e, 2021a
18	Allylhydroxybenzenes	Eugenol	04.003	2011
		1-Methoxy-4-(prop-1 (*trans*)-enyl)benzene (trans-anethole)	04.010	
22	Aryl-substituted primary alcohol, aldehyde, acid, ester and acetal derivatives	Cinnamyl alcohol	02.017	2017
		Cinnamyl acetate	09.018	

[4] 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.

[5] 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

[6] 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.

CG	Chemical Group	Product (EU register name)	FLAVIS No	EFSA opinion*, Year
23	Benzyl alcohols, aldehydes, acids, esters and acetals	Benzyl alcohol	02.010	2012f, 2019, FAF
		Benzyl acetate	09.014	2012f
		Benzyl butyrate	09.051	
		Methyl benzoate	09.725	
		Ethyl benzoate	09.726	
		Benzyl benzoate	09.727	
		Methyl salicylate	09.749	
		Benzyl salicylate	09.752	
		Prenyl benzoate[(a)]	09.693	2010b, CEF
		Geranyl benzoate[(a)]	09.767	2009, AFC
		Methyl 2-methoxybenzoate[(a)]	09.796	JECFA
		(*Z*)-Hex-3-enyl benzoate[(a)]	09.806	JECFA
25	Phenol derivatives containing ring-alkyl, ring-alkoxy and side-chains with an oxygenated functional group	2-Methoxy-4-vinylphenol	04.009	2012g
		4-Methoxyphenol	04.028	
26	Aromatic ethers including anisole derivatives	1-Methoxy-4-methylbenzene	04.015	2012h
		1,2-Dimethoxybenzene[(a)]	04.062	JECFA
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	Limonene[(a),(d)]	01.001	2008b, AFC
		d-Limonene	01.045	2015b
		l-Limonene	01.046	
		Pin-2(10)-ene (β-pinene)	01.003	2016b
		Pin-2(3)-ene (α-pinene)	01.004	
		β-Caryophyllene	01.007	
		Myrcene	01.008	
		δ-Cadinene[(a),(e)]	01.021	2011, CEF
		β-Cubebene[(a),(e)]	01.030	
		δ-Elemene[(a)]	01.039	
		Germacra-1(10),4(14),5-triene (δ-Germacrene)[(a),(e)]	01.042	
		3,7,10-Humulatriene[(a,e)]	01.043	
		α-Muurulene[(a),(e)]	01.052	2015b, CEF
		β-Bourbonene[(a)]	01.024	2015c, CEF
		α-Farnesene[(a)]	01.040	
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 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) before 2000 are not required to be re-evaluated by EFSA.
(b): A mixture of (*E*)- and (*Z*)-nerolidol was evaluated [02.018] (EFSA FEEDAP Panel, 2012b).
(c): A mixture of cis- and trans-linalool oxide (5-ring) was evaluated [13.140].
(d): JECFA and EFSA evaluated d-limonene [01.045] (EFSA, 2008b). d-limonene [01.045] and l-limonene [01.046] were also evaluated for use in feed (EFSA FEEDAP Panel, 2015b).
(e): 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, 2010c). No longer authorised for use as flavours in food.

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[7] in support of the authorisation request for the use of ylang ylang oil as a feed additive.

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

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings. The applicant submitted a written agreement to use the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 6.[8]

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 in animal feed. The Executive Summary of the EURL report can be found in Annex A.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of ylang ylang oil is in line with the principles laid down in Regulation (EC) No 429/2008[10] and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA SC, 2009), Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012i), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012l), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017d), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance on the use of the benchmark dose approach in risk assessment (EFSA SC, 2017), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA SC, 2019b) 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, 2021b).[11]

3. Assessment

The additive under assessment, ylang ylang oil, is an essential oil obtained by steam distillation from the flowers of *C. odorata* (Lam) Hook f. & Thomson and is intended for use as a sensory additive (functional group: flavouring compounds) in feed and water for drinking for all animal species.

3.1. Origin and extraction

Cananga odorata is a perennial tropical tree native of South-East Asia countries (e.g. Philippines and Malaysia) and it also occurs naturally in Australia and on several Pacific islands. It belongs to the Annonaceae family. The essential oils extracted from the flowers of the tree have been used mainly in

[7] FEED dossier reference: FAD-2010-0218.
[8] Technical dossier/Supplementary information/Letter dated 29/04/2021.
[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0218-BDG06.doc_.pdf
[10] 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.
[11] https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf

cosmetic and food industry but also in traditional medicine in Asian countries (to treat malaria, stomach ailments, asthma, gout and rheumatism) (Tan et al., 2015).

The essential oil is obtained by steam distillation of the flowers from *C. odorata* Hook f. & Thomson *forma genuina*. The essential oil is then separated from the condensed water by decantation.

3.2. Characterisation

3.2.1. Characterisation of ylang ylang oil

The essential oil under assessment is a light amber to brown, clear, mobile, transparent liquid with characteristic odour. In five batches of the additive (all originating from Comoros), the density (20°C) ranged between 944 and 945 kg/m^3 (specification: 930–970 kg/m^3), the refractive index (20°C) between 1.503 and 1.508 (specification: 1.496–1.509).[12] Ylang ylang oil is identified with the single Chemical Abstracts Service (CAS) number 8006-81-3, the European Inventory of Existing Commercial Chemical Substances (EINECS) number 281-092-1, Flavor Extract Manufacturers Association (FEMA) 3199, and Council of Europe (CoE) number 103.

The product specifications are based on the standard developed by the International Organisation for Standardization (ISO) 3063:2004 for ylang ylang oil,[13] which were adapted to reflect the concentrations of the main components of the essential oil, analysed by gas chromatography with flame ionisation detection (GC-FID) and expressed as % of gas chromatographic peak area (% GC area).

These components and their specifications are germacra-1(10),4(14),5-triene (9.5–28%), α-farnesene (3–21%), linalool (2–19%), benzyl acetate (0.5–14%), benzyl benzoate (4.2–10%) and β-caryophyllene (4–17%, selected as phytochemical marker). Analysis of five batches of the additive by GC-FID showed compliance with these specifications: benzyl benzoate (2.7–3%), benzyl acetate (6.7–7.1%), β-caryophyllene (7.2–7.8%), linalool (4.9–5.6%), α-farnesene (13.2–17.7%) and germacra-1(10),4(14),5-triene (17.9–17.7%).[14]

Compliance with specifications was also demonstrated by gas chromatography–mass spectrometry (GC–MS) analysis (Table 2).[15] When analysed by GC–MS, the six compounds included in the specifications account for about 55.6% on average (range 51.1–59.0%) of the % GC area. According to ISO, the oil of ylang ylang 'is not generally collected as a whole oil, but in five successive fractions during the course of distillation. These five fractions, known respectively as "Extra super", "Extra", "First", "Second" and "Third", are the oils usually found in the trade' (ISO, 2004). The oil under assessment is similar to the ISO's first and second fractions as defined by ISO.

Table 2: Major constituents of the essential oil from the flowers of *Cananga odorata* (Lam.) Hook.f. & Thomson as defined based on ISO standard (3063:2004): specifications and batch to batch variation based on the analysis of five 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 EU register name	CAS No	FLAVIS No	% GC area		
			Specifications	Mean[(a)]	Range
Germacra-1(10),4(14),5-triene	23986-74-5	01.042	9.5–28	17.5	14.1–22.1
α-Farnesene	502-61-4	01.040	3–21	13.4	11.2–16.6
Linalool	78-70-6	02.013	2–19	4.3	3.7–4.9
Benzyl acetate	140-11-4	09.014	0.5–14	6.4	5.1–6.8
Benzyl benzoate	120-51-4	09.727	4.2–10	6.7	5.6–7.2
β-Caryophyllene	87-44-5	01.007	4–17	7.1	5.7–7.9
Total				55.6	51.1–59.0

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

[12] Technical dossier/Supplementary information January 2021/Annex_II_ SIn_Ylang Ylang_oil_COA_chromatograms.
[13] Technical dossier/Supplementary information January 2021/Annex III_SIn_Reply_ylang_ylang_oil_ISO. Essential oil obtained by steam distillation of the fresh flowers of *Cananga odorata* (Lam.) Hook. f. et Thomson forma *genuina*, of the Annonaceae family, growing mainly in Madagascar, Mayotte and Comores.
[14] Technical dossier/Supplementary information January 2021/Sin reply_ylang_ylang_oil/GC-FID analysis.
[15] Technical dossier/Supplementary information January 2021/Annex_II_ SIn_Reply_Ylang Ylang_oil_COA_chromatograms.

The applicant provided the full characterisation of the five batches obtained by GC–MS.[16] In total, up to 93 peaks were detected in the chromatogram, 86 of which were identified and accounted on average for 98.6% of the GC area. Besides the six compounds indicated in the product specifications, 45 other compounds were detected at individual levels ≥ 0.1% and are listed in Table 3. These 51 compounds together account on average for 97.4% (range 96.6–98.0%) of the GC area. The remaining 35 compounds (ranging between 0.01% and 0.09%) and accounting for 1.18% are listed in the footnote.[17]

Table 3: Other constituents of the essential oil from the flowers of *Cananga odorata* (Lam.) Hook.f. & Thomson accounting for > 0.1% of the composition (based on the analysis of five 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 EU register name	CAS No	FLAVIS No	% GC area	
			Mean[(a)]	Range
3,7,11-Trimethyldodeca-2,6,10-trienyl acetate	29548-30-9	09.818	4.08	3.51–4.67
Benzyl salicylate	118-58-1	09.752	3.82	3.03–5.21
Geranyl acetate	105-87-3	09.011	3.33	2.41–3.82
δ-Cadinene	29350-73-0	01.021	3.13	2.83–3.98
Methyl benzoate	93-58-3	09.725	2.85	2.33–3.28
Cinnamyl acetate	103-54-8	09.018	2.74	2.25–2.93
α-Cadinol	481-34-5	–	2.32	2.07–2.75
3,7,10-Humulatriene	6753-98-6	01.043	2.28	1.91–2.44
1-Methoxy-4-methylbenzene	104-93-8	04.015	1.92	1.46–2.31
(*E,E*)-Farnesol	106-28-5	–	1.71	1.36–2.00
γ-Muurolene	30021-74-0	–	1.46	1.37–1.63
α-Copaene	3856-25-5	–	1.31	1.14–1.58
τ-cadinol	5937-11-1	–	1.29	0.97–1.74
α-Muurolene	10208-80-7	01.052	0.93	0.84–1.19
γ-Cadinene	39029-41-9	–	0.87	0.78–1.11
γ-Amorphene	6980-46-7	–	0.83	0.75–1.07
δ-Cadinol (isomer 2)	19435-97-3	–	0.51	0.43–0.63
τ-muurolol	19912-62-0	–	0.50	0.43–0.64
(*Z,E*)-α-Farnesene	26560-14-5	–	0.50	0.28–0.64
Bicyclogermacrene	67650-90-2	–	0.44	0.37–0.55
Junenol	472-07-1	–	0.41	0.33–0.53
β-Caryophyllene epoxide	1139-30-6	16.043	0.39	0.20–0.6
β-Elemene	33880-83-0	–	0.37	0.19–0.49
β-Copaene	18252-44-3	–	0.36	0.32–0.41
Cubenol	21284-22-0	–	0.30	0.22–0.40
Prenyl benzoate	5205-11-8	09.693	0.29	0.26–0.32
α-Cadinene	24406-05-1	–	0.25	0.20–0.36
Prenyl acetate	1191-16-8	09.692	0.24	0.21–027
(*E*)-isoeugenol	5932-68-3	–	0.22	0.06–0.37
β-Cubebene	13744-15-5	01.030	0.22	0.15–0.29

[16] Technical dossier/Supplementary information January 2021/Annex_II_ SIn_Reply_Ylang Ylang_oil_COA_chromatograms.

[17] Additional constituents: constituents (n = 12) between < 0.1 and ≥ 0.05%: guaiol, *trans*-anethol, spathulenol, geranyl benzoate, rosifoliol, 1,8-cineole, germacrene B, δ-elemene, (-)-α-elemol, eugenol, benzyl alcohol and (Z)-hex-3-enyl benzoate; constituents (n = 15) between < 0.05 and > 0.01%: α-terpineol, hexyl acetate, nonanal, methyl salicylate, phenethyl acetate, β-pinene, (3*E*)-hexenyl acetate, nitrophenyl ethane, 4-methylphenol, benzyl butyrate, myrcene, limonene, *trans*-linalool oxide, cinnamyl alcohol and 6-methylhept-5-en-2-one; constituents (n = 8) between < 0.01 and > 0.003%: 1,2-dimethoxybenzene, 2-methoxy-4-vinylphenol, estragole, 3,5-dimethylbenzaldehyde, isopentyl acetate, *cis*-linalool oxide, butyl acetate and 2-methylbutyl acetate.

Constituent EU register name	CAS No	FLAVIS No	% GC area	
			Mean[(a)]	Range
trans-Cadina-1,4-diene	38758-02-0	–	0.21	0.18–0.25
α-Cubebene	17699-14-8	–	0.16	0.14–0.22
cis-Muurola-4(15),5-diene	157477-72-0	–	0.15	0.14–0.17
Geraniol	106-24-1	02.012	0.15	0.10–0.18
Cadina-3,5-diene	267665-20-3	–	0.13	0.12–0.16
β-Cadinene	523-47-7	–	0.13	0.12–0.15
α-Ylangene	14912-44-8	–	0.13	0.11–0.16
δ-Cadinol (isomer 1)	19435-97-3	–	0.13	0.10–0.18
(*E*)-Nerolidol	40716-66-3	02.232	0.12	0.08–0.20
3-Methylbut-3-enyl acetate	5205-07-2	09.655	0.12	0.10–0.13
Methyl 2-methoxybenzoate	606-45-1	09.796	0.11	0.03–0.16
β-Bourbonene	5208-59-3	01.024	0.10	0.07–0.12
Ethyl benzoate	93-89-0	09.726	0.10	0.05–0.26
1,10-di-epi-Cubenol	73365-77-2	–	0.10	0.07–0.15
α-Pinene (Pin-2(3)-ene)	80-56-8	01.004	0.10	0.07–0.12
Total			41.8	38.7–45.5

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

The applicant performed a literature search regarding substances of concern and chemical composition of the plant species *C. odorata* and its preparations.[18] No substances of concern were identified. The presence of safrole and isosafrole in essential oils (including ylang ylang oil) from the aerial parts of *C. odorata* has been reported in the EFSA Compendium (EFSA, 2012).[19] Methyleugenol has been detected in essential oils from the flowers of *C. odorata* from Colombia obtained by combined steam distillation and solvent extraction. The percentage of methyleugenol in the oil was found to depend on the parts of the flower used and the different stages of flower development (Stashenko et al., 1993, 1995, also reported in the review by Tan et al., 2015). In the most recent paper by the Stashenko's group, where different extraction techniques were compared, methyleugenol was not detected in essential oils obtained by steam distillation, whereas it was present in oils obtained by simultaneous distillation-extraction and supercritical fluid extraction with carbon dioxide (Stashenko et al., 1996). The presence of safrole and isosafrole (and estragole) has not been reported in Colombian oils by Tan et al. (2015). Safrole, isosafrole and methyleugenol were not detected in the additive under assessment (limit of detection: 0.002%). Estragole was detected in all five batches (0.006–0.008%).

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), organochloride pesticides, organophosphorous pesticides, aflatoxins B1, B2, G1, G2 and ochratoxin A. However, no data have been provided on the presence of these impurities. Since ylang ylang 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] However, no data supporting this statement were provided.

[18] Technical dossier/Supplementary information January 2021/Literature search_ylang ylang_oil.
[19] Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals
[20] Technical dossier/Section II.

3.2.4. Conditions of use

Ylang ylang oil is intended to be added to feed for all animal species without withdrawal. The applicant is proposing the levels as in Table 4. The additive is also proposed for use in water for drinking, however, no use level has been proposed by the applicant.

Table 4: Conditions of use for the essential oil from the flowers of *Cananga odorata* (Lam.) Hook.f. & Thomson: maximum proposed use levels in complete feed for the different target species

Animal category	Use level (mg/kg feed)
Chickens for fattening	1
Laying hens	1.5
Turkeys for fattening	1.5
Piglets	2
Pigs for fattening	2.5
Sows	3
Veal calves (milk replacer)	5
Cattle for fattening	4.5
Dairy cows	3
Sheep/goat	4.5
Horses	4.5
Rabbits	1.5
Fish	5
Dogs	5
Cats	4.5
Ornamental fish	5

3.3. Safety

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

Many of the components of ylang ylang oil, accounting for about 82% of the GC peak areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for food[21] and feed[22] uses. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Five compounds, δ-cadinene [01.021], β-cubebene [01.030], germacra-1(10),4(14),5-triene [01.042], 3,7,10-humulatriene [01.043] and α-muurulene [01.052], 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, 2010c). 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 (34) have not been previously assessed for use as flavourings. The FEEDAP Panel notes that most of them (22) are aliphatic mono- or sesquiterpenes structurally related

[21] 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.

[22] 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

to flavourings already assessed in CGs 6, 8 and 31 and a similar metabolic and toxicological profile is expected.[23] These lipophilic compounds, accounting for about 10% of the GC area, are expected to be rapidly absorbed from the gastrointestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2016b,c, 2021b).

The following sections focus on estragole and the other 11 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.

3.3.1. Absorption, distribution, metabolism and excretion of estragole

Estragole is a lipophilic compound and as such readily and completely absorbed from the gastrointestinal tract. Phase I metabolism is catalysed by cytochromes P450 (CYP450) enzymes mainly in the liver. Demethylation of the 4-methoxygroup with formation of 4-allylphenol is followed by conjugation with glucuronic acid or sulfate and renal excretion. Oxidation of the allyl-side chain leads to estragole-2′,3′-epoxide, which is hydrolysed to the corresponding diol with subsequent glucuronidation and excretion. Both metabolic pathways represent detoxification of estragole. The formation of genotoxic metabolites is initiated by oxidation of the side chain with formation of 1′-hydroxy-estragole. Sulfate-conjugation of the hydroxyl group leads to 1′-sulfooxyestragole, which is highly unstable and breaks down to form a highly reactive carbonium ion, which can react covalently with DNA (as reviewed in EMA, 2019).

The metabolism of estragole was evaluated in experimental animals with special focus on the formation of its proximate metabolite, 1′-hydroxyestragole, and the influence of the dose administered on the quantity excreted in urine (Zangouras et al., 1981; Anthony et al., 1987). When ^{14}C-estragole (4-[^{14}C-methoxyl]-allylbenzene) was given in low doses to rodents it was mainly excreted as $^{14}CO_2$ in exhaled air as a result of demethylation and only a minor portion in urine in the form of several metabolites resulting from hydroxylation in 1′-C and epoxidation at 2′,3′-C followed by ring hydrolysis. In a single study found in two volunteers orally given 100 μg of methoxy-^{14}C-estragole, 1′-hydroxyestragole quantified in urine of both individuals was 0.2% and 0.4% of the dose; the majority of the dose was excreted in expired air as $^{14}CO_2$ in the first 8 h (Sangstar et al., 1987). Metabolites identified in urine indicate that estragole follows a similar biotransformation profile in rats, mice, and humans. There are no studies in human volunteers with high doses of estragole, but in rats and in mice it is consistently shown that as doses increase the urinary levels of 1′-estragole as glucuronide significantly increases.

3.3.2. Genotoxicity and carcinogenicity

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 SC, 2019b).

The genotoxic potential for 11 substances (spathulenol, δ-cadinol isomer 1, 1,10-di-epi-cubenol, υ-cadinol, τ-muurolol, δ-cadinol isomer 2, α-cadinol, junenol, cubenol, 3,5-dimethylbenzaldehyde and nitrophenyl ethane) was predicted using the QSAR Toolbox.[24] No structural alerts were found for spathulenol, δ-cadinol, 1,10-di-epi-cubenol and cubenol. The prediction for δ-cadinol was considered to cover also δ-cadinol isomer 1, υ-cadinol, τ-muurolol, δ-cadinol isomer 2 and α-cadinol. Structural alerts for junenol, 3,5-dimethylbenzaldehyde and nitrophenyl ethane were due to the presence of the menthol, aldehyde group and arenes, respectively. For these compounds, the mutagenicity (Ames test) prediction was made by read-across analyses of data available for similar substances (i.e. analogues obtained by categorisation). Categories were defined using general mechanistic and endpoint profilers as well as empirical profilers. Mutagenicity read-across-based predictions were found consistently negative for all categories of analogues. On this basis, the alerts raised for junenol, 3,5-dimethylbenzaldehyde and nitrophenyl ethane were discounted.

[23] Twelve components, (*E*,*E*)-farnesol, (*E*)-nerolidol, guaiol, rosifoliol, (*E*)-isoeugenol, (*Z*,*E*)-a-farnesene, b-elemene, a-copaene, b-copaene, b-cadinene, g-cadinene, and a-cadinene) representing about 5.2% of the % GC area are structurally related to compounds already authorised for use in food and feed as flavourings. Ten additional constituents (a-cubebene, a-ylangene, b-bourbonene, cadina-3,5-diene, *cis*-muurola-4(15),5-diene, g-muurolene, g-amorphene, a-muurolene, bicyclogermacrene and *trans*-cadina-1,4-diene), representing on average 4.7% of % GC area, are allocated to CG 31.

[24] Technical dossier/Supplementary information January 2021/Annex_VI_Sin reply_ylang_ylang_oil_QSAR.

Estragole

Ylang ylang oil contains trace amounts of estragole (range: 0.006–0.008%), a compound with experimentally proven genotoxicity and carcinogenicity in rodents as reviewed in the references Scientific Committee on Food (2001) and EMA (2019).

Estragole was included in the diet of female CD-1 mice at 0, 2.3 or 4.6 g/kg diet for 12 months. At least 50% of the animals in the exposed groups developed hepatic tumours by 18 months,[25] which were diagnosed as hepatomas type A (hepatocellular adenomas) or type B (hepatocellular adenocarcinomas) or mixed types A and B. The animals which were fed with the control diet did not show any hepatic tumour (Miller et al., 1983).

Van den Berg et al. (2011) performed an evaluation of the available evidence using the benchmark dose (BMD) approach and found that the application of dose-response modelling on the long-term chronic toxicity study (Miller et al., 1983) using hepatocellular carcinomas as a response, yielded a BMD lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) of 3.3 mg estragole/kg body weight (bw) per day. However, the FEEDAP Panel notes that there is high uncertainty in derivation of a $BMDL_{10}$ for estragole from a carcinogenicity study in CD-1 mice. This strain of mice spontaneously develops a high incidence of hepatocellular adenomas and carcinomas, and the relevance of these tumours for human risk assessment is questionable. In addition, BMD modelling with only two dose-levels is adding extra uncertainty in the derivation of the $BMDL_{10}$ value.

Miller et al. (1983) also investigated the possible carcinogenic activity of a variety of p-allylalkoxybenzenes in newborn male mice, injected intraperitoneally (i.p.) with nine different compounds at day 1, 8, 15 and 22 after birth. Among these, estragole, safrole and methyleugenol induced a significant number of hepatomas at 13 months, whereas anethol, elemicin, myristicin, dillapiole, parsley apiole and eugenol did not under the limited conditions of the study.

In another experiment using the same treatment protocol, DNA was isolated from the liver of the treated mice and the occurrence and quantity of DNA adducts was investigated (Phillips et al., 1984). The highest amount of DNA-adducts was observed with methyleugenol, estragole and safrole (73, 30 and 15 pmol/mg DNA, respectively). The yield of DNA adducts with myristicin, elemicin and dillapiole were 7.8, 2.7 and 1.2 pmol/mg DNA and the correspondent values for parsley apiol and anethol where below the LOQ of 1 pmol/mg DNA. No adducts at all were observed for eugenol. The incidence of DNA adducts correlated to the tumour incidence observed in the experiment by Miller et al. (1983). Two other studies on the induction of DNA adducts in liver of adult mice after i.p. injection of alkenylbenzenes (Randerath et al., 1984) and in human hepatoma cells in culture (Zhou et al., 2007) confirmed methyleugenol as the most potent derivative. The two in vivo studies resulted in the same order of potency (i.e. methyleugenol > safrole > estragole > elemicin > dillapiole). In the *in vitro* study, estragole was more potent than safrole.

The carcinogenicity of methyleugenol was investigated in a 2-year National Toxicology Program (NTP) carcinogenicity study in rats and mice (NTP, 2000) using doses of 0, 37, 75 or 150 mg/kg bw per day in both species and a higher dose of 300 mg/kg bw per day in rats. Rats of both sexes receiving methyleugenol had dose-related increased incidences of hepatocellular carcinomas and neuroendocrine tumours of the glandular stomach.[26] Higher incidences of kidney neoplasms, malignant mesothelioma, mammary gland fibroadenoma and subcutaneous fibroma and fibrosarcoma were observed in male rats only.[27] Increased incidence of hepatocellular carcinomas was seen in both sexes of mice although the incidence was not related to dose. Neuroendocrine tumours of the glandular stomach were also observed in male mice but only at the highest dose. The NTP concluded that there was clear evidence for the carcinogenicity of methyleugenol in rats and mice.

Suparmi et al. (2019) performed an evaluation of the available evidence using the BMD approach and found that dose-response modelling, applying model averaging, as recommended by the EFSA Scientific Committee (EFSA SC, 2017) on the long-term chronic toxicity study (NTP, 2000) using

[25] Incidence of hepatomas in female mice (0/50, 25/50, 35/50).

[26] Male rats: hepatocellular adenoma (5/50, 12/50, 23/50, 38/50, 32/50), hepatocellular carcinoma (2/50, 3/50, 14/50, 25/50, 36/50), hepatocellular adenoma or carcinoma combined (7/50, 14/50, 28/50, 43/50, 45/50), hepatocholangioma or hepatocolangiocarcinoma (0/50, 0/50, 1/50, 2/50, 13/50); glandular stomach (0/50, 0/50, 0/50, 7/50, 4/50). Female rats: hepatocellular adenoma (1/50, 8/50, 11/49, 33/49, 43/50), hepatocellular carcinoma (0/50, 0/50, 4/49, 8/49, 22/50), hepatocellular adenoma or carcinoma combined (1/50, 8/50, 14/49, 34/49, 43/50), hepatocholangioma or hepatocolangiocarcinoma (0/50, 0/50, 0/50, 3/50, 13/17); glandular stomach (0/50, 1/50, 25/50, 34/50, 41/50).

[27] Males rats: kidney neoplasms (4/50, 6/50, 17/50,13/50, 20/50), malignant mesothelioma (1/50, 3/50, 5/50, 12/50, 5/50), mammary gland fibroadenoma (5/50, 5/50, 15/50, 13/50, 6/50), subcutaneous fibroma or fibrosarcoma (1/50, 12/50, 8/50, 8/50, 4/50).

hepatocellular carcinomas in male rats as a response, yielded a $BMDL_{10}$ of 22.2 mg/kg bw per day. Based on the above considerations on the relative potency of *p*-allylalkoxybenzenes, the FEEDAP Panel selects the $BMDL_{10}$ derived from the rat study with methyleugenol, with three test doses and derived applying model averaging, as reference point for the assessment group p-allylalkoxybenzenes.

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%), 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 18 assessment groups, corresponding to the CGs 1, 2, 3, 4, 5, 6, 8, 13, 15, 16, 17, 18, 22, 23, 25, 26, 31 and 32, as defined in Annex I of Regulation (EC) No 1565/2000. For CG 31 ('aliphatic and aromatic hydrocarbons'), the application of subassessment groups as defined in Flavouring Group Evaluation 25 (FGE.25) and FGE.78 is applied (EFSA CEF Panel, 2015b,c). 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, 2017c). 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 chickens for fattening, the species with the highest ratio of feed intake/body weight per day, are shown in Table 5.

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification. For some components in the assessment group toxicological data were available to derive no observed adverse effect level (NOAEL) values. 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 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 octyl acetate [09.007] and ethyl acetate [09.001] in CG 1 (EFSA FEEDAP Panel, 2013), 2-ethylhexan-1-ol [02.082] in CG 2 (EFSA FEEDAP Panel, 2012a), citral [05.020] in CG 3 (EFSA FEEDAP Panel, 2016a), linalool [02.013] and terpineol[28] [02.230] in CG 6 (EFSA FEEDAP Panel, 2012b), 1,8-cineole in CG 16 (EFSA FEEDAP Panel, 2012e, 2021a), eugenol [04.003] and *trans*-anethole [04.010] in CG 18 (EFSA FEEDAP Panel, 2011), cinnamaldehyde [05.014] in CG 22 (EFSA FEEDAP Panel, 2017), benzyl alcohol [02.010] (EFSA FAF Panel, 2019) and methyl salicylate [09.749] in CG 23 (EFSA FEEDAP Panel, 2012f), benzene-1,3-diol [04.047] in CG 25 (EFSA FEEDAP Panel, 2012g), 1-methoxy-4-methylbenzene [04.015] in CG 26 (EFSA FEEDAP Panel, 2012h), myrcene [01.008] and β-caryophyllene in CG 31 (EFSA FEEDAP Panel, 2016b), and β-caryophyllene oxide in CG 32 (EFSA CEF Panel, 2014).

For the compounds belonging to CG 1, read-across was also applied using the NOAEL of 120 mg/kg bw per day for octyl acetate [09.007] to butyl acetate [09.004] and to nonanal [05.025], whereas read-across was applied using the NOAEL of 900 mg/kg bw per day for ethyl acetate [09.001] to extrapolate to hexyl acetate [09.006].

Similarly, read-across was also applied using the NOAEL of 50 mg/kg bw per day for 2-ethylhexan-1-ol [02.082] to isopentyl acetate [09.006] and to 2-methylbutyl-acetate [09.286] in CG 2.

[28] Terpineol is a mixture of four isomers: a-terpineol [02.014], a mixture of (*R*)-(+)-a-terpineol and (*S*)-(–)-a-terpineol, b-terpineol, g-terpineol and 4-terpinenol [02.072].

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 geraniol [02.012] and geranyl acetate [09.011], in CG 3.

The NOAEL of 127 mg/kg bw per day for hex-3(cis)-enyl acetate was extrapolated to (3*E*)-hexenyl acetate [09.928] in CG 4.

For α-terpineol [02.072] in CG 6, the reference point was selected based on the NOAEL of 250 mg/kg For α-terpineol [02.072] in CG 6, 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].

Read-across was also applied in CG 22 using the NOAEL of 275 mg/kg bw per day for cinnamaldehyde [05.014] to cinnamyl alcohol [02.017] and cinnamyl acetate [09.018].

The NOAEL of 400 mg/kg bw per day for benzyl alcohol was applied to all benzoates and benzyl esters, whereas the NOAEL of 5 mg/kg bw per day for isopentyl salicylate [09.751] was extrapolated to benzyl salicylate [09.752] in CG 23.

The group NOAEL of 36 mg/kg per bw per day for benzene-1,3-diol [04.047] was assigned to 4-methylphenol [04.028] and 2-methoxy-4-vinylphenol [04.009] in CG 25.

The NOAELs for the representative compounds of CG 31, myrcene [01.008] and β-caryophyllene [01.007] were applied using read-across to the compounds within sub-assessment group II (α-farnesene [01.040] and (*Z*,*E*)-α-farnesene) and group V (α-pinene [01.004], β-pin-ene [01.003], α-copaene, β-bourbonene [01.024], β-copaene, β-cadinene, γ-cadinene, δ-cadinene [01.021], α-cadinene) (EFSA CEF Panel, 2015b,c), respectively.

For the remaining compounds,[29] 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, 2017c).

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 SC, 2019). A MOET > 100 allowed for interspecies- and intra-individual variability (as in the default 10x10 uncertainty factor). The compounds resulting individually in an MOE > 50,000, listed in the footnote,[30] were not further considered in the assessment group as their contribution to the MOE(T) is negligible.

The approach to the safety assessment of ylang ylang oil for the target species was done through calculations for chickens for fattening, the species with the highest ratio of feed intake/body weight as representing the worst-case scenario at the use level of 1 mg/kg complete feed (Table 5).

[29] Prenyl acetate, 3,7,11-trimethyldodeca-2,6,10-trienyl acetate, 3-methylbut-3-enyl acetate, 6-methylhept-5-en-2-one, (-)-α-elemol, (*E*)-nerolidol, spathulenol, δ-cadinol isomer 1, guaiol, rosifoliol, 1,10-di-epi-cubenol, τ-cadinol, τ-muurolol, delta-cadinol isomer 2, α-cadinol, junenol, cubenol, *cis*-linalool oxide, trans-linalool oxide, phenethyl acetate, (*E*)-isoeugenol, benzyl alcohol, methyl benzoate, 3,5-dimethylbenzaldehyde, benzyl acetate, ethyl benzoate, methyl 2-methoxybenzoate, benzyl butyrate, (*Z*)-hex-3-enyl benzoate, benzyl benzoate, geranyl benzoate, 1,2-dimethoxybenzene, δ-elemene, β-elemene, α-cubebene, α-ylangene, β-cubebene, cadina-3,5-diene, *cis*-muurola-4(15),5-diene, γ-muurolene, γ-amorphene, bicyclogermacrene, α-muurolene, *trans*-cadina-1,4-diene, 3,7,10-humulatriene, germacra-1(10),4(14),5-triene, germacrene B, nitrophenyl ethane.

[30] Compounds included in the assessment groups but not reported in the table: hexyl acetate, nonanal and butyl acetate (CG 1); isopentyl acetate and 2-methylbutyl acetate (CG 2); geranyl acetate, (*E*,*E*)-farnesol and geraniol (CG 3); (3*E*)-hexenyl acetate (CG4); 6-methylhept-5-en-2-one (CG 5); a-terpineol (CG 6); *cis*-linalool oxide (CG 13); phenethyl acetate (CG 15); 1,8-cineole (CG 16); *trans*-anethole and eugenol (CG 18); cinnamyl acetate and cinnamyl alcohol (CG 22); benzyl benzoate, benzyl acetate, benzyl salicylate, methyl benzoate, prenyl benzoate, methyl 2-methoxybenzoate, ethyl benzoate, geranyl benzoate, (*Z*)-Hex-3-enyl benzoate, methyl salicylate, benzyl butyrate and 3,5-dimethylbenzaldehyde (CG 23); 4-methylphenol and 2-methoxy-4-vinylphenol (CG 25); 1,2-dimethoxybenzene (CG 26); (*Z*,*E*)-a-farnesene and myrcene (CG 31, II); limonene (CG 31, III); d-cadinene, a-copaene, g-cadinene, b-copaene, a-cadinene, b-cubebene, b-cadinene and b-bourbonene (CG 31, V); b-caryophyllene epoxide (CG 32).

Table 5: Compositional data, intake values (calculated for chickens for fattening at 1 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of ylang ylang 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[b]**	**NOAEL[c]**	**MOE**	**MOET**
Constituent	–	**%**	**mg/kg**	**mg/kg bw per day**	–	**mg/kg bw per day**	–	–
CG 3								
3,7,11-Trimethyldodeca-2,6,10-trienyl acetate	09.818	4.67	0.047	0.0042	I	*3*	716	
Prenyl acetate	09.692	0.24	0.003	0.0002	I	*3*	12,241	
MOET CG 3								711
CG 4								
3-Methylbut-3-enyl acetate	09.655	0.13	0.001	0.0001	I	*3*	25,510	
CG 6								
Linalool	02.013	4.86	0.049	0.0044	(I)	**117**	26,833	
α-Cadinol	–	2.75	0.027	0.0025	I	*3*	1,216	
τ-Cadinol	–	1.74	0.017	0.0016	I	*3*	1,921	
δ-Cadinol isomer 2	–	0.63	0.006	0.0006	I	*3*	5,347	
τ-Muurolol	–	0.64	0.006	0.0006	I	*3*	5,189	
δ-Cadinol isomer 1	–	0.18	0.002	0.0002	I	*3*	18,361	
(*E*)-Nerolidol	02.232	0.20	0.002	0.0002	I	*3*	17,050	
1,10-di-epi-cubenol	–	0.15	0.001	0.0001	III	*0.15*	1,129	
Guaiol	–	0.11	0.001	0.0001	I	*3*	31,826	
Spathulenol	–	0.14	0.001	0.0001	I	*3*	24,392	
Rosifoliol	–	0.08	0.001	0.0001	I	*3*	41,256	
(-)-α-Elemol	02.149	0.07	0.001	0.0001	I	*3*	48,431	
MOET CG 6								348
CG 8								
Juneol	–	0.53	0.005	0.0005	I	*3*	6,305	
Cubenol	–	0.40	0.004	0.0004	III	*0.15*	418	
MOET CG 8								392
CG 13								
trans-Linalool oxide	–	0.02	0.0002	0.00002	II	*0.91*	46,076	
CG 17								
(*E*)-isoeugenol	–	0.37	0.004	0.0003	I	*3*	9,032	
CG 26								
1-Methoxy-4-methylbenzene	04.015	2.31	0.023	0.0021	(I)	50	24,153	
CG 31, II (Acyclic alkanes)								
α-Farnesene	01.040	16.63	0.166	0.015	(I)	44	2,947	
CG 31, III (Cyclohexene hydrocarbons)								
β-Elemene	–	0.49	0.005	0.0004	I	*3*	6,848	
δ-Elemene	01.039	0.08	0.001	0.0001	I	*3*	40,262	
MOET CG 31, III								5,825

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS No**	**Max conc. in the oil**	**Max feed conc.**	**Intake[a]**	**Cramer Class[b]**	**NOAEL[c]**	**MOE**	**MOET**
Constituent	–	**%**	**mg/kg**	**mg/kg bw per day**	–	**mg/kg bw per day**	–	–
CG 31, V (Bi-, tricyclic, non-aromatic hydrocarbons)								
β-Caryophyllene	01.007	7.88	0.079	0.0071	(I)	*222*	31,390	
γ-Muurolene	–	1.63	0.016	0.0015	I	*3*	2,046	
α-Muurolene	–	1.19	0.012	0.0011	I	*3*	2,811	
γ-Amorphene	–	1.07	0.011	0.0010	I	*3*	3112	
Bicyclogermacrene	–	0.55	0.005	0.0005	I	*3*	6,098	
trans-Cadina-1,4-diene	–	0.25	0.002	0.0002	I	*3*	13,529	
α-Cubebene	–	0.22	0.002	0.0002	I	*3*	15,543	
cis-Muurola-4 (15),5-diene	–	0.17	0.002	0.0002	I	*3*	19,317	
Cadina-3,5-diene	–	0.16	0.002	0.0001	I	*3*	20,886	
α-Ylangene	–	0.16	0.002	0.0001	I	*3*	20,337	
MOET CG 31, V								597
CG 31, VI (macrocyclic non-aromatic hydrocarbons)								
Germacra-1(10),4 (14),5-triene	01.042	22.13	0.221	0.0199	I	*3*	151	
3,7,10-Humulatriene	01.043	2.44	0.024	0.0022	I	*3*	1370	
Germacrene B	–	0.09	0.001	0.0001	I	*3*	38,411	
MOET CG 31, VI								136
Others								
Nitrophenyl ethane	–	0.04	0.0004	0.00004	III	*0.15*	4,177	

(a): Intake calculations for the individual components are based on the use level of 1 mg/kg in complete feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point (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): When a NOAEL value is available or read-across is applied, the allocation to the Cramer class is put into parentheses.
(c): 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 ≥ 136. Therefore, no safety concern was identified for the ylang ylang oil when used as a feed additive for chickens for fattening at the proposed use levels (1 mg/kg complete feed).

From the lowest MOET of 136 resulting for the assessment group CG 31, VI (macrocyclic non aromatic hydrocarbons) for chickens for fattening, the MOET was calculated for the other target species considering the respective daily feed intake and conditions of use (Table 6).

Table 6: Combined margin of exposure (MOET) for the assessment group 'macrocyclic non-aromatic hydrocarbons' (CG 31, VI) calculated for the different target animal categories at the proposed use level

Animal category	Body weight (kg)	Feed intake (g DM/day)	Use level (mg/kg feed)	Lowest MOET
Chickens for fattening	2	158	1	136
Laying hens	2	106	1.5	135
Turkeys for fattening	3	176	1.5	121
Piglets	20	880	2	122
Pigs for fattening	60	2,200	2.5	116
Sows	175	5,280	3	119
Veal calf (milk replacer)	100	1,890	5	121
Cattles for fattening	400	8,000	4.5	119
Dairy cows	650	20,000	3	116
Sheep/goats	60	1,200	4.5	119
Horses	400	8,000	4.5	119
Rabbits	2	100	1.5	143
Salmon	0.12	2.1	5	119
Dogs	15	250	5	126
Cats	3	60	4.5	119
Ornamental fish	0.012	0.054	5	430

DM: dry matter.

Table 6 showed a MOET above the value of 100 for all animal species. Owing to the unusually low capacity for glucuronidation in cats (Court and Greenblatt, 1997; Lautz et al., 2021), safe concentration in complete feed for this species should be reduced to 1 mg/kg to ensure a MOET > 500.

The FEEDAP Panel concludes that the use of ylang ylang oil at the maximum proposed use levels in feed is safe for all animal species, except cats, for which the calculated safe concentration in complete feed is 1 mg/kg. No specific proposals have been made by the applicant for the use level in water for drinking.

The Panel considers that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed (EFSA FEEDAP Panel, 2010).

Estragole

Low concentrations of estragole were detected in all batches of the additive under assessment (average: 0.007%, range: 0.006–0.008%). The use of ylang ylang oil at the proposed use levels in feed for the different target species (ranging from 1 to 5 mg/kg complete feed, see Section 3.2.2), would result in concentrations ranging from 0.08 to 0.40 μg estragole/kg complete feed.

The maximum daily intake of estragole was calculated at the maximum proposed use level of the additive in feed for the different target animal categories and considering the maximum analysed value in the additive. The calculated intake value was 0.002 μg/kg bw per day for cats and ornamental fish and ranged between 0.007 and 0.008 μg/kg bw per day for the other target species.

When the estimated exposures for the different animal categories are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day derived for methyleugenol by Suparmi et al. (2019) from a rodent carcinogenicity study (NTP, 2000, see Section 3.2.2), a MOE of at least 2,600,000 (range 2,664,000–12,210,000) is calculated. The magnitude of this MOE is indicative of a low concern for the target species (see Appendix A).

Conclusions on safety for the target species

The FEEDAP Panel concludes that ylang ylang oil is safe up to the maximum proposed use levels in complete feed of 1 mg/kg for chickens for fattening, 1.5 mg/kg for laying hens, turkeys for fattening and rabbits, 2 mg/kg for piglets, 2.5 mg/kg for pigs for fattening, 3 mg/kg for sows, 4.5 mg/kg for

cattle for fattening, sheep, goats, horses, 5 mg/kg for veal calves (milk replacer), fish (salmon), dogs and ornamental fish. For cats, the calculated safe concentration in complete feed is 1 mg/kg.

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

3.3.4. Safety for the consumer

Ylang ylang 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) cites values of 0.0001 mg/kg bw per day (FEMA 3119). Fenaroli's also reports use levels in food and beverages in the range of 1 mg/kg up to 5 mg/kg (Burdock, 2009).

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 ylang ylang oil are expected to be extensively metabolised and excreted in the target species. Also for estragole, the available data indicate that it is absorbed, metabolised and rapidly excreted and is not expected to accumulate in animal tissues and products, consequently residues in food products are unlikely (see Section 3.3.1). Therefore, a relevant increase of the uptake of the individual constituents by humans consuming products of animal origin is not expected.

Considering the reported human exposure due to direct use of ylang ylang oil in food (Burdock, 2009) it is unlikely that the consumption of products from animals given ylang ylang oil at the proposed maximum use level would increase human background exposure. Consequently, no safety concern would be expected for the consumer from the use of ylang ylang oil up to the highest safe use level in feed for the target animals.

3.3.5. Safety for the user

No specific data were provided by the applicant regarding the safety of the additive for users. However, published reports (Bleasel et al., 2002; de Groot and Schmidt, 2016) have identified ylang ylang oil as a potential contributor for skin allergy and dermatitis and on this basis, it should be considered a dermal sensitiser. This is reinforced by the classification assigned on the safety data sheets.[31]

Ylang ylang oil should be considered as irritant to skin and eyes. Although there is no evidence for respiratory toxicity, it might be a respiratory sensitiser, since it is considered a dermal sensitiser.

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

3.3.6. Safety for the environment

Cananga odorata is not a European native species. Therefore, the safety for the environment is assessed based on the individual components of the essential oil.

The major components (β-caryophyllene, benzyl benzoate, benzyl acetate, benzyl salicylate, linalool, geranyl acetate, methyl benzoate, cinnamyl acetate, 1-methoxy-4-methylbenzene) and additional 23 components (prenyl acetate, ethyl benzoate, geraniol, trans-anethole, 1,8-cineole, α-pinene, eugenol, benzyl alcohol, nonanal, hexyl acetate, α-terpineol, phenethyl acetate, methyl salicylate, β-pinene, 4-methylphenol, benzyl butyrate, myrcene, cinnamyl alcohol, 6-methylhept-5-en-2-one, isopentyl acetate, 2-methoxy-4-vinylphenol, butyl acetate, 2-methylbutyl acetate) accounting together for 40.4% of the composition of the oil, have been evaluated by EFSA as sensory additives for animal feed and 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 (see Table 1).

The applicant provided evidence that 3,7,10-humulatriene, germacra-1(10),4(14),5-triene, τ-cadinol, α-cadinol, γ-muurolene, γ-amorphene and α-muurolene occur naturally in plants commonly found in

[31] Technical dossier/Supplementary Information January 2021/Annex_VIII_ylang_ylang_oil_MSDS. Serious eye damage/eye irritation (H319, category 2), hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1).

Europe at concentrations considerably higher than those resulting from the use of the oil at the proposed levels in feed and would therefore not raise safety concern for the environment.[32]

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 in CG 6 and CG 31 for use in animal feed (EFSA FEEDAP Panel, 2012b, 2015b, 2016b) 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 6 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

The oil from the flowers of *C. odorata* (Lam) Hook.f. & Thomson is listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference number 3119 (ylang ylang oil).

Since the oil from the flowers of *C. odorata* is recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

4. Conclusions

Since ylang ylang oil from *C. odorata* (Lam.) Hook.f. & Thomson may be produced from plants of different origins and by various processes resulting in preparations with different composition and toxicological profiles, the following conclusions apply only to ylang ylang oil which contains $\leq$ 0.008% estragole and is produced by steam distillation from the flowers of *C. odorata* (Lam.) Hook.f. & Thomson.

The FEEDAP Panel concludes that ylang ylang oil from the flowers of *C. odorata* is safe up to the maximum proposed use levels in complete feed of 1 mg/kg for chickens for fattening, 1.5 mg/kg for laying hens, turkeys for fattening and rabbits, 2 mg/kg for piglets, 2.5 mg/kg for pigs for fattening, 3 mg/kg for lactating sows and dairy cows, 4.5 mg/kg for cattle for fattening, sheep, goats, horses, 5 mg/kg for veal calves (milk replacer), dogs, salmons and ornamental fish. For cats, the calculated safe concentration in complete feed is 1 mg/kg. The FEEDAP Panel considers that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

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

The essential oil under assessment should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser. When handling the essential oil, exposure of unprotected users to estragole cannot be excluded. Therefore, to reduce the risk, the exposure of the users should be minimised.

Ylang ylang 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. Recommendation

The specification should ensure that the estragole concentration should be as low as possible and should not exceed 0.008% of the essential oil.

6. 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

[32] https://www.vcf-online.nl/VcfHome.cfm

Date	Event
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*
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
07/01/2021	Reception of supplementary information from the applicant (partial submission)
09/11/2021	The application was split and a new EFSA-Q-2021-00596 was assigned to the preparation included in the present assessment. Scientific assessment re-started for the preparation included in the present assessment
27/01/2022	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

DM	dry matter
EEIG	European economic interest grouping
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
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
ISO	International standard organisation
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
MOE	margin of exposure
MOET	combined margin of exposure (total)
NOAEL	no observed adverse effect level
PPR	EFSA Panel on Plant Protection Products and their Residues
TTC	threshold of toxicological concern
UF	uncertainty factor
WHO	World Health Organization

Appendix A – Estragole: Maximum daily intake and margin of exposure for the different target species

The maximum daily intake of estragole for the different target species and categories was calculated based on

- the default values for body weight and feed intake (EFSA FEEDAP Panel, 2017b)
- the maximum proposed use level of the additive in feed for the different target animal categories (ranging from 1 to 5 mg/kg complete feed) and
- assuming that estragole is present at a concentration corresponding to the maximum analysed value in the additive (0.008%).

According to the General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021),[33] 'for substances for which carcinogenicity studies in rodents are available, from which a $BMDL_{10}$ can be derived, the MOE approach (EFSA, 2005; EFSA SC, 2012) can be applied. Similarly to human risk assessment, a combined (total) margin of exposure (MOET) with a magnitude of $\geq$ 10,000, when comparing estimated exposure to genotoxic and/or carcinogenic substances with a $BMDL_{10}$ from a rodent carcinogenicity study, would be indicative of a low concern for the target species (EFSA SC, 2019a)'.

The margin of exposure (MOE) for each animal category is calculated as the ratio of the reference point (the $BMDL_{10}$ of 22.2 mg/kg bw per day, see Section 3.3.2) to the intake.

The maximum daily intake of estragole for the different target animal categories and the corresponding MOE are reported in Table A.1.

Table A.1: Target animal intake of estragole (as μg/kg bw per day) and margin of exposure (MOE) calculated at the maximum proposed use level of the additive in feed for target animal category and considering the maximum analysed value in the additive

Animal category	Daily feed intake	Body weight	Use level	Estragole intake[(a)]	MOE[(b)]
	kg DM/day	kg	mg/kg	μg/kg bw per day	
Chickens for fattening	0.158	2	1	0.007	3,091,139
Laying hens	0.106	2	1.5	0.007	3,071,698
Turkeys for fattening	0.176	3	1.5	0.008	2,775,000
Piglets	0.88	20	2	0.008	2,775,000
Pigs for fattening	2.2	60	2.5	0.008	2,664,000
Lactating sows	5.28	175	3	0.008	2,697,917
Veal calf (milk replacer)	1.89	100	5	0.008	2,775,000
Cattles for fattening	8	400	4.5	0.008	2,713,333
Dairy cows	20	650	3	0.008	2,645,500
Sheep/goats	1.2	60	4.5	0.008	2,713,333
Horses	8	400	4.5	0.008	2,713,333
Rabbits	0.1	2	1.5	0.007	3,256,000
Salmons	0.0021	0.12	5	0.008	2,790,857
Dogs	0.25	15	5	0.008	2,930,400
Cats	0.06	3	1	0.002	12,210,000
Ornamental fish	0.00054	0.012	5	0.002	10,853,333

(a): The values of estragole in feed is calculated considering the maximum analysed value in the additive.
(b): The MOE estragole is calculated as the ratio of the reference point (BMDL10) to the intake.

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

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for 18 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.