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)

EFSA Panel on Additives, Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Mojca Fasmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Andrew Chesson, Johannes Westendorf, Paola Manini, Fabiola Pizzo and Birgit Dusemund

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.

© 2022 Wiley-VCH Verlag GmbH & Co. KgaA on behalf of the European Food Safety Authority.

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

**Requestor:** European Commission

**Question number:** EFSA-Q-2010-01296 (EFSA-Q-2021-00596)

**Correspondence:** feedap@efsaneuropa.eu
**Panel members:** Giovanna Azimonti, Vasileios Bampidis Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

**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](https://ess.efsa.europa.eu/doi/doiweb/doisearch).

**Acknowledgements:** The Panel wishes to acknowledge the contribution of Joana Revez and Elisa Pettenati to this opinion.

**Suggested citation:** EFSA FEEDAP Panel (EFSA Panel on Additives, Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos MDL, Christensen H, Faßmon Durjava M, Koubá 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, 2022. Scientific Opinion on the 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). EFSA Journal 2022;20(2):7159, 28 pp. [https://doi.org/10.2903/j.efsa.2022.7159](https://doi.org/10.2903/j.efsa.2022.7159)

**ISSN:** 1831-4732

© 2022 Wiley-VCH Verlag GmbH & Co. KgaA on behalf of the European Food Safety Authority.

This is an open access article under the terms of the Creative Commons Attribution-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.

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

Abstract ................................................................................................................................................... 1

1. Introduction................................................................................................................................... 4
   1.1. Background and Terms of Reference ....................................................................................... 4
   1.2. Additional information ............................................................................................................. 4

2. Data and methodologies ................................................................................................................. 7
   2.1. Data ............................................................................................................................................ 7
   2.2. Methodologies ............................................................................................................................. 7

3. Assessment .................................................................................................................................... 7
   3.1. Origin and extraction .................................................................................................................... 7
   3.2. Characterisation ............................................................................................................................. 8
   3.2.1. Characterisation of ylang ylang oil ............................................................................................ 8
   3.2.2. Impurities ................................................................................................................................... 10
   3.2.3. Shelf-life .................................................................................................................................... 10
   3.2.4. Conditions of use ...................................................................................................................... 11
   3.3. Safety ........................................................................................................................................... 11
   3.3.1. Absorption, distribution, metabolism and excretion of estragole .............................................. 12
   3.3.2. Genotoxicity and carcinogenicity ............................................................................................... 12
   3.3.3. Safety for the target species ........................................................................................................ 14
   3.3.4. Safety for the consumer ............................................................................................................. 19
   3.3.5. Safety for the user ....................................................................................................................... 19
   3.3.6. Safety for the environment ......................................................................................................... 19
   3.4. Efficacy ......................................................................................................................................... 20

4. Conclusions.................................................................................................................................... 20

5. Recommendation ........................................................................................................................... 20

6. Documentation as provided to EFSA/Chronology ........................................................................... 20

References ............................................................................................................................................... 21

Abbreviations ........................................................................................................................................... 25

Appendix A – Estragole: Maximum daily intake and margin of exposure for the different target species .... 27

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, Magnoliales, Piperales .................................................................................... 28
1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003 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) 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. 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 and feed uses together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000 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 | α, 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-ethyl acetate(a) | 09.655    | 2010a, CEF          |
|    |                                                                                 | (3E)-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    | 2015a, CEF          |
|    |                                                                                 | (-)-α-Elemol(b)             | 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 | Allyhydroxybenzenes                                                             | 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, 21.10.2012, 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.
| Chemical Group | Product (EU register name) | FLAVIS No | EFSA opinion*, Year |
|----------------|----------------------------|-----------|---------------------|
| **23** Benzyl alcohols, aldehydes, acids, esters and acetics | 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 acetics containing saturated aldehydes | Limonene\(^{(a),(d)}\) | 01.001 | 2008b, AFC |
| | d-Limonene | 01.045 | 2015b |
| | l-Limonene | 01.046 | |
| | Pin-2(10)-ene (\(\beta\)-pinene) | 01.003 | 2016b |
| | Pin-2(3)-ene (\(\alpha\)-pinene) | 01.004 | |
| | \(\beta\)-Caryophyllene | 01.007 | |
| | Myrcene | 01.008 | |
| | \(\delta\)-Cadinene\(^{(a),(e)}\) | 01.021 | 2011, CEF |
| | \(\beta\)-Cubebene\(^{(a),(e)}\) | 01.030 | |
| | \(\delta\)-Elemene\(^{(a)}\) | 01.039 | |
| | Germacr-1(10),4(14),5-triene (\(\delta\)-Germacrene)\(^{(a),(e)}\) | 01.042 | |
| | 3,7,10-Humulatriene\(^{(a),(e)}\) | 01.043 | |
| | \(\alpha\)-Muurulene\(^{(a),(e)}\) | 01.052 | 2015b, CEF |
| | \(\beta\)-Bourbonene\(^{(a)}\) | 01.024 | 2015c, CEF |
| | \(\alpha\)-Farnesene\(^{(a)}\) | 01.040 | |
| **32** Epoxides | \(\beta\)-Caryophyllene epoxide\(^{(a)}\) | 16.043 | 2014, CEF |

\(^{(a)}\): FEEDAP opinion unless otherwise indicated.
\(^{(b)}\): 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.
\(^{(c)}\): A mixture of (\(E\))- and (\(Z\))-nerolidol was evaluated [02.018] (EFSA FEEDAP Panel, 2012b).
\(^{(d)}\): A mixture of cis- and trans-linalool oxide (5-ring) was evaluated [13.140].
\(^{(e)}\): 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 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.

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 flavours Group (BDG 06) – Laurales, Magnoliales, Piperales in animal feed. The Executive Summary of the EURL report can be found in Annex A.

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 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, 2021).

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...
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/m³ (specification: 930–970 kg/m³), the refractive index (20°C) between 1.503 and 1.508 (specification: 1.496–1.509). 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, 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 germacre-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 (4–7%, 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 germacre-1(10),4(14),5-triene (17.9–17.7%).

Compliance with specifications was also demonstrated by gas chromatography–mass spectrometry (GC-MS) analysis (Table 2). 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.

| Constituent | EU register name | CAS No. | FLAVIS No. | % GC area |
|-------------|------------------|---------|------------|-----------|
|             |                  |         |            | Specification | Mean (a) | Range |
| Germacre-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.

**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%.

---

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. 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.

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-Trimethyl-dodeca-2,6,10-trienyl acetate | 29548-30-9 | 09.818 | 4.08 | 3.51–4.67 |
| Benyl 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 | 01.054 | 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 | 01.021 | 1.71 | 1.36–2.00 |
| γ-Murolene | 30021-74-0 | 01.088 | 1.46 | 1.37–1.63 |
| α-Copaene | 3856-25-5 | 01.093 | 1.31 | 1.14–1.58 |
| τ-cadinol | 5937-11-1 | 01.093 | 1.29 | 0.97–1.74 |
| α-Murolone | 10208-80-7 | 01.052 | 0.93 | 0.84–1.19 |
| γ-Cadinene | 39029-41-9 | 01.093 | 0.87 | 0.78–1.11 |
| γ-Amorphene | 6980-46-7 | 01.093 | 0.83 | 0.75–1.07 |
| δ-Cadinol (isomer 2) | 19435-97-3 | 01.093 | 0.51 | 0.43–0.63 |
| τ-murolol | 19912-62-0 | 01.093 | 0.50 | 0.43–0.64 |
| (Z,E)-α-Farnesene | 26560-14-5 | 01.093 | 0.50 | 0.28–0.64 |
| Bicyclogermacrene | 67650-90-2 | 01.093 | 0.44 | 0.37–0.55 |
| Junenol | 472-07-1 | 01.093 | 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 | 01.093 | 0.37 | 0.19–0.49 |
| β-Copaene | 18252-44-3 | 01.093 | 0.36 | 0.32–0.41 |
| Cubenol | 21284-22-0 | 01.093 | 0.30 | 0.22–0.40 |
| Prenyl benzoate | 5205-11-8 | 09.693 | 0.29 | 0.26–0.32 |
| α-Cadinene | 24406-05-1 | 01.093 | 0.25 | 0.20–0.36 |
| Prenyl acetate | 1191-16-8 | 09.692 | 0.24 | 0.21–0.27 |
| (E)-isoeugenol | 5932-68-3 | 01.093 | 0.22 | 0.06–0.37 |
| β-Cubebene | 13744-15-5 | 01.093 | 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, rosilol, 1,8-cineole, germacrene B, α-elemene, (--)α-elemol, eugenol, benzyl alcohol and (Z)-hex-3-3-enyl benzoate; constituents (n = 15) between < 0.05 and > 0.01%: α-terpineol, hexyl acetate, nonanal, methyl salicylate, phenethyl acetate, β-pinene, (3E)-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 > 0.005%: 1,2-dimethoxybenzene, 2-methoxy-4-vinylphenol, estragole, 3,5-dimethylbenzaldehyde, isopentyl acetate, cis-linalool oxide, butyl acetate and 2-methylbutyl acetate.
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](https://www.efsa.europa.eu/en/data-report/compendium-botanicals)
20 Technical dossier/Section II.

---

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

EU: European Union; CAS no. Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers. (a): Mean calculated on five batches.
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, \(\delta\)-cadinene [01.021], \(\beta\)-cubebene [01.030], germacr-1(10),4(14),5-triene [01.042], 3,7,10-humulatriene [01.043] and \(\alpha\)-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](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. 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-methoxy group 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'-sulfoxyestragole, 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 14C-estragole (4-[14C-methoxy]-allylbenzene) was given in low doses to rodents it was mainly excreted as 14CO2 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-14C-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 14CO2 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, α-cadinol, τ-muurolol, δ-cadinol isomer 2, α-cadinol, junenol, cubenol, 3,5-dimethylbenzaldehyde and nitrophenyl ethane) was predicted using the QSAR Toolbox. 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, τ-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)-isoegenol, (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 (α-cubebeene, α-ylangene, b-bourbonene, cadina-3,5-diene, cis-muurola-1(15),5-diene, g-muurolene, g-amorphone, a-muurolene, bicyclogermacrene and trans-cadina-1,4-diene), representing on average 4.2% 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% (BMDL10) 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 BMDL10 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 BMDL10 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 apiole and anethol were 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 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), hepatocannabinoid or hepatocannabinoid-adenoma (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), hepatocannabinoid or hepatocannabinoid-adenoma (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<sub>10</sub> 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<sub>10</sub> 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<sup>28</sup> [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.

---

<sup>28</sup> 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 (3E)-hexenyl acetate [09.028] 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-pinene [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, 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, were not further considered in the assessment group as their contribution to the MOET(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-trimethylundeca-2,6,10-trienyl acetate, 3-methylbut-3-enyl acetate, 6-methylhept-5-en-2-one, (E)-α-elemol, (E)-nerolidol, spathulenol, α-cadinol isomer 1, guaiol, rosilol, 1,10-di-epi-cubenol, α-cadinol, α-muurolol, δ-cadinol isomer 2, α-cadinol, junenol, cubenol, cis-linalool oxide, trans-linalool oxide, phenethyl acetate, (E)-isoegenol, 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-cubebeine, γ-ylangene, β-cubebene, cadina-3,5-diene, cis-muurola-4(15),5-diene, γ-muurolene, γ-amorphene, bicyclogermacrene, α-muurolene, trans-cadin-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, nonanl and butyl acetate (CG 1); isopentyl acetate and 2-methylbutyl acetate (CG 2); geranyl acetate, (E,E)-farnesol and geraniol (CG 3); (3E)-hexenyl acetate (CG4); 6-methylhept-5-en-2-one (CG 5); α-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); δ-cadinene, α-copaene, γ-cadinene, β-copaene, α-cubebebe, β-cubebene, β-cadinene and β-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 |
|---------------------------|----------|------------------------|----------------------|
|                          | FLAVIS No | Max conc. in the oil | Max feed conc. | Intake\(^{(a)}\) | mg/kg bw per day | Cramer Class\(^{(b)}\) | NOAEL\(^{(c)}\) | MOE | MOET |
| **Assessment group**      |          | % | mg/kg | mg/kg bw per day | – | – | – | – | – |
|**Constituent**            |          |   |       |                   |   |   |   |   |   |
| CG 3 3,7,11-Trimethylododeca-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 |   |   |
| β-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 |   |   |
| δ-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) |          |   |       |                   |   |   |   |   |   |
| γ-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 |
As shown in Table 5, for all the assessment groups, the MOET was \( \geq 136 \). 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).

| Essential oil composition | Exposure | Hazard characterisation | Risk characterisation |
|---------------------------|----------|------------------------|----------------------|
| Assessment group          | FLAVIS No | Max conc. in the oil | Max feed conc. | Intake (\( \text{mg/kg bw per day} \)) | Cramer Class (\( \text{(b)} \)) | NOAEL (\( \text{(c)} \)) | MOE | MOET |
| Constituent               |          | %                     | mg/kg            | – | mg/kg bw per day | – | – |
| CG 31, V (Br, tricyclic, non-aromatic hydrocarbons) | | | | | | | |
| \( \beta \)-Caryophyllene | 01.007   | 7.88                  | 0.079            | 0.0071 | (I) | 222 | 31,390 |
| \( \gamma \)-Murolene     | –        | 1.63                  | 0.016            | 0.0015 | I | 3 | 2,046 |
| \( \alpha \)-Murolene     | –        | 1.19                  | 0.012            | 0.0011 | I | 3 | 2,811 |
| \( \gamma \)-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 |
| \( \alpha \)-Cubebeene    | –        | 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 |
| \( \alpha \)-Ylangene     | –        | 0.16                  | 0.002            | 0.0001 | I | 3 | 20,337 |
| MOET CG 31, V              |          | 597                   |                  | | | |
| CG 31, VI (macrocyclic non-aromatic hydrocarbons) | | | | | | | |
| Germacre-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                     |          | 136                  |                  | | | |
| 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 \( \geq 136 \). 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 is safe for all animal species, except cats, for which the calculated safe concentration in complete feed ranges 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 analytically detected 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 BMDL10 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 (preylnl 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, germacr-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 ≤ 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 sensitizer. 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, Magnoliaceae, Piperaceae for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFACEEIG) 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                                                                                 |

References

Anthony A, Caldwell J, Hutt AJ and Smith RL, 1987. Metabolism of estragole in rat and mouse and influence of dose size on excretion of the proximate carcinogen 1'-hydroxyestragole. Food and Chemical Toxicology, 25, 799–806.

van den Berg SJPL, Restani P, Boersma MG, Delmulle L and Rietjens IMCM, 2011. Levels of genotoxic and carcinogenic compounds in plant food supplements and associated risk assessment. Food and Nutrition Sciences, 2, 989–1010.

Bleasel N, Tate B and Rademarker M, 2002. Allergic contact dermatitis following exposure to essential oils. Australian Journal of Dermatology, 43, 211–213.

Burdock GA, 2009. Fenaroli's handbook of flavor ingredients. 6th Edition. CRC Press, Taylor & Francis Group, Boca Raton, FL. 243, 2019-2030 pp.

Court MH and Greenblatt DJ, 1997. Molecular basis for deficient acetaminophen glucuronidation in cats. An interspecies comparison of enzyme kinetics in liver microsomes. Biochemical Pharmacology, 53, 1041–1047.

EFSA (European Food Safety Authority), 2008a. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request from the Commission on Flavouring Group Evaluation 6, Revision 1 (FGE.06Rev 1): Flavouring Group Evaluation 6, Revision 1 (FGE.06Rev1): straight- and branched-chain aliphatic unsaturated primary alcohols, aldehydes, carboxylic acids, and esters from chemical groups 1 and 4 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). EFSA Journal 2008;6(5):616, 75 pp. https://doi.org/10.2903/j.efsa.2008.616

EFSA (European Food Safety Authority), 2008b. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from Commission on Flavouring Group Evaluation 87, (FGE.87) bicyclic secondary alcohols, ketones and related esters. EFSA Journal 2008;918, 109 pp. https://doi.org/10.2903/j.efsa.2008.918
EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (AFN) on a request from the Commission on FGE54Rev1 Consideration of benzyl derivatives evaluated by JECFA (57th meeting) structurally related to benzyl alcohols, benzaldehydes, a related acetol, benzoic acids and related esters evaluated by EFSA in FGE.06Rev1 (2009). EFSA Journal 2009;10:25, 73 pp. https://doi.org/10.2903/j.efsa.2008.1025

EFSA (European Food Safety Authority), 2012. Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements. EFSA Journal 2012;10(5):2663, 60 pp. https://doi.org/10.2903/j.efsa.2012.2663

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2010a. Scientific Opinion on Flavouring Group Evaluation 62, Revision 1 (FGE.62Rev1): consideration of linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids, and related esters evaluated by JECFA (61st and 68th meeting) structurally related to branched- and straight-chain unsaturated carboxylic acids and esters of these with aliphatic saturated alcohols evaluated by EFSA in FGE.05Rev2 (2010) and to straight- and branched-chain aliphatic unsaturated primary alcohols, aldehydes, carboxylic acids, and esters evaluated by EFSA in FGE.06Rev1 (2008). EFSA Journal 2010;8(11):1407, 47 pp. https://doi.org/10.2903/j.efsa.2010.1407

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2010b. Scientific Opinion on Flavouring Group Evaluation 20, Revision 2 (FGE.20Rev2): benzyl alcohols, benzaldehydes, a related acetol, benzoic acids, and related esters from chemical groups 23 and 30. EFSA Journal 2010;8(7):1405, 136 pp. https://doi.org/10.2903/j.efsa.2010.1405

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2010c. Guidance on the data required for the risk assessment of flavourings. EFSA Journal 2010;8(6):1623, 38 pp. https://doi.org/10.2903/j.efsa.2010.1623

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011. Scientific Opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2): aliphatic hydrocarbons from chemical group 31. EFSA Journal 2011;9(6):2177, 126 pp. https://doi.org/10.2903/j.efsa.2011.2177

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Scientific Opinion on Flavouring Group Evaluation 82, Revision 1 (FGE.82Rev1): consideration of epoxides evaluated by the JECFA (65th meeting). EFSA Journal 2014;12(6):3708, 32 pp. https://doi.org/10.2903/j.efsa.2014.3708

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015a. Scientific Opinion on Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3): aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8. EFSA Journal 2015;13(5):4118, 115 pp. https://doi.org/10.2903/j.efsa.2015.4118

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015b. Scientific Opinion on Flavouring Group Evaluation 25, Revision 3 (FGE.25Rev3): aliphatic hydrocarbons from chemical group 31. EFSA Journal 2015;13(4):4069, 116 pp. https://doi.org/10.2903/j.efsa.2015.4069

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015c. Scientific Opinion on Flavouring Group Evaluation 78, Revision 2 (FGE.78Rev2): consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic hydrocarbons evaluated by EFSA in FGE.25Rev3. EFSA Journal 2015;13(4):4067, 72 pp. https://doi.org/10.2903/j.efsa.2015.4067

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Fürst P, Gürler T, Gundert-Remy U, Hussy T, Mennes W, Moldeus P, Oskarsson A, Shah R, Waalkens-Berendsen I, Wölflé D, Boon P, Crebelli R, Di Domenico A, Filipić M, Mortensen A, Van Loveren H, Woutersen R, Gergelova P, Giarola A, Lodi F and Frutos Fernandez MJ, 2019. Scientific Opinion on the re-evaluation of benzyl alcohol (E 1519) as food additive. EFSA Journal 2019;17(10):5876, 25 pp. https://doi.org/10.2903/j.efsa.2019.5876

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010. Statement on the use of feed additives authorised/applied for use in feed when supplied via water. EFSA Journal 2010;8(12):1956, 9 pp. https://doi.org/10.2903/j.efsa.2010.1956

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Scientific Opinion on the safety and efficacy of allylhydroxybenzenes (chemical group 18) when used as flavourings for all animal species. EFSA Journal 2011;9(12):2440, 14 pp. https://doi.org/10.2903/j.efsa.2011.2440

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Scientific Opinion on the safety and efficacy of branched-chain primary aliphatic alcohols/aldehydes/ acids, acetals and esters with esters containing branched-chain alcohols and acetals containing branched-chain aldehydes (chemical group 2) when used as flavourings for all animal species. EFSA Journal 2012;10(10):2927, 26 pp. https://doi.org/10.2903/j.efsa.2012.2927

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Scientific opinion on the safety and efficacy of aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers (chemical group 6) when used as flavourings for all animal species. EFSA Journal 2012;10(11):2966, 25 pp. https://doi.org/10.2903/j.efsa.2012.2966
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Opinion on the safety and efficacy of furanones and tetrahydrofurfuryl derivatives: 4-hydroxy-2,5-dimethylfuran-3(2H)-one, 4,5-dihydro-2-methylfuran-3(2H)-one, 4-acetoxo-2,5-dimethylfuran-3(2H)-one and linalool oxide (chemical Group 13) when used as flavourings for all animal species. EFSA Journal 2012;10(7):2786, 16 pp. https://doi.org/10.2903/j.efsa.2012.2786

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012d. Scientific Opinion on the safety and efficacy of phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxycetic acids and related esters (chemical group 15) when used as flavourings for all animal species. EFSA Journal 2012;10(3):2625, 16 pp. https://doi.org/10.2903/j.efsa.2012.2625

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012e. Scientific Opinion on the safety and efficacy of aliphatic and alicyclic ethers (chemical group 16) when used as flavourings for all animal species. EFSA Journal 2012;10(11):2967, 17 pp. https://doi.org/10.2903/j.efsa.2012.2967

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012f. Scientific Opinion on the safety and efficacy of benzyl alcohols, aldehydes, acids, esters and acetals (chemical group 23) when used as flavourings for all animal species. EFSA Journal 2012;10(7):2785, 30 pp. https://doi.org/10.2903/j.efsa.2012.2785

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012g. Scientific Opinion on the safety and efficacy of phenol derivatives containing ring-alkyl, ring-alkoxy and side-chains with an oxygenated functional group (chemical group 25) when used as flavourings for all species. EFSA Journal 2012;10(2):2573, 19 pp. https://doi.org/10.2903/j.efsa.2012.2573

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012h. Scientific Opinion on the safety and efficacy of aromatic ethers including anisole derivatives (chemical group 26) when used as feed additives for all animal species. EFSA Journal 2012;10(5):2678, 19 pp. https://doi.org/10.2903/j.efsa.2012.2678

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012i. Guidance for the preparation of dossiers for sensory additives. EFSA Journal 2012;10(1):2534, 26 pp. https://doi.org/10.2903/j.efsa.2012.2534

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012j. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of straight-chain primary aliphatic alcohols/aldehydes/acid, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes (chemical group 01) when used as flavourings for all animal species. EFSA Journal 2013;11(4):3169, 35 pp. https://doi.org/10.2903/j.efsa.2013.3169

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015a. Scientific opinion on the safety and efficacy of saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols belonging chemical group 5 when used as flavourings for all animal species. EFSA Journal 2015;13(11):4268, 21 pp. https://doi.org/10.2903/j.efsa.2015.4268

EFSA Feedap Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015b. Scientific Opinion on the safety and efficacy of aliphatic and aromatic hydrocarbons (chemical group 31) when used as flavourings for all animal species. EFSA Journal 2015;13(3):4053, 22 pp. https://doi.org/10.2903/j.efsa.2015.4053

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016a. Scientific opinion on the safety and efficacy of (α,β)-unsaturated straight-chain and branched-chain aliphatic primary alcohols, aldehydes, acids and esters belonging to chemical group 3 when used as flavourings for all animal species. EFSA Journal 2016;14(6):4512, 21 pp. https://doi.org/10.2903/j.efsa.2016.4512

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016b. Scientific opinion on the safety and efficacy of aliphatic and aromatic hydrocarbons (chemical Group 31) when used as flavourings for all animal species and categories. EFSA Journal 2016;14(1):4339, 17 pp. https://doi.org/10.2903/j.efsa.2016.4339

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016c. Scientific opinion on the safety and efficacy of saturated alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols from chemical group 8 when used as flavourings for all animal species. EFSA Journal 2016;14(6):4475, 26 pp. https://doi.org/10.2903/j.efsa.2016.4475

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017. Scientific opinion on the safety and efficacy of αr-substituted primary alcohol, aldehyde, acid, ester and acetal derivatives belonging to chemical group 22 when used as flavourings for all animal species. EFSA Journal 2017;15(1):4672, 21 pp. https://doi.org/10.2903/j.efsa.2017.4672
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Koubia M, López-Alonso M, López Puente S, Mantovanì A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017a. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Koubia M, López-Alonso M, López Puente S, Mantovanì A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017b. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Koubia M, López-Alonso M, López Puente S, Mantovanì A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017c. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Koubia M, López-Alonso M, López Puente S, Mantovanì A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML, 2017d. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Koubia M, López-Alonso M, López Puente S, Mantovanì A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2018. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos ML, Christensen H, Dusemund B, Koubia M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brock T, Knecht J, Kolar B, Beelen P, Padovani L, Tarrés-Call J, Vettori MV and Azimonti G, 2019. Guidance on the assessment of the safety of feed additives for the environment. EFSA Journal 2019;17(4):5648, 78 pp. https://doi.org/10.2903/j.efsa.2019.5648

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Koubia M, Faison Durjava 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, Galobart J, Manini P, Pizzo F and Dusemund B, 2021a. Scientific Opinion on the safety and efficacy of feed additives consisting of expressed lemon oil and its fractions from Citrus limon (L.) Osbeck and of lime oil from Citrus aurantifolia (Christm.) Swingle for use in all animal species. EFSA Journal 2021;19(4):6548, 55 pp. https://doi.org/10.2903/j.efsa.2021.6548

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2021b. General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic. Endorsed by the FEEDAP Panel during the 153rd Plenary meeting of 17-18 March 2021. Available online: https://www.efsa.europa.eu/en/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf

EFSA SC (EFSA Scientific Committee), 2009. Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements, on request of EFSA. EFSA Journal 2009;7(9):1249, 19 pp. https://doi.org/10.2903/j.efsa.2009.1249pp

EFSA Scientific Committee, 2012. Scientific Opinion on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed. EFSA Journal 2012;10(3):2578, 5 pp. https://doi.org/10.2903/j.efsa.2012.2578

EFSA SC (EFSA Scientific Committee), Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen KH, More S, Mortensen A, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turk D, Aerts M, Bodin L, Davis A, Edler L, Gundert-Remy U, Sand S, Slob W, Bottex B, Abrahamtes JC, Marques DC, Kass G and Schlatter JR, 2017. Update: guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 41 pp. https://doi.org/10.2903/j.efsa.2017.4658

EFSA SC (EFSA Scientific Committee), More SJ, Bampidis V, Benford D, Bennekou SH, Bragard C, Halldorsson TJ, Hernández-Jerez AF, Koutsoumanis K, Naegeli H, Schlatter JR, Silano V, Nielsen SS, Schrenk D, Turk D, Younges M, Benfenati E, Castle L, Cedergren N, Hardy A, Laskowski R, Leblanc JC, Kortenkamp A, Ragas AD, Posthuma L, Swendsen C, Solecki R, Testai E, Dujardin B, Kass GEN, Manini P, Jedd MZ, Dorne J-L and Hogstrand C, 2019a. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal 2019;17(3):5634, 77 pp. https://doi.org/10.2903/j.efsa.2019.5634
EFSAS (EFSA Scientific Committee), More S, Bampidis V, Benford D, Boesten J, Bragard C, Halldorsson T, Hernandez-Jerez A, Hougaard-Benkeou S, Koutsoumanis K, Naegeli H, Nielsen SS, Schrenk D, Silano V, Turk D, Younes M, Aquilina G, Crebelli R, Gürtler R, Hirscher-Ernst K, Mosesso P, Nielsen E, Solecki R, Carfi M, Martino C, Maurici D, Parra Morte J and Schlatter J, 1999b. Statement on the genotoxic assessment of chemical mixtures. EFSAS Journal 2019;17(1):5519, 11 pp. https://doi.org/10.2903/j.efsa.2019.5519

EMA (European Medicines Agency), 2019. Public statement on the use of herbal medicinal products containing estragole. 2nd Draft, Revision 1, EMA/HPMC/137212/2005 Rev 1. Committee on Herbal Medicinal Products (HPMC). Available online: https://www.ema.europa.eu/en/documents/other/second-draft-revision-1-public-statement-use-herbal-medicinal-products-containing-estragole_en.pdf

de Groot AC and Schmidt E, 2016. Essential oils, Part IV: contact allergy. Dermatitis, 7/8, 27, 170–175.

ISO (International Organization for Standardization), 2004. Oil of ylang-ylang [Cananga Odorata (Lam.) Hook. f. et Thomson forma genuine]. Reference number ISO 3063:2004(E), Switzerland.

Lautz LS, Jeddi MZ, Girolami F, Nebbia C and Dorne JLCM, 2021. Metabolism and pharmacokinetics of pharmaceuticals in cats (Felis sylvestris catus) and implications for the risk assessment of feed additives and contaminants. Toxicology Letters, 338, 114–127.

Miller EC, Swanson AB, Phillips DH, Fletcher TL, Liem A and Miller JA, 1983. Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Research, 43, 1124–1134.

NTP (National Toxicology Program), 2000. NTP Technical Report on the Toxicology and carcinogenesis studies of methyleugenol (CAS NO. 93-15-2) in F344/N rats and B6C3F1 mice (gavage study). NTP, Technical Report Series, 491, 1–420. Available online: https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/lt491.pdf

Phillips D, Reddy MV and Randerath K, 1984. 32P- Postlabelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes. II. Newborn male B6C3F1 mice. Carcinogenesis, 5, 1623–1628.

Randerath K, Haglund RE, Phillips DH and Reddy MV, 1984. 32P- Postlabelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes. I. Adult female CD-1 mice. Carcinogenesis, 5, 1613–1622.

Sangster SA, Caldwell AJ, Hutt A, Anthony A and Smith RL, 1987. The metabolic disposition of [methoxy-14C]-labelled trans-anethole, estragole and p-propylanisole in human volunteers. Xenobiotica, 17, 1223–1232.

Scientific Committee on Food (SCF), 2001. Opinion of the Scientific Committee on Food on Estragole (1-allyl-4-methoxybenzene) - 583 SCF/CS/FLAV/FLAVOUR/6 ADD 2 Final, European Commission Health and Consumer Protection Directorate-General, 26.09.2001, pp. 1-10. Available online: https://ec.europa.eu/food/system/files/2016-10/fs_food-improvement-agents_flavourings-out104.pdf

Stashenko E, Martínez JR, MacKu C and Shibamoto T, 1993. HRGC and GC-MS analysis of essential oil from Colombian ylang–ylang (Cananga odorata Hook fil. et Thomson, forma genuina). Journal of High Resolution Chromatography, 16, 441–444.

Stashenko EE, Torres W and Martinez Morales JR, 1995. A study of the compositional variation of the essential oil of ylang-ylang (Cananga odorata Hook fil. et Thomson, forma genuina) during flower development Morales. Journal of High Resolution Chromatography, 18, 101–104.

Stashenko EE, Prada NQ and Martinez Morales JR, 1996. HRGC/FID/NPD and HRGC/MSD study of Colombian ylang-ylang (Cananga odorata) oils obtained by different extraction techniques. Journal of High Resolution Chromatography, 19, 353–358.

Suparmi S, Ginting AJ, Mariyam S, Wesseling S and Rietjens IMCM, 2019. Levels of methyleugenol and eugenol in instant herbal beverages available on the Indonesian market and related risk assessment. Food and Chemical Toxicology, 125, 467–478.

Tan LTH, Lee LH, Yin WF, Chan CK, Kadir HA, Chan KG and Goh BH, 2015. Traditional uses, phytochemistry, and bioactivities of Cananga odorata (Ylang-Ylang). Evidence-Based Complementary and Alternative Medicine, Volume 2015, Article ID 986314, 1–30. https://doi.org/10.1155/2015/986314

Zangoureas A, Caldwell J, Hutt AJ and Smith RL, 1981. Dose-dependent conversion of estragole in the rat and mouse to the carcinogenic metabolite 1-hydroxyestragole. Biochemical Pharmacology, 30, 1383.

Zhou GD, Moorthy B, Bi J, Donnelly KC and Randerath K, 2007. DNA adducts from alkoxylallylbenzene herb and spice constituents in cultured human (HepG2) cells. Environmental and Molecular Mutagenesis, 48, 715–721.

**Abbreviations**

| Abbreviation | Meaning |
|-------------|---------|
| 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 |
| Abbreviation | Description |
|--------------|-------------|
| 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 ≥ 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 kg DM/day | Body weight kg | Use level mg/kg | Estragole intake(a) µg/kg bw per day | MOE(b) |
|----------------------------------|-----------------------------|----------------|-----------------|-------------------------------------|--------|
| 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, Magnioales, Piperales

The Botanically Defined Flavourings – Group 6 BDG 06 (Laurales, Magnioales, 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.