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# Safety and efficacy of feed additives consisting of essential oils derived from the flower buds or the leaves of *Syzygium aromaticum* (L.) Merr. & L.M. Perry (clove bud oil and clove leaf oils) for all animal species (FEFANA asbl)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Mojca 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, Josef Schlatter, Johannes Westendorf, Yvette Dirven, Paola Manini and Birgit Dusemund

## Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of essential oils from the flower buds or the leaves of *Syzygium aromaticum* (L.) Merr. & L.M. Perry: clove bud oil, clove leaf oil and a β-caryophyllene-rich fraction of clove leaf oil (CCL oil), when used as sensory additives in feed and water for drinking for all animal species. Clove oils contain methyleugenol (up to 0.13%). The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the use of clove oils was of low concern for long-living and reproductive animals and of no concern for target species for fattening at the following concentrations in complete feed: 25–50 mg/kg for clove bud oil, 28–100 mg/kg for clove leaf oil and 20 mg/kg for CCL oil. The FEEDAP Panel considered that the use in water for drinking alone or in conjunction with use in feed should not exceed the daily amount that is considered of low or no concern when consumed via feed alone. No concerns for consumers were identified following the use of clove oils up to the highest safe level in feed. The additives under assessment should be considered as irritant to skin and eyes and the respiratory tract and as skin sensitisers. When handling the essential oils, exposure of unprotected users to methyleugenol may occur. Therefore, to reduce the risk, the exposure of the users should be minimised. The use of clove oils at the proposed use level in feed was not expected to pose a risk for the environment. Since clove bud oil and clove leaf oil are recognised to flavour food and their function in feed would be essentially the same, no demonstration of efficacy was considered necessary.

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**Keywords:** sensory additives, flavouring compounds, *Syzygium aromaticum* (L.) Merr. & L.M. Perry, clove bud oil, clove leaf oil, eugenol, β-caryophyllene

**Requestor:** European Commission

**Question number:** EFSA-Q-2010-01282 (New EFSA-Q-2023-00397)

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## Table of contents

# 1. Introduction

## 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. In addition, Article 10(2) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of 7 years after the entry into force of this Regulation.

The European Commission received a request from the Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation/re-evaluation of 18 preparations (namely geranium oil, geranium rose oil, eucalyptus oil, eucalyptus tincture, clove oil, clove tincture, broom teatree oil, purple loosetrife tincture, tea tree oil, melaleuca cajuputi oil, niaouli oil, allspice oil, bay oil, pomegranate bark extract, bambusa tincture, citronella oil, lemongrass oil and vetiveria oil) belonging to botanically defined group (BDG) 07 – *Geraniales*, *Myrtales*, *Poales* when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). During the assessment, the applicant withdrew the application for four preparations (namely broom teatree oil, geranium oil, bay oil and vetiveria oil[3]). These preparations were deleted from the register of feed additives.[4] During the course of the assessment, this application was split and the present opinion covers only one out of the initial 18 preparations under application: clove oil from the flower buds or the leaves of *Syzygium aromaticum* (L.) Merr. & L.M. Perry[5] for all animal species. During the assessment, the applicant clarified that three types of additives fall into the definition "clove oil", i.e., clove bud oil, clove leaf oil and a β-caryophyllene-rich fraction of clove leaf oil. The three preparations from *S. aromaticum* will be assessed individually.

The remaining 13 preparations belonging to botanically defined group (BDG) 07 – *Geraniales, Myrtales, Poales* under application are assessed in separate opinions.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 21 December 2010.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the clove oil from the flower buds or leaves the of *S. aromaticum*, when used under the proposed conditions of use (see Sections 3.2.1.3, 3.3.1.3 and 3.4.1.3).

## 1.2. Additional information

An essential oil from *Syzygium aromaticum* L. (clove oil) 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.

There is no specific EU authorisation for any *S. aromaticum* preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008[6] flavouring preparations

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

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

[3] On 27 February 2019, EFSA was informed by the applicant about the withdrawal of the applications on broom teatree oil, geranium oil, bay oil and vetiveria oil.

[4] Register of feed additives, Annex II, withdrawn by OJ L162, 10.05.2021, p. 5.

[5] Accepted name: *Syzygium aromaticum* (L.) Merr. & L.M. Perry; synonyms: *Eugenia caryophyllata* Thunb.; *Caryophyllus aromaticus* L.; *Eugenia caryophyllus* (Spreng.) Bullock & S.G.Harrison.

[6] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Regulation (EC) No 1601/91 of the Council, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34.

produced from food, may be used without an evaluation and approval as long as 'they do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer, and their use does not mislead the consumer'.

'Clove (Caryophylli flos)' are described in a monograph of the European Pharmacopoeia 11.0 (PhEur, 2022a). They are defined as the whole flower buds of *S. aromaticum* (L.) Merr. et L.M.Perry (Syn. *Eugenia caryophyllus* (Spreng.) Bullock et S.G.Harrison) dried until they become reddish-brown. They have a minimum content of 150 mL/kg of essential oil.

'Clove oil (Caryophylli floris aetheroleum)' is described in a monograph of the European Pharmacopoeia 11.0 (PhEur, 2022b). It is defined as the essential oil obtained by steam distillation from the dried flower buds of *S. aromaticum* (L.) Merr. et L.M.Perry (Syn. *E. caryophyllus* (Spreng.) Bullock et S.G.Harrison.

In 1998, the Committee for Veterinary Medicinal Products of the European Medicines Agency (EMA) published a report on Caryophylli aetheroleum, the volatile oil obtained from *S. aromaticum* (L.) Merr. & Perry (EMA, 1998).

For *S. aromaticum* (L.) Merill et L.M. Perry, floris aetheroleum, the European Medicines Agency (EMA) issued a community herbal monograph for human medicinal use (EMA, 2011a), an assessment report on *S. aromaticum* (L.) Merill et L.M. Perry, flos and *S. aromaticum* (L.) Merill et L.M. Perry, floris aetheroleum (EMA, 2011b) and an addendum to the assessment report (EMA, 2020).

Many of the individual components of the essential oils have been already assessed as chemically defined flavourings for use in feed and food by the FEEDAP Panel, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) and the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). The flavouring compounds currently authorised for feed[7] and/or food[8] use, together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000[9], and the corresponding EFSA opinion are listed 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 (common name) | FLAVIS No | EFSA opinion,* Year |
|---|---|---|---|---|
| 05 | Saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols | 6-Methylhept-5-en-2-one | 07.015 | 2021a |
| 06 | Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers | Linalool | 02.013 | 2012a |
| 14 | Furanones and tetrahydrofurfuryl derivatives | 5-Methylfurfural | 13.001 | 2016a |
| | | Furfural | 13.018 | |
| 17 | Propenylhydroxybenzenes | Isoeugenol[(a)] | 04.004 | 2012b |
| 18 | Allylhydroxybenzenes | Eugenol | 04.003 | 2011 |
| | | Eugenyl acetate | 09.020 | |
| | | 4-Allylphenol[(b)] | 04.058 | EFSA, 2009 (AFC) |

[7] European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03.pdf

[8] 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.

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

| CG | Chemical Group | Product – EU register name (common name) | FLAVIS No | EFSA opinion,* Year |
|---|---|---|---|---|
| 23 | Benzyl alcohols, aldehydes, acids, esters and acetals | Benzyl alcohol | 02.010 | 2012c |
| | | Benzaldehyde | 05.013 | |
| | | Benzyl benzoate | 09.727 | |
| | | Methyl salicylate | 09.749 | |
| 31 | Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes | d-Limonene | 01.045 | 2015 |
| | | Pin-2(10)-ene (β-pinene) | 01.003 | 2016b |
| | | Pin-2(3)-ene (α-pinene) | 01.004 | |
| | | β-Caryophyllene | 01.007 | |
| | | Valencene | 01.017 | |
| | | δ-Cadinene[(b),(c)] | 01.021 | 2011, CEF |
| | | 1(5),11-Guaiadiene[(b),(c)] | 01.023 | |
| | | δ-Germacrene[(b),(c)] | 01.042 | |
| | | 3,7,10-Humulatriene[(b),(c)] | 01.043 | |
| | | α-Muurulene[(b),(c)] | 01.052 | |
| | | β-Bourbonene[(b)] | 01.024 | 2015a, CEF |
| | | α-Farnesene[(b)] | 01.040 | |
| 32 | Epoxides | β-Caryophyllene oxide | 16.043 | 2014, CEF |

*: FEEDAP opinion unless otherwise indicated.
(a): EFSA evaluated isoeugenol [04.004], a mixture of *(E)*- and *(Z)*-isomers (EFSA FEEDAP Panel, 2012b).
(b): Evaluated for use in food only. According to Regulation (EC) 1565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.
(c): Evaluated applying the 'Procedure' described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). No longer authorised for use as flavours in food, as the additional toxicity data requested (EFSA CEF Panel, 2011) were not submitted and the CEF Panel was unable to complete its assessment (EFSA CEF Panel, 2015b).

## 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[10] in support of the authorisation request for the use of clove oils from *S. aromaticum* as a feed additive. The dossier was received on 8/6/2023 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00397.[11]

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.

Some of the components of the essential oils under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings (CDGs). 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 07, including the current one under assessment.[12]

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phytochemical markers in the additives. The evaluation report is related to the methods of analysis for each feed additive included the group BDG 07 (Geraniales, Myrtales, Poales). In particular, the EURL recommended a method based on gas chromatography coupled with flame ionisation detection (GC–FID) for the determination of the phytochemical marker *eugenol* in the feed additives clove leaf oil (eugenol type) and clove bud oil, and of the phytochemical marker *β-caryophyllene* in the feed additive *clove leaf oil* (β-caryophyllene type).[13]

[10] FEED dossier reference: FAD-2010-0219.
[11] The original application EFSA-Q-2010-01282 was split on 7/6/2023 and a new EFSA-Q-2023-00397 was generated.
[12] Technical dossier/Supplementary information February 2023/Letter dated 31/01/2023.
[13] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0219_en

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of clove oils from *S. aromaticum* is in line with the principles laid down in Regulation (EC) No 429/2008[14] 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, 2012d), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012e), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), 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), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA SC, 2019c) and General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021b).[15]

# 3. Assessment

The additives under assessment, clove bud oil, clove leaf oil and a β-caryophyllene-rich fraction of clove leaf oil (hereinafter referred to as CCL oil), are derived from the flower buds or leaves of *S. aromaticum* (L.) Merr. & L.M. Perry and are intended for use as sensory additives (functional group: flavouring compounds) in feed and water for drinking for all animal species.

## 3.1. Origin and extraction

*S. aromaticum* (L.) Merr. & L.M. Perry (synonym: *Eugenia caryophyllata* Thunb.) is a small evergreen tree belonging to the myrtle (Myrtaceae) family. It is native to the Maluki islands (Moluccas or spice islands) of Indonesia but, because of its commercial importance, it is now widely cultivated in other parts of the world with similar climatic conditions. The tree is the source of 'cloves', the immature flower buds harvested just before flowering and dried, which are widely used as a spice and as a source of flavour and fragrance in many consumer products. The medicinal use of cloves includes the dental or oromucosal use of their essential oil as an analgesic in the treatment of toothache and is also described in Chinese and Ayurvedic systems. Essential oils are obtained either from the flower buds (clove bud oil) or from the leaves (clove leaf oil) of *S. aromaticum*.

Clove bud oil is obtained by steam distillation of the dried flower buds of *S. aromaticum* sourced from Indonesia. The volatile constituents are condensed and then separated from the aqueous phase by decantation.

Clove leaf oil is obtained by steam distillation of the leaves of *S. aromaticum* (sourced from Madagascar and Indonesia). The volatile constituents are condensed and then separated from the aqueous phase by decantation.

CCL oil is obtained by fractional distillation of clove leaf oil itself, resulting in an enriched composition in β-caryophyllene and other hydrocarbons (e.g. 3,7,10-humulatriene, α-copaene and δ-cadinene).

## 3.2. Clove bud oil

### 3.2.1. Characterisation of clove bud oil

The essential oil under assessment is a clear yellow to light amber, slightly viscous liquid with a characteristic odour. In seven batches of the additive, the refractive index (20°C) ranged between

[14] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

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

1.534 and 1.537 (specification: 1.528–1.537), the specific gravity (20°C) between 1.0559 and 1.0593 and the optical rotation (20°C) between −0.64 and −1.0 (five batches).[16] 'Clove oil' is identified with the single Chemical Abstracts Service (CAS) number 8000-34-8, the European Inventory of Existing Chemical Substances (EINECS) number 284-638-7 and the Council of Europe (CoE) number 188. Clove bud oil is identified by the Flavor Extract Manufacturers Association (FEMA) number 2323.

For clove bud oil, the product specifications are based on the standard developed by the International Organisation for Standardization (ISO) 3142:1997 for 'Oil of clove buds',[17] adapted to reflect the concentrations of selected volatile components. Three compounds are specified as shown in Table 2, with eugenol selected as the phytochemical marker. Analysis of seven batches of the additive showed compliance with these specifications when analysed by GC–FID and expressed as percentage of gas chromatographic peak area (% GC area).[18]

**Table 2:** Major constituents of clove bud oil from the buds of *Syzygium aromaticum* (L.) Merr. & L.M. Perry as defined by specifications and batch to batch variation based on the analysis of seven batches by gas chromatography with flame ionisation detector. 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 | | |
|---|---|---|---|---|---|
| | | | Specification[(a)] | Mean | Range |
| Eugenol | 97-53-0 | 04.003 | 67–85 | 82.7 | 81.78–84.00 |
| β-Caryophyllene | 87-44-5 | 01.007 | 2–16 | 6.7 | 5.89–7.21 |
| Eugenyl acetate | 93-28-7 | 09.030 | 5–15 | 8.5 | 7.90–9.09 |

EU: European Union; CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.
(a): Specifications defined based on GC–FID analysis.

The applicant provided a full characterisation of the seven batches obtained by gas chromatography–mass spectrometry (GC–MS).[19] In total, up to 30 constituents were detected, 28 of which were identified and accounted on average for 99.33% (99.05–99.82%) of the % GC area. The three compounds indicated in the product specifications accounted for 96.2% on average (range 95.6–96.8%) of the GC area. Besides the three compounds indicated in the product specifications, 11 other compounds were detected at individual levels ≥ 0.05% and are listed in Table 3. These 14 compounds together account on average for 99.15% (98.93–99.59%) of the % GC area. The remaining 14 compounds identified (ranging between 0.002% and 0.04%) and accounting for 0.18% of the % GC area are listed in the footnote.[20] Based on the available data on the characterisation, clove bud oil is considered a fully defined mixture (EFSA SC, 2019a).

**Table 3:** Constituents of clove bud oil from the buds of *Syzygium aromaticum* (L.) Merr. & L.M. Perry accounting for ≥ 0.05% of the composition (based on the analysis of seven batches by gas chromatography–mass spectrometry). 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 | Range |
| Eugenol | 97-53-0 | 04.003 | 75.06 | 73.03–75.96 |
| β-Caryophyllene | 87-44-5 | 01.007 | 13.20 | 12.21–14.11 |
| Eugenyl acetate | 93-28-7 | 09.030 | 7.92 | 7.40–8.49 |

[16] Technical dossier/Supplementary information March 2023/Annex_II_SIn_Reply_clove_oil_COA_chrom.
[17] Technical dossier/Supplementary information March 2023/Annex_V_SIn_reply_clove_oil_ ISO_3142_1997.
[18] Technical dossier/Supplementary information March 2023/Anne_III_Sin_reply_clove-oil composition.
[19] Technical dossier/Supplementary information March 2023/ Annex_II_SIn_Reply_clove_oil_COA_chrom.
[20] Additional constituents:constituents (n = 7) between < 0.05 and ≥ 0.01%: furfural, isocadinene, 1-epi-cubenol, cadina-1,4-diene, alloaromadendrene, 5-methylfurfural and linalool;constituents (n = 7) between < 0.01 and ≥ 0.002%: 6-methylhept-5-en-2-one, ethyl benzoate, benzyl alcohol, benzaldehyde, d-limonene, α-pinene and β-pinene.

| Constituent<br>EU register name | CAS No | FLAVIS No | % GC area | |
|---|---|---|---|---|
| | | | Mean | Range |
| 3,7,10-Humulatriene | 6753-98-6 | 01.043 | 1.13 | 1.03–1.23 |
| β-Caryophyllene epoxide | 1139-30-6 | 16.043 | 0.76 | 0.45–1.09 |
| α-Copaene | 3856-25-5 | – | 0.40 | 0.34–0.52 |
| Humulene oxide II | 19888-34-7 | – | 0.38 | 0.09–1.09 |
| α-Cubebene | 17699-14-8 | – | 0.17 | 0.04–0.49 |
| Methyl salicylate | 119-36-8 | 09.749 | 0.16 | 0.08–0.25 |
| Calamenene | 483-77-2 | – | 0.13 | 0.10–0.19 |
| 4-Allylphenol | 501-92-8 | 04.058 | 0.13 | 0.10–0.14 |
| δ-Cadinene | 29350-73-0 | 01.021 | 0.12 | 0.09–0.15 |
| Methyleugenol | 93-15-2 | – | 0.08 | 0.04–0.13 |
| *trans*-Cadina-1(6),4-diene | 20085-11-4 | – | 0.07 | 0.07–0.08 |
| Total | | | 99.15 | 98.93–99.59[(a)] |

EU: European Union; CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.
(a): The values given for the total are the lowest and the highest values of the sum of the components in the seven batches analysed.

The applicant performed a literature search for information on the chemical composition of *S. aromaticum* and its preparations and the presence of compounds of known concern.[21] Among the compounds identified in the essential oil from the leaves and the flower buds of *S. aromaticum*, eugenol (up to 90%) and eugenyl acetate (up to 17%) are reported in the EFSA Compendium of botanicals (EFSA, 2012).[17] Several references (Elzayyat et al., 2018; Gooderham et al., 2020; Selles et al., 2020) reported the presence of methyleugenol (0.01–0.04%) in clove bud oils and one reference (Gooderham et al., 2020) in a clove leaf oil (methyleugenol 0.01%) similar in composition to the oils under assessment. The presence of estragole was reported in three references, however in products that are different from the clove bud oil, the clove leaf oil and the CCL oil under assessment.

Methyleugenol (0.039–0.128%) was detected by GC–MS in all the seven batches of clove bud oil under assessment, whereas estragole was not detected (limit of detection (LOD): 0.01%).

The applicant consulted the online database on Volatile Compounds in Food (VCF).[22] The presence of other substances of concern was not reported.

#### 3.2.1.1. Impurities

The applicant referred to the 'periodic testing' of some representative flavourings premixtures for mercury, cadmium, lead, arsenic, fluoride, dioxins and polychlorinated biphenyls (PCBs), organo-chloride pesticides, organo-phosphorus pesticides, aflatoxins (B1, B2, G1, G2) and ochratoxin A. However, no data have been provided on the presence of these impurities. Since clove bud oil is produced by steam distillation, the likelihood of any measurable carry-over of all the above-mentioned elements is considered low, except for mercury.

#### 3.2.1.2. Shelf life

The shelf-life of clove bud oil 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).[23] However, no data supporting this statement were provided.

#### 3.2.1.3. Conditions of use

Clove bud oil is intended to be added to feed or water for drinking for all animal species without withdrawal. The maximum proposed use level in complete feed for all animal species is 50 mg/kg, except for laying hens and rabbits, for which a maximum use level of 28.5 and 30.5 mg/kg complete feed is proposed, respectively. No use level has been proposed by the applicant for use in water for drinking.

[21] Technical dossier/Supplementary information March 2023/Literature search_clove_oil.
[22] https://www.vcf-online.nl/VcfHome.cfm
[23] Technical dossier/Section II.

### 3.2.2. Safety

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

Many of the components of clove bud oil, accounting for more than 98.5% of the GC peak areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for use in food[8] without limitations and for use in feed[7] at individual use levels higher than those resulting from the intended use of the essential oil in feed, except for eugenol and eugenyl acetate in poultry. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2). An update of the absorption distribution, metabolism and excretion (ADME) of eugenol and eugenyl acetate in poultry is presented in Section 3.2.2.1.

One compound listed in Tables 1, 3, 7, 10-humulatriene [01.043] has been evaluated in Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2) by applying the procedure described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). For this compound, for which there is no concern for genotoxicity, EFSA requested additional subchronic toxicity data (EFSA CEF Panel, 2011). In the absence of such data, the EFSA CEF Panel was unable to complete its assessment (EFSA CEF Panel, 2015b). As a result, this compound is not authorised for use as flavour in food. For this compound, in the absence of toxicity data, the FEEDAP Panel applies the threshold of toxicological concern (TTC) approach or read-across from structurally related substances, as recommended in the Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a).

Eight components of clove bud oil, accounting on average for 1.12% of the GC area, have not been previously assessed for use as flavourings. The FEEDAP Panel notes that six of them are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 31 and for which a similar metabolic and toxicological profile is expected.[24] These six lipophilic compounds, accounting together for about 0.70% 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, 2015, 2016b). Another compound, 1-epi-cubenol, is a tertiary alcohol structurally related to the compounds already evaluated in CG 6, and is expected to be absorbed, metabolised and excreted (EFSA FEEDAP Panel, 2012a). The last compound, humulene oxide II, is an oxygenated terpene and is structurally related to compounds that have been evaluated for use in food and/or feed.

The additive contains methyleugenol (0.039–0.128%). For the ADME and the toxicology of methyleugenol, reference is made to the safety evaluation made by the FEEDAP Panel in the EFSA opinion on laurel leaf oil (EFSA FEEDAP Panel, 2023).

#### 3.2.2.1. Absorption distribution, metabolism and excretion of eugenol and eugenyl acetate in poultry

Both in rodents and humans, eugenol and eugenyl acetate are rapidly absorbed from the gastrointestinal tract. Eugenol is conjugated in the liver, and the resulting glucuronide and sulfate conjugates are subsequently excreted in the urine. Eugenyl acetate is hydrolysed to eugenol and the corresponding carboxylic acid (WHO, 2006). To a lesser extent, both compounds are metabolised to polar metabolites, which are also conjugated and eliminated, primarily in the urine. Only small amounts (< 1%) of eugenol are excreted unchanged. In its former assessment on CG 18, the Panel noted that the metabolic pathways involved in the biotransformation of eugenol and eugenyl acetate are common to mammalian species, but no data were available concerning their metabolic fate in poultry. Therefore, the FEEDAP Panel concluded that the efficient metabolism of eugenol and eugenyl acetate in mammals and the subsequent rapid excretion of the metabolites preclude their accumulation in tissues and transfer to products. However, the lack of data on metabolism and residues in poultry precluded an assessment of consumer exposure from this source. However, phase I (CYP450 monooxygenase families, epoxide hydrolases) and phase II enzymes (glucuronide- sulfate- and glutathione-transferases) involved in the biotransformation of several classes of compounds including CG 18 compounds are also expressed in birds as already described (EFSA FEEDAP Panel, 2013, 2016b,c). Therefore, birds can also be assumed to have the ability to metabolise and excrete eugenol as mammals and there is no evidence that the compound or its metabolites would accumulate in tissues and cause a concern for consumer safety.

[24] Calamenene, cadina-1,4-diene, α-copaene, isocadinene, trans-cadina-1(6),4-diene, alloaromadendrene.

### 3.2.2.2. Safety for the target species

Tolerance studies in the target species and/or toxicological studies in laboratory animals 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 and that 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 (EFSA SC, 2019a).

As the distilled fraction of clove bud oil is a fully defined mixture (the identified components represent about 99% of the % CG area, see Section 3.2.1), the FEEDAP Panel applied a component-based approach to assess the safety for target species. Methyleugenol, a substance for which a concern for genotoxicity has been identified, is assessed separately.

*Components other than methyleugenol*

Based on considerations related to structural and metabolic similarities, the components were allocated to seven assessment groups, corresponding to the chemical groups (CGs) 5, 6, 14, 18, 23, 31 and 32, as defined in Annex I of Regulation (EC) No 1565/2000. For chemical group 31 ('aliphatic and aromatic hydrocarbons'), sub-assessment groups as defined in FGE.25 and FGE.78 were applied (EFSA CEF Panel, 2015a,b). The allocation of the components to the (sub-)assessment groups is shown in Table 4 and in the corresponding footnote.

For each component in the assessment group, exposure of target animals was estimated considering the use levels in feed, the percentage of the component in the oil and the default values for feed intake according to the guidance on the safety of feed additives for target species (EFSA FEEDAP Panel, 2017b). Default values on body weight (bw) 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/bw per day, are shown in Table 4.

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification (Cramer et al., 1978). 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. If justified, extrapolation from a known NOAEL of a component of an assessment group to the other components of the group with no available NOAEL was made. If sufficient evidence was available for the members of a (sub-) assessment group, a (sub-)assessment group NOAEL was derived.

Toxicological data for sub-chronic studies, from which NOAEL values could be derived, were available for 6-methylhept-5-en-2-one [07.015] in CG 5 (EFSA FEEDAP Panel, 2021a), linalool [02.013] in CG 6 (EFSA FEEDAP Panel, 2012a), furfural [13.018] in CG 14 (EFSA FEEDAP Panel, 2016a), eugenol [04.003] (EFSA FEEDAP Panel, 2011), methyl salicylate [09.749] in CG 23 (EFSA FEEDAP Panel, 2012c) and for the representative compounds for sub-assessment groups of CG 31, d-limonene [01.045] and β-caryophyllene [01.007] (EFSA FEEDAP Panel, 2015, 2016b) and β-caryophyllene oxide [16.043] (EFSA CEF Panel, 2014). In addition, for benzyl alcohol the EFSA Panel on Food Additives and Flavourings (FAF) established an acceptable daily intake (ADI) of 4 mg/kg bw per day based on a NOAEL of 400 mg/kg bw per day from a carcinogenicity study in rats (EFSA FAF Panel, 2019).

Considering the structural and metabolic similarities in CG 18, read-across was applied using the NOAEL of 300 mg/kg bw per day for eugenol [04.003] to extrapolate to eugenyl acetate [09.020]. Similarly, read-across was also applied using the NOAEL of 400 mg/kg bw per day for benzyl alcohol [02.010] to extrapolate to ethyl benzoate [09.276] and to benzaldehyde [05.013][25] in CG 23.

The NOAEL of 222 mg/kg bw per day for β-caryophyllene [01.007] was applied using read-across to the compounds within CG 31 sub-assessment group V (α-copaene, α-cubebene, δ-cadinene [01.021], alloroarmadendrene, α-pinene [01.004] and β-pinene [01.003]) (EFSA CEF Panel, 2015a,b). Read-across was also applied from β-caryophyllene [01.007] to 3,7,10-humulatriene [01.043] in CG 31,

[25] The NOAEL of 400 mg/kg bw per day for benzyl alcohol was halved to take into account the higher reactivity of aldehyde in read-across.

VI. The NOAEL of 222 mg/kg bw per day for β-caryophyllene [01.007] was halved to take into account the uncertainty in read-across.

The NOAEL of 109 mg/kg bw per day for β-caryophyllene oxide [16.043] was extrapolated to humulene oxide II in CG 32.

For the remaining compounds,[26] toxicity studies performed with the compounds under assessment with derived NOAEL values were not available and read-across was not possible. Therefore, the threshold TTC approach was applied (EFSA FEEDAP Panel, 2017b). All these compounds belong to Cramer class I except 5-methylfurfural (Cramer class II).

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, Munro et al., 1996). Reference points selected for each compound are shown in Table 4.

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, 2019a). A MOET > 100 allowed for interspecies differences and intraspecies variability (as in the default 10 × 10 uncertainty factor). The compounds resulting individually in an MOE > 50,000 were not further considered in the assessment group as their contribution to the MOE(T) is negligible. They are listed in the footnote.[27]

The approach to the safety assessment of clove bud oil for the target species is summarised in Table 4. The calculations were done for chickens for fattening, the species with the highest ratio of feed intake/body weight and represent the worst-case scenario at the use level of 50 mg/kg complete feed.

**Table 4:** Compositional data, intake values (calculated for chickens for fattening at 50 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of clove bud oil classified according to assessment groups

| **Essential oil composition** | | | **Exposure** | | **Hazard characterisation** | | **Risk characterisation** | |
|---|---|---|---|---|---|---|---|---|
| **Assessment group** | **FLAVIS No** | **Highest conc. in the oil** | **Highest feed conc.** | **Intake[(a)]** | **Cramer class[(b)]** | **NOAEL[(c)]** | **MOE** | **MOET** |
| **Constituent** | – | **%** | **mg/kg** | **mg/kg bw per day** | – | **mg/kg bw per day** | – | – |
| **CG 6** | | | | | | | | |
| 1-epi-Cubenol | – | 0.04 | 0.020 | 0.0018 | I | *3* | 1,671 | |
| **CG 14** | | | | | | | | |
| Furfural | 13.018 | 0.06 | 0.031 | 0.0028 | (II) | **54** | 19,404 | |
| 5-Methylfurfural | 13.001 | 0.02 | 0.011 | 0.0009 | II | *0.91* | 965 | |
| | | | | | | | | 920 |
| **CG 18** | | | | | | | | |
| Eugenol | 04.003 | 76.0 | 37.98 | 3.4097 | (I) | **300** | 88 | |
| Eugenyl acetate | 09.020 | 14.1 | 7.056 | 0.6334 | (I) | 300 | 474 | |
| 4-Allylphenol | 04.058 | 0.14 | 0.072 | 0.0065 | I | *3* | 464 | |
| | | | | | | | | 64 |
| **CG 23** | | | | | | | | |
| Methyl salicylate | 09.749 | 0.25 | 0.125 | 0.0112 | (I) | 50 | 4,474 | |

[26] 3-epi-Cubenol (CG 6); 5-methylfurfural (CG 14); 4-allylphenol (CG 18); calamenene (CG 31, IVe); trans-cadina-1(6),4-diene, isocadinene and cadina-1,4-diene (CG 31, V).

[27] 6-Methylhept-5-en-2-one (CG 5); linalool (CG 6); benzaldehyde, benzyl alcohol and ethyl benzoate (CG 23); d-limonene (CG 31, III); alloroaromadendrene, α-pinene and β-pinene (CG 31, V); δ-cadinene and 10,10-dimethyl-2,6-dimethylenebicyclo [7.2.0]undecane (CG 31, V).

| Essential oil composition | | | Exposure | | Hazard characterisation | | Risk characterisation | |
|---|---|---|---|---|---|---|---|---|
| **Assessment group** | **FLAVIS No** | **Highest conc. in the oil** | **Highest feed conc.** | **Intake[a]** | **Cramer class[b]** | **NOAEL[c]** | **MOE** | **MOET** |
| **Constituent** | – | **%** | **mg/kg** | **mg/kg bw per day** | – | **mg/kg bw per day** | – | – |
| **CG 31, IVe** (Benzene hydrocarbons, alkyl) | | | | | | | | |
| Calamenene | – | 0.19 | 0.094 | 0.0084 | I | *3* | 356 | |
| **CG 31, V** (Bi-, tricyclic, non-aromatic hydrocarbons) | | | | | | | | |
| β-Caryophyllene | 01.007 | 8.49 | 4.245 | 0.3810 | (I) | **222** | 583 | |
| α-Copaene | – | 0.52 | 0.260 | 0.0233 | (I) | 222 | 9,530 | |
| α-Cubebene | – | 0.49 | 0.245 | 0.0219 | (I) | 222 | 10,114 | |
| δ-Cadinene | 01.021 | 0.15 | 0.073 | 0.0066 | (I) | 222 | 33,875 | |
| trans-Cadina-1(6),4-diene | – | 0.08 | 0.039 | 0.0035 | I | *3* | 857 | |
| Isocadinene | – | 0.05 | 0.027 | 0.0024 | I | *3* | 1,238 | |
| Cadina-1,4-diene | – | 0.05 | 0.024 | 0.0021 | I | *3* | 1,422 | |
| MOET CG 31, V | | | | | | | | 216 |
| **CG 31, VI** | | | | | | | | |
| 3,7,10-Humulatriene | 01.043 | 1.23 | 0.614 | 0.0551 | (I) | 111[d] | 2,014 | |
| **CG 32** | | | | | | | | |
| β-Caryophyllene oxide | 16.043 | 1.09 | 0.544 | 0.0488 | (III) | **109** | 2,232 | |
| Humulene oxide II | – | 1.09 | 0.544 | 0.0488 | (III) | 109 | 2,232 | |
| | | | | | | | | 1,116 |

(a): Intake calculations for the individual components are based on the use level of 50 mg/kg in 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 (no observed adverse effect level, 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.
(d): The NOAEL of 222 mg/kg bw per day for β-caryophyllene was halved to take into account the uncertainty in read-across.

As shown in Table 4, for the assessment group CG 18, which includes the major component eugenol, the MOET was < 100 at the proposed use level (50 mg/kg feed). From the lowest MOET of 64 for chickens for fattening, the MOET for CG 18 compounds was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 5.

**Table 5:** Combined margin of exposure (MOET) for CG 31,V calculated for the different target animal categories at the proposed use level in feed

| Animal category | Body weight (kg) | Feed intake (g DM/day) | Proposed use level (mg/kg feed) | Lowest MOET CG 31,V | Maximum safe use level (mg/kg feed)[a] |
|---|---|---|---|---|---|
| Chicken for fattening | 2 | 158 | 50 | 64 | 32 |
| Laying hen | 2 | 106 | 28.5 | 176 | 28.5 |
| Turkey for fattening | 3 | 176 | 50 | 86 | 43 |
| Piglet | 20 | 880 | 50 | 115 | 50 |
| Pig for fattening | 60 | 2,200 | 50 | 137 | 50 |
| Sow lactating | 175 | 5,280 | 50 | 169 | 50 |
| Veal calf (milk replacer) | 100 | 1,890 | 50 | 287 | 50 |
| Cattle for fattening | 400 | 8,000 | 50 | 253 | 50 |

| Animal category | Body weight (kg) | Feed intake (g DM/day) | Proposed use level (mg/kg feed) | Lowest MOET CG 31,V | Maximum safe use level (mg/kg feed)[(a)] |
|---|---|---|---|---|---|
| Dairy cows | 650 | 20,000 | 50 | 163 | 50 |
| Sheep/goat | 60 | 1,200 | 50 | 253 | 50 |
| Horse | 400 | 8,000 | 50 | 253 | 50 |
| Rabbit | 2 | 100 | 30.5 | 166 | 30.5 |
| Salmon | 0.12 | 2.1 | 50 | 281 | 50 |
| Dog | 15 | 250 | 50 | 297 | 50 |
| Cat | 3 | 60 | 50 | 253[(b)] | 25 |
| Ornamental fish | 0.012 | 0.054 | 50 | 1,011 | 50 |

(a): Complete feed containing 88% DM, milk replacer 94.5% DM.
(b): The minimum MOET considered to be of no concern for cats should be increased to 500 because of the reduced capacity of glucuronidation of compounds.

Table 5 shows that the MOET exceeds the value of 100 for all animal categories except poultry species for fattening. For these species the maximum safe use levels in feed were calculated to ensure a MOET $\geq$ 100. Because glucuronidation is an important metabolic pathway to facilitate the excretion of the components of the essential oil and considering that cats have a low capacity for glucuronidation (Court and Greenblatt, 1997; Lautz et al., 2021), the use of clove bud oil as additive in cat feed needs a wider margin of exposure. A MOET of 500 is considered adequate. The maximum safe levels in feed (without considering the presence of methyleugenol) are shown in Table 5.

The FEEDAP Panel considers that the use in water for drinking the use in water for drinking alone or in conjunction with use in feed should not exceed the daily amount that is considered safe when consumed via feed alone.

*Methyleugenol*

Methyleugenol belongs to the group of *p*-allylalkoxybenzenes and is a genotoxic carcinogen. 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, 2021b), different reference points and a different magnitude of the MOE are applied for long-living and reproductive animals (including those animals reared for laying/breeding/reproduction) and for short-living animals. Short-living animals are defined as those animals raised for fattening whose lifespan under farming conditions makes it very unlikely that they develop cancer as a result of the exposure to genotoxic and/or carcinogenic substances in the diet.

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

For short-living animals, genotoxicity and carcinogenicity endpoints are not considered biologically relevant, therefore a lower magnitude of the MOE ($>$ 100) when comparing estimated exposure with a reference point based on non-neoplastic endpoints is considered adequate (EFSA FEEDAP Panel, 2021b). The FEEDAP Panel identified a NOAEL of 10 mg/kg bw per day for non-neoplastic lesions (changes in organ weight[28] and function, including effects on liver[29] and the glandular stomach[30]) from a 90-day study in mice with methyleugenol (NTP, 2000; EFSA FEEDAP Panel, 2023).

Low concentrations of methyleugenol were detected in all batches of the additive under assessment (average: 0.078%, range: 0.039–0.128%). The use of clove bud oil at the levels in feed which were

[28] Increases in absolute liver weights of rats (at doses of 100 mg/kg of higher in males and at doses of 300 mg/kg of higher in females) and mice (at 30, 100 and 300 mg/kg in males and at 300 mg/kg in females) and the increase in testis weight of rats administered 1,000 mg/kg.
[29] Cytologic alteration, cytomegaly, Kupffer cell pigmentation, bile duct hyperplasia and foci of cellular alteration.
[30] Incidences of atrophy and chronic inflammation of the mucosa of the glandular stomach were significantly increased in rats administered 300 or 1,000 mg/kg; the incidences of lesions of the glandular stomach were increased in one or more groups administered 30 mg/kg or greater.

considered safe for the different target species, without considering the presence of methyleugenol, ranges from 25 to 50 mg/kg complete feed (see Table 5). These levels correspond to methyleugenol concentrations ranging from 0.032 to 0.064 mg/kg complete feed. The highest daily intake of methyleugenol in μg/kg bw was calculated for the different target animal categories considering the highest analysed value in the additive (0.128%). The calculated intake values range between 0.33 μg/kg bw per day (in ornamental fish) and 3.7 μg/kg bw per day (in chickens for fattening) (see Appendix A).

When the estimated exposures for long-living and reproductive animals are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day, derived by Suparmi et al. (2019) from a rodent carcinogenicity study (NTP, 2000), a MOE ranging between 9,921 and 67,833 is calculated for long living-animals. When comparing the exposure of short-living animals to the reference point based on non-neoplastic endpoints, a magnitude of the MOE > 100, is obtained for all species (see Appendix A).

The magnitude of the MOE is indicative of a low concern for long-living and reproductive animals and of no concern for species for fattening.

#### 3.2.2.3. Conclusions on safety for the target species

The conclusions of the FEEDAP Panel on the maximum safe concentrations in complete feed of clove bud oil are summarised in Table 6.

**Table 6:** Maximum safe concentrations of clove bud oil in complete feed (mg/kg) for all animal species and categories

| Animal categories | Maximum feed concentration of low[(a)]/no concern[(b)] (mg/kg complete feed)[(c)] |
|---|---|
| **Long-living and reproductive animals[(a)]** | |
| Laying hens and other laying/reproductive birds including animals reared for laying/reproduction and ornamental birds | 28.5 |
| Sows and all pigs (Suidae) for reproduction including animals reared for reproduction | 50 |
| Dairy cows and other ruminants and camelids for milk production and reproduction including animals reared for milk production/reproduction | 50 |
| Sheep/goats | 50 |
| Horses and other Equidae | 50 |
| Rabbits | 30.5 |
| Dogs | 50 |
| Cats | 25 |
| Ornamental fish | 50 |
| **Short-living animals (species for fattening)[(b)]** | |
| Chickens for fattening and minor poultry for fattening | 32 |
| Turkey for fattening | 43 |
| Pigs for fattening | 50 |
| Piglets and other Suidae species for meat production | 50 |
| Veal calves (milk replacer) | 50 |
| Cattle for fattening and other ruminants for fattening and camelids at the same physiological stage | 50 |
| Sheep/goats | 50 |
| Horses and other Equidae | 50 |
| Rabbits | 30.5 |
| Salmonids and minor fin fish | 50 |
| Any other species | 25 |

(a): Based on a MOE > 10,000 for long-living and reproductive animals, calculated as the ratio of the reference point (BMDL10 of 22.2 mg/kg bw per day) to the combined intake.
(b): Based on a MOE > 100 for short living animals (species for fattening), calculated as the ratio of the reference point (NOAEL of 10 mg/kg bw per day) to the combined intake.
(c): Complete feed containing 88% DM, milk replacer 94.5% DM.

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

#### 3.2.2.4. Safety for the consumer

Cloves (immature flower buds) and their preparations including ethanolic extracts are added to a wide range of food categories as spice or for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites values of 0.43 mg/kg bw per day for cloves, 0.045 mg/kg bw per day for clove bud oil and 0.038 mg/kg bw per day for clove leaf oil.

The majority of the individual constituents of the clove bud oil 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, Section 1.2).

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 clove bud oil are expected to be extensively metabolised and excreted in the target species.

Although the FEEDAP Panel could not conclude on the safety for the consumer of eugenol when used as a feed additive in poultry species at the proposed use level of 25 mg/kg complete feed in its previous assessment (EFSA FEEDAP Panel, 2011), the inclusion of 22–33 mg eugenol/kg complete feed to poultry species is considered of no concern for the consumer (see Section 3.2.2.1). For methyleugenol, the available data indicate that it is absorbed, metabolised and rapidly excreted and is not expected to accumulate in animal tissues and products at the levels present in the additive (EFSA FEEDAP Panel, 2023).

Considering the above and the reported human exposure due to direct use of clove bud oil in food (Burdock, 2009), it is unlikely that consumption of products from animals given clove bud oil up to the highest safe use level in feed would significantly increase human background exposure.

No safety concern would be expected for the consumer from the use of the clove bud oil up to the highest proposed use level in feed.

#### 3.2.2.5. Safety for the user

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

The applicant produced a safety data sheet[31] for clove bud oil, where hazards for users have been identified.

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

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

#### 3.2.2.6. Safety for the environment

Although *S. aromaticum* L. is not a native species to Europe, eugenol, β-caryophyllene and eugenyl acetate, the most abundant components in the essential oil are naturally occurring in European plants. Therefore, the use of the clove bud oil under the proposed conditions of use in animal feed is not expected to pose a risk to the environment.

## 3.3. Clove leaf oil

### 3.3.1. Characterisation of clove leaf oil

The essential oil under assessment is a clear yellow to amber, slightly viscous liquid with a characteristic spice odour. In four batches of the additive, the refractive index (20 °C) ranged between 1.5315 and 1.5323 (specification: 1.5303–1.540).[16] 'Clove oil' is identified with the CAS number 8000-34-8,[32] the EINECS number 284-638-7, and the CoE number 188. Clove leaf oil is identified by the FEMA number 2325.

[31] Technical dossier/ Supplementary Information March 2023/Annex_Xc_SIn reply_clove_bud_oil_MSDS. Aspiration hazard (H304, category 1), serious eye damage/irritation (H319; category 2); skin sensitisation (H317, category 1B) in accordance with the criteria outlined in Annex I of 1272/2008/EC (CLP/EU-GHS).

[32] The following entries were found at https://echa.europa.eu/home for 'Clove leaf oil': EINECS 616-969-3; CAS 8015-97-2.

For clove leaf oil, the product specifications are based on the standard developed by the International Organisation for Standardization (ISO) 3141:1997 for 'Oil of clove leaves',[33] adapted to reflect the concentrations of selected volatile components. Three compounds contribute to the specifications, eugenol (80–95%, selected as the phytochemical marker), β-caryophyllene (4–17%) and eugenyl acetate (≤ 1%). Analysis of two batches of the additive showed compliance with these specifications when analysed by GC–FID and expressed as % GC area.[34]

The applicant provided a full characterisation of six batches by GC–MS.[16] In total, up to 41 constituents were detected, 34 of which were identified and accounted on average for 99.41% (98.67–99.93%) of the % GC area. The three compounds indicated in the product specifications account for about 96.8% on average (range 94.6–98.4%) of the GC area. Besides the three compounds indicated in the product specifications, 13 other compounds were detected at individual levels ≥ 0.05% and are listed in Table 7. These 16 compounds together account on average for 99.3% (98.4–99.9%) of the % GC area. The remaining 18 compounds (ranging between 0.005% and 0.04%) and accounting for 0.12% of the % GC area are listed in the footnote.[35] Based on the available data on the characterisation, clove leaf oil is considered a fully defined mixture (EFSA SC, 2019a).

**Table 7:** Constituents of clove leaf oil from the leaves of *Syzygium aromaticum* (L.) Merr. & L.M. Perry accounting for ≥ 0.05% of the composition (based on the analysis of six batches by gas chromatography–mass spectrometry). 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<br>EU register name | CAS No | FLAVIS No | % GC area | |
|---|---|---|---|---|
| | | | Mean | Range |
| Eugenol | 97-53-0 | 04.003 | 82.60 | 78.68–86.01 |
| β-Caryophyllene | 87-44-5 | 01.007 | 13.45 | 11.10–14.85 |
| Eugenyl acetate | 93-28-7 | 09.030 | 0.70 | 0.02–1.47 |
| 3,7,10-Humulatriene | 6753-98-6 | 01.043 | 1.60 | 1.10–2.03 |
| β-Caryophyllene epoxide | 1139-30-6 | 16.043 | 0.44 | 0.09–1.03 |
| Octane | 111-65-9 | – | 0.32 | 0.11–0.52 |
| Caryophyllene alcohol | 56747-96-7 | – | 0.24 | 0.24[(a)] |
| α-Copaene | 3856-25-5 | – | 0.21 | 0.06–0.30 |
| β-Cadinene | 523-47-7 | – | 0.11 | 0.09–0.13 |
| Nonane | 111-84-2 | – | 0.11 | 0.05–0.16 |
| β-Elemene | 33880-83-0 | – | 0.07 | 0.07[(a)] |
| 4-Allylphenol | 501-92-8 | 05.058 | 0.06 | 0.06[(a)] |
| Calamenene | 483–77-2 | – | 0.06 | 0.05–0.07 |
| Benzyl benzoate | 120-51-4 | 09.727 | 0.06 | 0.06[(a)] |
| Humulene oxide II | 19888-34-7 | – | 0.05 | 0.01–0.08 |
| Germacra-1(10),4(14),5-triene | 23986-74-5 | 01.042 | 0.05 | 0.05[(a)] |
| Total | | | 2.53 | 99.50–99.90[(b)] |

EU: European Union; CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.
(a): Compound detected in only one batch.
(b): The values given for the Total (range) are the lowest and the highest values of the sum of the components in the six batches analysed.

The applicant performed a literature search for information on the chemical composition of *S. aromaticum* and its preparations and the presence of compounds of known concern (see Section 3.2.1).

[33] Technical dossier/Supplementary information March 2023/Annex_V_SIn_reply_clove_oil_ ISO_3141_1997.
[34] Technical dossier/Supplementary information March 2023/Annex_III_SIn_Reply_clove_oil_composition GC–FID analysis: eugenol (82.6%), β-caryophyllene (13.3%) and α-eugenyl acetate (0.66%).
[35] Additional constituents:constituents (n = 11) between < 0.05 and ≥ 0.02%: methyl salicylate, (3E)-3-ethylidene-3a-methyl-2,4,5,6,7,7a-hexahydro-1H-indene, alloaromadendrene, α-farnesene, (Z)-isoeugenol, valencene, furfural, coniferyl alcohol, methyleugenol, (3E,10Z)-oxacyclotrideca-3,10-diene-2,7-dione and δ-cadinene.constituents (n = 7) between <0.02 and ≥0.005%: cubebol, cadina-1,4-diene, 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane, 1(5),11-guaiadiene, linalool, 6-methylhept-5-en-2-one and 5-methylfurfural.

Methyleugenol (0.010–0.030%) was detected by GC–MS in four out of the six batches of the clove leaf oil, whereas estragole was not detected (LOD: 0.01%).

#### 3.3.1.1. Impurities

The applicant referred to the 'periodic testing' of some representative flavourings premixtures for mercury, cadmium, lead, arsenic, fluoride, dioxins and polychlorinated biphenyls (PCBs), organo-chloride pesticides, organo-phosphorus pesticides, aflatoxins (B1, B2, G1, G2) and ochratoxin A. However, no data have been provided on the presence of these impurities. Since clove leaf oil is produced by steam distillation, the likelihood of any measurable carry-over of all the above-mentioned elements is considered low, except for mercury.

#### 3.3.1.2. Shelf life

The shelf-life of clove leaf oil 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).[23] However, no data supporting this statement were provided.

#### 3.3.1.3. Conditions of use

Clove leaf oil is intended to be added to feed and water for drinking for all animal species without a withdrawal period. The maximum proposed use level in complete feed for all target species is 100 mg/kg. For the use in water for drinking the applicant proposed maximum use levels of 40 mg/kg for poultry, calves and rabbits, 20 mg/kg for piglets, pigs for fattening and sows and 10 mg/kg for cattle for fattening, dairy cows, sheep/goats and horses.

### 3.3.2. Safety

The assessment of safety of clove leaf oil is based on the maximum use level proposed by the applicant (100 mg/kg complete feed).

Many of the components of clove leaf oil, accounting on average for about 99% of the GC peak area, have been previously assessed and considered safe for use as flavourings, and are currently authorised for use in food[8] without limitations and for use in feed[7] at individual use levels higher than those resulting from the intended use of the essential oil in feed except for eugenol and eugenyl acetate in poultry (see Section 3.2.2.1). The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Two compounds listed in Table 1, germacra-1(10),4(14),5-triene [01.042] and 3,7,10-humulatriene [01.043], have been evaluated in Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2) by applying the procedure described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). For these compounds, for which there is no concern for genotoxicity, EFSA requested additional subchronic toxicity data (EFSA CEF Panel, 2011). In the absence of such data, the EFSA CEF Panel was unable to complete its assessment (EFSA CEF Panel, 2015b). As a result, these compounds are not authorised for use as flavour in food. For these compounds, in the absence of toxicity data, the FEEDAP Panel applies the threshold of TTC approach or read-across from structurally related substances, as recommended in the Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a).

Sixteen components of clove leaf oil, accounting on average for about 1.0% of the % GC area, have not been previously assessed for use as flavourings. The FEEDAP Panel notes that nine of them are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 31 and for which a similar metabolic and toxicological profile is expected.[36] These nine lipophilic compounds, accounting together for about 0.96% 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, 2015, 2016b). Two compounds, *(Z)*-isoeugenol and humulene oxide II, are structurally related to authorised feed flavourings.

The remaining five compounds, cubebol, caryophyllene alcohol, (3E,10Z)-oxacyclotrideca-3,10-diene-2,7-dione, coniferyl alcohol and (3E)-3-ethylidene-3a-methyl-2,4,5,6,7,7a-hexahydro-1H-indene were screened with the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure–Activity Relationship (QSAR) Toolbox. No alert was identified for *in vitro* mutagenicity, for

[36] Octane, nonane, β-elemene, calamenene, α-copaene, alloroaromadendrene, cadina-1,4-diene, 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane and β-cadinene (CG 31).

genotoxic and non-genotoxic carcinogenicity and for other toxicity endpoints for cubebol, caryophyllene alcohol and (3E)-3-ethylidene-3a-methyl-2,4,5,6,7,7a-hexahydro-1H-indene, whereas for (3E,10Z)-oxacyclotrideca-3,10-diene-2,7-dione, structural alerts were due to the presence of ester group and for coniferyl alcohol to the presence of the phenol group. For these substances, predictions of mutagenicity by Ames test (with and without metabolic activation) were made by 'read-across' analyses of data available for similar substances to the target compounds (i.e. analogues obtained by categorisation). Mutagenicity read-across-based relevant predictions were found negative for both substances.[37] On this basis, the alerts raised were discounted.

The additive contains methyleugenol (range: 0.010–0.030%). For the ADME and the toxicology of methyleugenol, reference is made to the safety evaluation made by the FEEDAP Panel in the EFSA opinion on laurel leaf oil (EFSA FEEDAP Panel, 2023).

#### 3.3.2.1. Safety for the target species

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

As the additive under assessment is a fully defined mixture (the identified components represent > 99.5% of the % GC area, see Section 3.3.1), the FEEDAP Panel applied a component-based approach to assess the safety for target species of the essential oil. The approach followed, i.e. the allocation of the components to the (sub-)assessment groups, the estimate of exposure for the target species, the identification of a reference point for each constituent (hazard characterisation) and the calculation of the MOET for each assessment group (risk characterisation), is described in Section 3.2.2.1. Methyleugenol, a substance for which a concern for genotoxicity has been identified is assessed separately.

*Components other than methyleugenol*

The components of clove leaf oil were allocated to nine assessment groups, corresponding to the chemical groups (CGs) 5, 6, 9, 14, 17, 18, 23, 31 and 32, as shown in Table 8 and in the corresponding footnote.

Toxicological data for sub-chronic studies, from which NOAEL values could be derived, were available for 6-methylhept-5-en-2-one [07.015] in CG 5 (EFSA FEEDAP Panel, 2021a), linalool [02.013] in CG 6 (EFSA FEEDAP Panel, 2012a), furfural [13.018] in CG 14 (EFSA FEEDAP Panel, 2016a), isoeugenol [04.004] in CG 17 (EFSA FEEDAP Panel, 2012b), eugenol [04.003] (EFSA FEEDAP Panel, 2011), methyl salicylate [09.749] in CG 23 (EFSA FEEDAP Panel, 2012c) and for the representative compounds for sub-assessment groups of CG 31, myrcene [01.008], d-limonene [01.045] and β-caryophyllene [01.007] (EFSA FEEDAP Panel, 2015, 2016b) and β-caryophyllene oxide [16.043] (EFSA CEF Panel, 2014). For benzyl benzoate [09.727] a NOAEL of 194 mg/kg bw per day has been identified for developmental and reproductive toxicity (Api et al., 2020).

Considering the structural and metabolic similarities in CG 18, read-across was applied using the NOAEL of 300 mg/kg bw per day for eugenol [04.003] to extrapolate to eugenyl acetate [09.020].

The NOAELs of 44, 250 and 222 mg/kg bw per day for the representative compounds of CG 31, myrcene [01.008], d-limonene [01.045] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within sub-assessment group II (α-farnesene [01.040], III (β-elemene) and V (α-copaene, alloroarmadendrene, δ-cadinene [01.021], 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0] undecane, valencene [01.017] and β-cadinene) (EFSA CEF Panel, 2015a,b). Read-across was also applied from β-caryophyllene [01.007] to 3,7,10-humulatriene [01.043] in CG 31, VI. The NOAEL of 222 mg/kg bw per day for β-caryophyllene [01.007] was halved to take into account the uncertainty in read-across.

The NOAEL of 109 mg/kg bw per day for β-caryophyllene oxide [16.043] was extrapolated to humulene oxide II in CG 32.

For the remaining compounds,[38] toxicity studies performed with the compounds under assessment with derived NOAEL values were not available and read-across was not possible. Therefore, the threshold of TTC approach was applied (EFSA FEEDAP Panel, 2017b).

The approach to the safety assessment of clove leaf oil for the target species is summarised in Table 8. The calculations were done for chickens for fattening, the species with the highest ratio of

[37] Technical dossier/Supplementary information March 2023/Annex XI_SIn_reply_clove_oil_QSAR.

[38] Cubebol, caryophyllene alcohol (CG 6); (3E,10Z)-oxacyclotrideca-3,10-diene-2,7-dione (CG 9); 5-methylfurfurale (CG 14); 4-allylphenol (CG 18); octane and nonane (CG 31, I); calamenene (CG 31, IVe); cadina-1,4-diene, 1(5),11-guaiadiene and (3E)-3-ethylidene-3a-methyl-2,4,5,6,7,7a-hexahydro-1H-indene (CG 31, V); 3,7,10-humulatriene and germacra-1(10),4(14),5-triene (CG 31, VI).

feed intake/body weight and represent the worst-case scenario at the use level of 100 mg/kg complete feed. The compounds resulting individually in an MOE > 50,000 were not further considered in the assessment group as their contribution to the MOE(T) is negligible.[39]

**Table 8:** Compositional data, intake values (calculated for chickens for fattening at 100 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of clove leaf oil classified according to assessment groups

| **Essential oil composition** | | | **Exposure** | | **Hazard characterisation** | | **Risk characterisation** | |
|---|---|---|---|---|---|---|---|---|
| **Assessment group** | **FLAVIS-No** | **Highest conc. in the oil** | **Highest feed conc.** | **Intake[a]** | **Cramer class[b]** | **NOAEL[c]** | **MOE** | **MOET** |
| **Constituent** | – | **%** | **mg/kg** | **mg/kg bw per day** | – | **mg/kg bw per day** | – | – |
| **CG 6** | | | | | | | | |
| Caryophyllene alcohol | – | 0.24 | 0.241 | 0.0216 | I | *3* | 139 | |
| Cubebol | – | 0.01 | 0.010 | 0.0009 | I | *3* | 3,342 | |
| MOET CG 06 | | | | | | | | 133 |
| **CG 9** | | | | | | | | |
| (3E,10Z)-Oxacyclotrideca-3,10-diene-2,7-dione | – | 0.02 | 0.020 | 0.0018 | III | *0.15* | 84 | |
| **CG 14** | | | | | | | | |
| Furfural | 13.018 | 0.03 | 0.029 | 0.0026 | (II) | **54** | 20,742 | |
| 5-Methylfurfural | 13.001 | 0.01 | 0.005 | 0.0004 | II | *0.91* | 2,027 | |
| | | | | | | | | 1,847 |
| **CG 17** | | | | | | | | |
| *(Z)*-Isoeugenol | – | 0.04 | 0.043 | 0.0039 | (I) | **75** | 19,429 | |
| Coniferyl alcohol | – | 0.03 | 0.025 | 0.0022 | I | *3* | 1,337 | |
| | | | | | | | | 1,251 |
| **CG 18** | | | | | | | | |
| Eugenol | 04.003 | 86.0 | 86.01 | 7.7214 | (I) | **300** | 39 | |
| Eugenyl acetate | 09.020 | 1.47 | 1.472 | 0.1321 | (I) | 300 | 2,270 | |
| 4-Allylphenol | 04.058 | 0.06 | 0.063 | 0.0057 | I | *3* | 530 | |
| | | | | | | | | 36 |
| **CG 23** | | | | | | | | |
| Benzyl benzoate | 09.727 | 0.06 | 0.060 | 0.0054 | (I) | **194** | 36,017 | |
| Methyl salicylate | 09.749 | 0.05 | 0.050 | 0.0045 | (I) | 50 | 11,139 | |
| | | | | | | | | 8,508 |
| **CG 31, I** | | | | | | | | |
| Octane | – | 0.52 | 0.520 | 0.0467 | I | *3* | 64 | |
| Nonane | – | 0.16 | 0.160 | 0.0144 | I | *3* | 209 | |
| | | | | | | | | 49 |
| **CG 31, II** (Acyclic alkanes) | | | | | | | | |
| α-Farnesene | 01.040 | 0.78 | 0.046 | 0.0041 | (I) | **44** | 10,655 | |
| **CG 31, III** (Cyclohexene hydrocarbons) | | | | | | | | |
| β-Elemene | – | 0.07 | 0.065 | 0.0058 | (I) | 250 | 42,843 | |
| **CG 31, IVe** (Benzene hydrocarbons, alkyl) | | | | | | | | |
| Calamenene | – | 0.07 | 0.070 | 0.0063 | I | *3* | 477 | |

[39] 6-Methylhept-5-en-2-one (CG 5); linalool (CG 6); β-cadinene, valencene, alloroaromadendrene, δ-cadinene and 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane (CG 31, V).

| Essential oil composition | | | Exposure | | Hazard characterisation | | Risk characterisation | |
|---|---|---|---|---|---|---|---|---|
| **Assessment group** | **FLAVIS-No** | **Highest conc. in the oil** | **Highest feed conc.** | **Intake[a]** | **Cramer class[b]** | **NOAEL[c]** | **MOE** | **MOET** |
| **Constituent** | – | **%** | **mg/kg** | **mg/kg bw per day** | – | **mg/kg bw per day** | – | – |
| **CG 31, V** (Bi-, tricyclic, non-aromatic hydrocarbons) | | | | | | | | |
| β-Caryophyllene | 01.007 | 14.85 | 14.85 | 1.3331 | (I) | **222** | 167 | |
| α-Copaene | – | 0.30 | 0.300 | 0.0269 | (I) | 222 | 8,243 | |
| (3E)-3-Ethylidene-3a-methyl-2,4,5,6,7,7a-hexahydro-1H-indene | – | 0.04 | 0.040 | 0.0036 | I | *3* | 835 | |
| Cadina-1,4-diene | – | 0.01 | 0.010 | 0.0009 | I | *3* | 3,342 | |
| 1(5),11-Guaiadiene | 01.023 | 0.04 | 0.010 | 0.0009 | I | *3* | 3,342 | |
| MOET CG 31, V | | | | | | | | 126 |
| **CG 31, VI** | | | | | | | | |
| 3,7,10-Humulatriene | 01.043 | 2.03 | 2.033 | 0.1825 | I | 111[d] | 608 | |
| Germacra-1(10),4(14),5-triene | 01.042 | 0.05 | 0.050 | 0.0045 | I | *3* | 668 | |
| MOET CG 31, V | | | | | | | | 318 |
| **CG 32** | | | | | | | | |
| β-Caryophyllene oxide | 16.043 | 1.03 | 1.028 | 0.0923 | (III) | **109** | 1,181 | |
| Humulene oxide II | – | 0.08 | 0.078 | 0.0070 | (III) | *109* | 15,566 | |
| | | | | | | | | 1,098 |

(a): Intake calculations for the individual components are based on the use level of 100 mg/kg in 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 (no observed adverse effect level, 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.
(d): The NOAEL of 222 mg/kg bw per day for β-caryophyllene was halved to take into account the uncertainty in read-across.

As shown in Table 8, for several assessment groups, the MOET was < 100 at the proposed use levels (100 mg/kg feed). The lowest MOET was calculated for CG 18, the chemical group which includes eugenol, the main compound of clove leaf oil. From the lowest MOET of 36 for chickens for fattening, the MOET for CG 18 compounds was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 9.

**Table 9:** Combined margin of exposure (MOET) for CG 18 calculated for the different target animal categories at the proposed use level in feed

| Animal category | Body weight (kg) | Feed intake (g DM/day) | Proposed use level (mg/kg feed) | Lowest MOET CG 18 | Maximum safe use level (mg/kg feed)[a] |
|---|---|---|---|---|---|
| Chicken for fattening | 2 | 158 | 100 | 36 | 36 |
| Laying hen | 2 | 106 | 100 | 54 | 54 |
| Turkey for fattening | 3 | 176 | 100 | 48 | 48 |
| Piglet | 20 | 880 | 100 | 65 | 65 |
| Pig for fattening | 60 | 2,200 | 100 | 77 | 77 |
| Sow lactating | 175 | 5,280 | 100 | 95 | 95 |
| Veal calf (milk replacer) | 100 | 1,890 | 100 | 162 | 100 |
| Cattle for fattening | 400 | 8,000 | 100 | 142 | 100 |

| Animal category | Body weight (kg) | Feed intake (g DM/day) | Proposed use level (mg/kg feed) | Lowest MOET CG 18 | Maximum safe use level (mg/kg feed)[a] |
|---|---|---|---|---|---|
| Dairy cows | 650 | 20,000 | 100 | 92 | 92 |
| Sheep/goat | 60 | 1,200 | 100 | 142 | 100 |
| Horse | 400 | 8,000 | 100 | 142 | 100 |
| Rabbit | 2 | 100 | 100 | 57 | 57 |
| Salmon | 0.12 | 2.1 | 100 | 158 | 100 |
| Dog | 15 | 250 | 100 | 167 | 100 |
| Cat | 3 | 60 | 100 | 142[b] | 28 |
| Ornamental fish | 0.012 | 0.054 | 100 | 569 | 100 |

(a): Complete feed containing 88% DM, milk replacer 94.5% DM.
(b): The minimum MOET considered to be of no concern for cats should be increased to 500 because of the reduced capacity of glucuronidation of compounds.

Table 9 shows that the MOET exceeds the value of 100 only for veal calves (milk replacer), cattle for fattening, sheep/goats, horses, salmon, dogs and ornamental fish. For the other species the maximum safe use levels in feed were calculated to ensure a MOET $\geq$ 100. Because glucuronidation is an important metabolic reaction to facilitate the excretion of the components of the essential oil and considering that cats have a low capacity for glucuronidation (Court and Greenblatt, 1997; Lautz et al., 2021), the use of clove leaf oil as additive in cat feed needs a wider margin of exposure. A MOET of 500 is considered adequate. The maximum safe levels in feed (without considering the presence of methyleugenol) are shown in Table 8.

In poultry, pigs and rabbits, the daily consumption of water by drinking is about two to three times the amount of feed DM ingested (EFSA FEEDAP Panel, 2017a). The applicant proposed a maximum use level of 20 mg/kg water for piglets, pigs for fattening and sows, which would ensure a comparable or lower exposure to the calculated maximum safe use level in feed. However, the proposed use level of 40 mg/kg water for poultry and rabbits would result in an exposure higher than that which is considered safe when consumed via feed.

For ruminants and horses, safe concentrations of an additive cannot be consistently extrapolated from feed to water using a fixed ratio of feed to water intake. However, considering that the proposed maximum use level in water for drinking for ruminants and horses, 10 mg/kg water, is 9 to 10 times lower than the maximum calculated safe concentrations in feed, the use of the additive at the proposed maximum use level in water for drinking is considered safe. In veal calves, the use of the additive at 40 mg/kg water can be considered safe only when added to the water for drinking but not to the water used to prepare the milk replacer.

The FEEDAP Panel considers that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered safe when consumed via feed alone.

*Methyleugenol*

Low concentrations of methyleugenol were detected in all batches of the additive under assessment (average: 0.023%, range: 0.010–0.030%). The use of clove leaf oil at the levels in feed which were considered safe for the different target species, without considering the presence of methyleugenol, ranges from 28 to 100 mg/kg complete feed (see Table 9). These levels correspond to methyleugenol concentrations ranging from 0.008 to 0.030 mg methyleugenol/kg complete feed. The highest daily intake of methyleugenol in μg/kg bw was calculated for the different target animal categories and considering the highest analysed value in the additive (0.030%). The calculated intake values range between 0.15 μg/kg bw per day (in ornamental fish) and 0.98 μg/kg bw per day (in laying hens, piglets and sows) (see Appendix B).

When the estimated exposures for the different animal categories are compared to the $BMDL_{10}$ of 22.2 mg methyleugenol/kg bw per day, derived by Suparmi et al. (2019) from a rodent carcinogenicity study (NTP, 2000), a MOE ranging between 22,753 and 144,711 is calculated for long-living and reproductive animals. When comparing the exposure of short-living animals to the reference point based on non-neoplastic endpoints, a magnitude of the MOET > 100, is obtained for all species (see Appendix B).

The magnitude of the MOE is indicative of a low concern for long-living and reproductive animals and of no concern for species for fattening.

### 3.3.2.2. Conclusions on safety for the target species

The conclusions of the FEEDAP Panel on the maximum safe concentrations in complete feed of clove leaf oil are summarised in Table 10.

**Table 10:** Maximum safe concentrations of clove leaf oil in complete feed (mg/kg) for all animal species and categories

| Animal categories | Maximum feed concentration of low[(a)]/no concern[(b)] (mg/kg complete feed)[(c)] |
|---|---|
| **Long-living and reproductive animals[(a)]** | |
| Laying hens and other laying/reproductive birds including animals reared for laying/reproduction and ornamental birds | 54 |
| Sows and other Suidae species for reproduction including animals reared for reproduction | 95 |
| Dairy cows and other ruminants and camelids for milk production and reproduction including animals reared for milk production/reproduction | 92 |
| Sheep/goats | 100 |
| Horses and other Equidae | 100 |
| Rabbits | 57 |
| Dogs | 100 |
| Cats | 28 |
| Ornamental fish | 100 |
| **Short-living animals (species for fattening)[(b)]** | |
| Chickens for fattening and minor poultry for fattening | 36 |
| Turkey for fattening | 48 |
| Pigs for fattening | 77 |
| Piglets and other Suidae species for meat production | 65 |
| Veal calves (milk replacer) | 100 |
| Cattle for fattening and other ruminants for fattening and camelids at the same physiological stage | 100 |
| Sheep/goats | 100 |
| Horses and other Equidae | 100 |
| Rabbits | 57 |
| Salmonids and minor fin fish | 100 |
| Any other species | 28 |

(a): Based on a MOE > 10,000 for long-living and reproductive animals, calculated as the ratio of the reference point (BMDL10 of 22.2 mg/kg bw per day) to the combined intake.
(b): Based on a MOE > 100 for short-living animals (species for fattening), calculated as the ratio of the reference point (NOAEL of 10 mg/kg bw per day) to the combined intake.
(c): Complete feed containing 88% DM, milk replacer 94.5% DM.

The proposed conditions of use of the additive in water for drinking for poultry and rabbits cannot be considered safe. When used in water for drinking, the proposed conditions of use are considered of low/no concern for pigs, calves, ruminants and horses, provided that the use in water for drinking alone or in conjunction with use in feed should not exceed the daily amount that is considered of low/no concern when consumed via feed alone.

### 3.3.2.3. Safety for the consumer

Considering the qualitative similarity in the composition of clove oils, the same considerations on the constituents apply to the assessment of the safety for the consumer (see Section 3.2.2.3).

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

### 3.3.2.4. Safety for the user

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

The applicant produced a safety data sheet[40] for clove leaf oil, where hazards for users have been identified.

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

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

#### 3.3.2.5. Safety for the environment

Although *S. aromaticum* is not a native species to Europe, eugenol, β-caryophyllene and eugenyl acetate, the most abundant components in the essential oil are naturally occurring in European plants. Therefore, the use of the clove leaf oil under the proposed conditions of use in animal feed is not expected to pose a risk to the environment.

## 3.4. β-Caryophyllene-rich fraction of clove leaf oil (CCL oil)

### 3.4.1. Characterisation of CCL oil

The essential oil under assessment is a clear yellow, mobile and slightly viscous liquid with a characteristic odour. In four batches of the additive, the refractive index (20°C) ranged between 1.4995 and 1.5001 (specification: 1.4890–1.5020).[16]

For CCL oil, the product specifications are based on the content of β-caryophyllene (70–90%), which is selected as the phytochemical marker. Analysis of two batches of the additive showed compliance with these specifications when analysed by GC-FID and expressed as % GC area (82%).[41]

The applicant provided a full characterisation of the five batches by GC–MS.[19] β-Caryophyllene accounted for 75% (range: 71.9–79.3%) on average of % GC area (Table 9). In total, up to 30 constituents were detected, 24 of which were identified and accounted on average for 98.1% (97.6–98.5%) of the % GC area. Besides β-caryophyllene, 19 other compounds were detected at individual levels ≥ 0.05% and are listed in Table 11. These 20 compounds together account on average for 98.0% (97.5–98.4%) of the % GC area. The remaining four compounds (ranging between 0.008 and 0.04%) and accounting for 0.09% of the % GC area are listed in the footnote.[42] Based on the available data on the characterisation, CCL oil is considered a fully defined mixture (EFSA SC, 2019a).

**Table 11:** Constituents of a β-caryophyllene-rich fraction of clove leaf oil (CCL oil) from the leaves of *Syzygium aromaticum* (L.) Merr. & L.M. Perry accounting for ≥ 0.05% of the composition (based on the analysis of five batches by gas chromatography–mass spectrometry). 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<br>EU register name | CAS No | FLAVIS No | % GC area | |
|---|---|---|---|---|
| | | | Mean | Range |
| β-Caryophyllene | 87-44-5 | 01.007 | 75.04 | 71.93–79.26 |
| 3,7,10-Humulatriene | 6753-98-6 | 01.043 | 13.25 | 10.77–15.06 |
| β-Caryophyllene epoxide | 1139-30-6 | 16.043 | 2.99 | 2.51–3.59 |
| α-Copaene | 3856-25-5 | – | 2.01 | 1.87–2.19 |
| δ-Cadinene | 29350-73-0 | 01.021 | 1.87 | 1.18–2.63 |
| α-Cubebene | 17699-14-8 | – | 0.51 | 0.47–0.56 |
| 10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0] undecane | 357414-37-0 | – | 0.50 | 0.43–0.59 |
| α-Selinene | 473-13-2 | – | 0.30 | 0.20–0.36 |

[40] Technical dossier/ Supplementary Information March 2023/Annex_VII_SIn reply_clove_leaf_oil_eug_MSDS. Aspiration hazard (H304, category 1), Hazards for eye damage/irritation (H319, category 2), skin sensitisation (H317, category 1B) in accordance with the criteria outlined in Annex I of 1272/2008/EC (CLP/EU-GHS).

[41] Technical dossier/Supplementary information March/EURL_Appendix_clove_oil. GC-FID analysis: β-caryophyllene (82.0%).

[42] Additional constituents: β-bourbonene, cloven, eugenol and γ-calcorene.

| Constituent<br>EU register name | CAS No | FLAVIS No | % GC area | |
|---|---|---|---|---|
| | | | Mean | Range |
| α-Farnesene | 502-61-4 | 01.040 | 0.29 | 0.19–0.43 |
| α-Muurolene | 10208-80-7 | 01.052 | 0.19 | 0.13–0.26 |
| β-Selinene | 17066-67-0 | – | 0.19 | 0.15–0.24 |
| Caryophyllene alcohol | 56747-96-7 | – | 0.19 | 0.19[(a)] |
| Humulene oxide II | 19888-34-7 | – | 0.18 | 0.11–0.23 |
| γ-Muurolene | 30021-74-0 | – | 0.18 | 0.13–0.21 |
| Alloaromadendrene | 25246-27-9 | – | 0.17 | 0.15–0.18 |
| β-Elemene | 33880-83-0 | – | 0.10 | 0.10[(a)] |
| 1(5),11-Guaiadiene | 3691-12-1 | – | 0.09 | 0.08–0.10 |
| 1-epi-Cubenol | 19912-67-5 | – | 0.06 | 0.04–0.08 |
| Seychellene | 20085-93-2 | – | 0.05 | 0.03–0.07 |
| Methyleugenol | 93-15-2 | – | 0.05 | 0.04–0.06 |
| Total | | | 98.00 | 97.51–98.43[(b)] |

EU: European Union; CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.
(a): Compound detected in only one batch.
(b): The values given for the Total are the lowest and the highest values of the sum of the components in the five batches analysed.

The applicant performed a literature search for information on the chemical composition of *S. aromaticum* and its preparations and the presence of compounds of known concern (see Section 3.2.1).

Methyleugenol (0.038–0.062%) was detected by GC–MS in all the five batches of CCL oil under assessment, whereas estragole was not detected (LOD: 0.01%).

#### 3.4.1.1. Impurities

The applicant referred to the 'periodic testing' of some representative flavourings premixtures for mercury, cadmium and lead, arsenic, fluoride, dioxins and polychlorinated biphenyls (PCBs), organo-chloride pesticides, organo-phosphorus pesticides, aflatoxins (B1, B2, G1, G2) and ochratoxin A. However, no data have been provided on the presence of these impurities. Since CCL oil is produced by steam distillation, the likelihood of any measurable carry-over of all the above-mentioned elements is considered low, except for mercury.

#### 3.4.1.2. Shelf life

The shelf-life of CCL oil 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).[23] However, no data supporting this statement were provided.

#### 3.4.1.3. Conditions of use

CCL oil is intended to be added to feed and water for drinking for all animal species without withdrawal. Maximum use levels in complete feed were proposed in Table 12. No use level has been proposed by the applicant for the use in water for drinking.

**Table 12:** Conditions of use for a β-caryophyllene-rich fraction of clove leaf oil (CCL oil): maximum proposed use levels in complete feed for all animal categories

| Animal category | Use level (mg/kg complete feed) |
|---|---|
| Chicken for fattening | 150 |
| Laying hen | 150 |
| Turkey for fattening | 150 |
| Piglet | 175 |
| Pig for fattening | 175 |
| Sow lactating | 175 |

| Animal category | Use level (mg/kg complete feed) |
|---|---|
| Veal calf (milk replacer) | 200 |
| Cattle for fattening | 200 |
| Dairy cow | 200 |
| Sheep/goat | 200 |
| Horse | 200 |
| Rabbit | 150 |
| Salmon (fish) | 150 |
| Dog | 50 |
| Cat | 50 |
| Ornamental fish | 50 |
| Others | 50 |

### 3.4.2. Safety

The assessment of safety of CLL oil is based on the maximum use levels proposed by the applicant for the species listed above (see Table 12).

As CCL oil is obtained by fractional distillation of clove leaf oil, to achieve an enriched composition in β-caryophyllene and other hydrocarbons (e.g. 3,7,10-humulatriene, α-copaene and δ-cadinene), the considerations made in Section 3.3.2 on clove leaf oil apply to the current assessment.

Many of the components of CCL oil, accounting for about 94% of the GC peak areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for use in food[8] without limitations and for use in feed[7] at individual use levels higher than those resulting from the intended use of the essential oil in feed. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Five compounds listed in Table 1, δ-cadinene [01.021], 1,(5),11-guauadiene [01.023], β-bourbonene [01.024], 3,7,10-humulatriene [01.043] and α-muurolene [01.052], have been evaluated in Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2) by applying the procedure described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). For these compounds, for which there is no concern for genotoxicity, EFSA requested additional subchronic toxicity data (EFSA CEF Panel, 2011). In the absence of such data, the EFSA CEF Panel was unable to complete its assessment (EFSA CEF Panel, 2015b). As a result, these compounds are not authorised for use as flavour in food. For these compounds, in the absence of toxicity data, the FEEDAP Panel applies the threshold of TTC approach or read-across from structurally related substances, as recommended in the Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a).

Fourteen components of CCL oil, accounting on average for 4.47% of the % GC area, have not been previously assessed for use as flavourings. The FEEDAP Panel notes that 11 of them are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 31 and for which a similar metabolic and toxicological profile is expected.[43] These 11 lipophilic compounds accounting together for about 4.06% 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, 2015, 2016b). The same consideration applies to caryophyllene alcohol. Another compound, 1-epi-cubenol, is a tertiary alcohol structurally related to the compounds already evaluated in CG 6, and is expected to be absorbed, metabolised and excreted (EFSA FEEDAP Panel, 2012a). The last compound, humulene oxide II, is an oxygenated terpene metabolite and is structurally related to compounds that have been evaluated for use in food and/or feed.

The additive contains methyleugenol (range: 0.038–0.062%). For the ADME and the toxicology of methyleugenol, reference is made to the safety evaluation made by the FEEDAP Panel in the EFSA opinion on laurel leaf oil (EFSA FEEDAP Panel, 2023).

[43] β-Elemene, γ-calacorene, α-cubebene, α-copaene, alloroaromadendrene, clovene, seychellene, γ-muurulene, 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane, β-selinene and α-selinene (CG 31).

### 3.4.2.1. Safety for the target species

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

As CCL oil is a fully defined mixture (the identified components represent about 98% of the % CG area, see Section 3.4.1), the FEEDAP Panel applied a component-based approach to assess the safety for target species. The approach followed, i.e. the allocation of the components to the (sub-) assessment groups, the estimate of exposure for the target species, the identification of a reference point for each constituent (hazard characterisation) and the calculation of the MOET for each assessment group (risk characterisation), is described in Section 3.2.2.1. Methyleugenol, a substance for which a concern for genotoxicity has been identified, is assessed separately.

*Components other than methyleugenol*

The components of CCL oil were allocated to four assessment groups, corresponding to the CGs 6, 18, 31 and 32, as shown in Table 13 and in the corresponding footnote.

Toxicological data for sub-chronic studies, from which NOAEL values could be derived, were available for eugenol [04.003] (EFSA FEEDAP Panel, 2011), for the representative compounds for sub-assessment groups of CG 31, myrcene [01.008], d-limonene [01.045] and β-caryophyllene [01.007] (EFSA FEEDAP Panel, 2015, 2016b) and β-caryophyllene oxide [16.043] (EFSA CEF Panel, 2014).

The NOAELs of 44, 250 and 222 mg/kg bw per day for the representative compounds of CG 31, myrcene [01.008], d-limonene [01.045] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within sub-assessment group II (α-farnesene [01.040], III (β-elemene) and V (δ-cadinene [01.021], α-copaene, α-cubebene, alloroarmadendrene, 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane, β-bourbonene, seychellene, γ-muurolene, β-selinene, α-selinene and α-muurolene) (EFSA CEF Panel, 2015a,b). Read-across was also applied from β-caryophyllene [01.007] to 3,7,10-humulatriene [01.043] in CG 31, VI. The NOAEL of 222 mg/kg bw per day for β-caryophyllene [01.007] was halved to take into account the uncertainty in read-across.

The NOAEL of 109 mg/kg bw per day for β-caryophyllene oxide [16.043] was extrapolated to humulene oxide II in CG 32.

For the remaining compounds,[44] toxicity studies performed with the compounds under assessment with derived NOAEL values were not available and read-across was not possible. Therefore, the TTC approach was applied (EFSA FEEDAP Panel, 2017b).

The compounds resulting individually in an MOE > 50,000 were not further considered in the assessment group as their contribution to the MOE(T) is negligible.[45]

The approach to the safety assessment of CLL oil for the target species is summarised in Table 13. The calculations were done for chickens for fattening, the species with the highest ratio of feed intake/ body weight and represent the worst-case scenario at the use level of 150 mg/kg complete feed.

**Table 13:** Compositional data, intake values (calculated for chickens for fattening at 150 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of CCL oil classified according to assessment groups

| **Essential oil composition** | | | **Exposure** | | **Hazard characterisation** | | **Risk characterisation** | |
|---|---|---|---|---|---|---|---|---|
| **Assessment group** | **FLAVIS No** | **Highest conc. in the oil** | **Highest feed conc.** | **Intake[(a)]** | **Cramer class[(b)]** | **NOAEL[(c)]** | **MOE** | **MOET** |
| **Constituent** | – | **%** | **mg/kg** | **mg/kg bw per day** | – | **mg/kg bw per day** | – | – |
| **CG 6** | | | | | | | | |
| Caryophyllene alcohol | – | 0.19 | 0.281 | 0.0252 | (I) | 222 | 8,816 | |
| 1-epi-Cubenol | – | 0.08 | 0.126 | 0.0113 | I | *3* | 265 | |
| MOET CG 06 | | | | | | | | 257 |

[44] 1-Epi-cubebol (CG 6); γ-calacorene (CG 31, IVe); 1(5),11-guaiadiene and clovene (CG 31, V).
[45] Eugenol (CG 18).

| Essential oil composition | | | Exposure | | Hazard characterisation | | Risk characterisation | |
|---|---|---|---|---|---|---|---|---|
| **Assessment group** | **FLAVIS No** | **Highest conc. in the oil** | **Highest feed conc.** | **Intake[a]** | **Cramer class[b]** | **NOAEL[c]** | **MOE** | **MOET** |
| **Constituent** | – | **%** | **mg/kg** | **mg/kg bw per day** | – | **mg/kg bw per day** | – | – |
| **CG 31, II** (Acyclic alkanes) | | | | | | | | |
| α-Farnesene | 01.040 | 0.29 | 0.639 | 0.0574 | (I) | **44** | 767 | |
| **CG 31, III** (Cyclohexene hydrocarbons) | | | | | | | | |
| β-Elemene | – | 0.43 | 0.639 | 0.0574 | (I) | 250 | 18,565 | |
| **CG 31, IVe** (Benzene hydrocarbons, alkyl) | | | | | | | | |
| γ-Calacorene | – | 0.02 | 0.023 | 0.0020 | I | *3* | 1,485 | |
| **CG 31, V** (Bi-, tricyclic, non-aromatic hydrocarbons) | | | | | | | | |
| β-Caryophyllene | 01.007 | 79.26 | 118.9 | 10.674 | (I) | **222** | 21 | |
| α-Cubebene | – | 0.56 | 0.834 | 0.0749 | (I) | 222 | 2,965 | |
| α-Copaene | – | 2.19 | 3.291 | 0.2954 | (I) | 222 | 751 | |
| Alloaromadendrene | – | 0.18 | 0.267 | 0.0240 | (I) | 222 | 9,262 | |
| δ-Cadinene | 01.021 | 2.63 | 3.948 | 0.3544 | (I) | 222 | 626 | |
| 10,10-Dimethyl-2,6-dimethylene bicyclo [7.2.0]undecane | – | 0.59 | 0.881 | 0.0790 | (I) | 222 | 2,809 | |
| 1(5),11-Guaiadiene | – | 0.10 | 0.150 | 0.0135 | I | *3* | 223 | |
| Clovene | – | 0.06 | 0.089 | 0.0079 | I | *3* | 378 | |
| β-Bourbonene | 01.024 | 0.04 | 0.063 | 0.0057 | (I) | 222 | 3,9253 | |
| Seychellene | – | 0.07 | 0.101 | 0.0090 | (I) | 222 | 24,606 | |
| γ-Muurolene | – | 0.21 | 0.317 | 0.0284 | (I) | 222 | 7,813 | |
| β-Selinene | – | 0.24 | 0.363 | 0.0326 | (I) | 222 | 6,812 | |
| α-Selinene | – | 0.36 | 0.542 | 0.0486 | (I) | 222 | 4,567 | |
| α-Muurolene | 01.052 | 0.26 | 0.387 | 0.0347 | (I) | 222 | 6,390 | |
| MOET CG 31, V | | | | | | | | 17 |
| **CG 31, VI** | | | | | | | | |
| 3,7,10-Humulatriene | 01.043 | 15.06 | 22.59 | 2.0278 | I | 111[d] | 55 | |
| **CG 32** | | | | | | | | |
| β-Caryophyllene oxide | 16.043 | 3.59 | 5.385 | 0.4834 | (III) | **109** | 225 | |
| Humulene oxide II | – | 0.23 | 0.351 | 0.0315 | (III) | 109 | 3,459 | |
| | | | | | | | | 212 |

(a): Intake calculations for the individual components are based on the use level of 150 mg/kg in 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 (no observed adverse effect level, 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.
(d): The NOAEL of 222 mg/kg bw per day for β-caryophyllene was halved to take into account the uncertainty in read-across.

As shown in Table 13, for the assessment group which includes the major component β-caryophyllene (CG 31, V), the MOET was < 100 at the proposed use levels (150 mg/kg feed). From the lowest MOET of 17 for chickens for fattening, the MOET for CG 31,V compounds was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 14.

**Table 14:** Combined margin of exposure (MOET) for CG 31,V calculated for the different target animal categories at the proposed use level in feed

| Animal category | Body weight (kg) | Feed intake (g DM/day) | Proposed use level (mg/kg feed) | Lowest MOET CG 31,V | Maximum safe use level (mg/kg feed)[(a)] |
|---|---|---|---|---|---|
| Chicken for fattening | 2 | 158 | 150 | 17 | 26 |
| Laying hen | 2 | 106 | 150 | 25 | 38 |
| Turkey for fattening | 3 | 176 | 150 | 23 | 34 |
| Piglet | 20 | 880 | 175 | 26 | 46 |
| Pig for fattening | 60 | 2,200 | 175 | 31 | 54 |
| Sow lactating | 175 | 5,280 | 175 | 38 | 67 |
| Veal calf (milk replacer) | 100 | 1,890 | 200 | 53 | 114 |
| Cattle for fattening | 400 | 8,000 | 200 | 50 | 101 |
| Dairy cows | 650 | 20,000 | 200 | 32 | 65 |
| Sheep/goat | 60 | 1,200 | 200 | 50 | 101 |
| Horse | 400 | 8,000 | 200 | 50 | 101 |
| Rabbit | 2 | 100 | 150 | 27 | 40 |
| Salmon | 0.12 | 2.1 | 150 | 75 | 112 |
| Dog | 15 | 250 | 50 | 237 | 50 |
| Cat | 3 | 60 | 50 | 201[(b)] | 20 |
| Ornamental fish | 0.012 | 0.054 | 50 | 806 | 50 |

(a): Complete feed containing 88% DM, milk replacer 94.5% DM.
(b): The minimum MOET considered to be of no concern for cats should be increased to 500 because of the reduced capacity of glucuronidation of compounds.

Table 14 shows that the MOET calculated at the maximum proposed use levels in feed exceeds the value of 100 only for dogs and ornamental fish. For the other species the maximum safe use levels in feed were calculated to ensure a MOET $\geq$ 100. Because glucuronidation is an important metabolic pathway to facilitate the excretion of the components of the essential oil and considering that cats have a low capacity for glucuronidation (Court and Greenblatt, 1997; Lautz et al., 2021), the use of CCL oil as additive in cat feed needs a wider margin of exposure. A MOET of 500 is considered adequate. The maximum safe levels in feed (without considering the presence of methyleugenol) are shown in Table 12.

The FEEDAP Panel considers that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered safe when consumed via feed alone.

*Methyleugenol*

Low concentrations of methyleugenol were detected in all batches of the additive under assessment (average: 0.050%, range: 0.038–0.062%). The use of CCL oil at the levels in feed which were considered safe for the different target species without considering the presence of methyleugenol, ranges from 20 to 112 mg/kg complete feed (see Table 12). These levels correspond to methyleugenol concentrations ranging from 0.012 to 0.069 mg/kg methyleugenol/kg complete feed. The highest daily intake of methyleugenol in μg/kg bw was calculated for the different target animal categories considering the highest analysed value in the additive (0.062%). The calculated intake values range between 0.16 μg/kg bw per day (in ornamental fish) and 1.45 μg/kg bw per day (in chickens for fattening) (see Appendix C).

When the estimated exposures for the different animal categories are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day, derived by Suparmi et al. (2019) from a rodent carcinogenicity study (NTP, 2000), an MOE ranging between 15,587 and 140,043 is calculated for long-living and reproductive animals. When comparing the exposure of short-living animals to the reference point based on non-neoplastic endpoints, a magnitude of the MOET > 100, is obtained for all species (see Appendix C).

The magnitude of the MOE is indicative of a low concern for long-living and reproductive animals and of no concern for species for fattening.

### 3.4.2.2. Conclusions on safety for the target species

The conclusions of the FEEDAP Panel on the maximum safe concentrations in complete feed of CCL oil are summarised in Table 15.

**Table 15:** Maximum safe concentrations of β-caryophyllene-rich fraction of clove leaf oil (CCL oil) in complete feed (mg/kg) for all animal species and categories

| Animal categories | Maximum feed concentration of low[(a)]/no concern[(b)] (mg/kg complete feed)[(c)] |
|---|---|
| **Long-living and reproductive animals[(a)]** | |
| Laying hens and other laying/reproductive birds including animals reared for laying/reproduction and ornamental birds | 38 |
| Sows and other Suidae species for reproduction including animals reared for reproduction | 67 |
| Dairy cows and other ruminants and camelids for milk production and reproduction including animals reared for milk production/reproduction | 65 |
| Sheep/goats | 101 |
| Horses and other Equidae | 101 |
| Rabbits | 40 |
| Dogs | 50 |
| Cats | 20 |
| Ornamental fish | 50 |
| **Short-living animals (species for fattening)[(b)]** | |
| Chickens for fattening and minor poultry for fattening | 26 |
| Turkey for fattening | 34 |
| Pigs for fattening | 54 |
| Piglets and other Suidae species for meat production | 46 |
| Veal calves (milk replacer) | 114 |
| Cattle for fattening and other ruminants for fattening and camelids at the same physiological stage | 101 |
| Sheep/goats | 101 |
| Horses and other Equidae | 101 |
| Rabbits | 40 |
| Salmonids and minor fin fish | 112 |
| Any other species | 20 |

(a): Based on a MOE > 10,000 for long-living and reproductive animals, calculated as the ratio of the reference point (BMDL10 of 22.2 mg/kg bw per day) to the combined intake.
(b): Based on a MOE > 100 for short-living animals (species for fattening), calculated as the ratio of the reference point (NOAEL of 10 mg/kg bw per day) to the combined intake.
(c): Complete feed containing 88% DM, milk replacer 94.5% DM.

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

### 3.4.2.3. Safety for the consumer

Considering the qualitative similarity in the composition of clove oils, the same considerations on the constituents apply to the assessment of the safety for the consumer (see Section 3.2.2.3).

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

#### 3.4.2.4. Safety for the user

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

The applicant produced a safety data sheet[46] for CCL oil, where hazards for users have been identified.

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

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

#### 3.4.2.5. Safety for the environment

Although *S. aromaticum* L. is not a native species to Europe, eugenol, β-caryophyllene and eugenyl acetate, the most abundant components in the essential oil are naturally occurring in European plants. Therefore, the use of the CCL oil under the proposed conditions of use in animal feed is not expected to pose a risk to the environment.

### 3.5. Efficacy

'Cloves' (immature flower buds) and related preparations are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009), by the Flavour and Extract Manufactures Association (FEMA) with the reference numbers 2327 (cloves), 2322 (clove bud extract), 2323 (clove bud oil), 2324 (clove bud oleoresin) and 2325 (clove leaf oil).

Since clove leaf oil and clove bud oil are recognised to flavour food and the function of the oils under assessment in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

## 4. Conclusions

Oils prepared from the flower buds or leaves of *S. aromaticum* (L.) Merr. & L.M. Perry may be produced from plants of different geographical origins and by various processes resulting in preparations with different composition and toxicological profiles. Therefore, the following conclusions apply only to oils produced by steam distillation from the flower buds or the leaves of *S. aromaticum*, which do not contain estragole (< 0.01%) and contain ≤ 0.13% methyleugenol (clove bud oil) or ≤ 0.03% methyleugenol (clove leaf oil) or ≤ 0.06% methyleugenol (β-caryophyllene-rich fraction of clove leaf oil, CCL oil).

The conclusions of the FEEDAP Panel on the maximum concentrations in complete feed of the three essential oils under assessment, which are considered of low concern for long-living and reproductive animals and of no concern for species for fattening are summarised as following:

| Animal categories | Maximum feed concentration of low[(a)]/no concern[(b)] (mg/kg complete feed)[(c)] | | |
|---|---|---|---|
| | Clove bud oil | Clove leaf oil | CCL oil |
| **Long-living and reproductive animals[(a)]** | | | |
| Laying hens and other laying/reproductive birds including animals reared for laying/reproduction and ornamental birds | 28.5 | 54 | 38 |
| Sows and other Suidae species for reproduction including animals reared for reproduction | 50 | 95 | 67 |
| Dairy cows and other ruminants and camelids for milk production and reproduction including animals reared for milk production/reproduction | 50 | 92 | 65 |
| Sheep/goats | 50 | 100 | 101 |
| Horses and other Equidae | 50 | 100 | 101 |
| Rabbits | 30.5 | 57 | 40 |
| Dogs | 50 | 100 | 50 |

[46] Technical dossier/ Supplementary Information March 2023/Annex_Xb_SIn reply_clove_leaf_oil_c50ar_MSDS. Aspiration hazard (H304, category 1), skin sensitisation (H317, category 1B) in accordance with the criteria outlined in Annex I of 1272/2008/EC (CLP/EU-GHS).

| Animal categories | Maximum feed concentration of low[a]/no concern[b] (mg/kg complete feed)[c] | | |
|---|---|---|---|
| | Clove bud oil | Clove leaf oil | CCL oil |
| Cats | 25 | 28 | 20 |
| Ornamental fish | 50 | 100 | 50 |
| **Short-living animals (species for fattening)[b]** | | | |
| Chickens for fattening and minor poultry for fattening | 32 | 36 | 26 |
| Turkey for fattening | 43 | 48 | 34 |
| Pigs for fattening | 50 | 77 | 54 |
| Piglets and other Suidae species for meat production | 50 | 65 | 46 |
| Veal calves (milk replacer) | 50 | 100 | 114 |
| Cattle for fattening and other ruminants for fattening and camelids at the same physiological stage | 50 | 100 | 101 |
| Sheep/goats | 50 | 100 | 101 |
| Horses and other Equidae | 50 | 100 | 101 |
| Rabbits | 30.5 | 57 | 40 |
| Salmonids and minor fin fish | 50 | 100 | 112 |
| Any other species | 25 | 28 | 20 |

(a): Based on a MOE > 10,000 for long-living and reproductive animals, calculated as the ratio of the reference point (BMDL10 of 22.2 mg/kg bw per day) to the combined intake.
(b): Based on a MOE > 100 for short-living animals (species for fattening), calculated as the ratio of the reference point (NOAEL of 10 mg/kg bw per day) to the combined intake.
(c): Complete feed containing 88% DM, milk replacer 94.5% DM.

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

For clove leaf oil, the proposed conditions of use of the additive in water for drinking for poultry and rabbits (40 mg/kg) cannot be considered safe. The proposed conditions of use for pigs (20 mg/kg), calves (40 mg/kg), ruminants and horses (10 mg/kg) are considered of low/no concern, provided that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered of low or no concern when consumed via feed alone.

No concerns for consumers were identified following the use of the additives at the use level considered of low or no concern in feed for the target animals.

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

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

Clove bud oil, clove leaf oil and its β-caryophyllene-rich fraction are recognised to flavour food. Since the function of the oils under assessment in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

## Recommendations

The specification for the additives should include for clove bud oil < 0.01% estragole and < 0.13% methyleugenol; for clove leaf oil < 0.01% estragole and < 0.03% methyleugenol; for β-caryophyllene-rich fraction of clove leaf oil (CCL oil) < 0.01% estragole and < 0.06% methyleugenol.

## Documentation provided to EFSA/Chronology

| Date | Event |
|---|---|
| **28/10/2010** | Dossier received by EFSA. Botanically defined flavourings from Botanical Group 07 – Geraniale, Myrtales, Poales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG) |
| **09/11/2010** | Reception mandate from the European Commission |
| **21/12/2010** | Application validated by EFSA – Start of the scientific assessment |
| **22/03/2011** | Comments received from Member States |
| **01/04/2011** | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: analytical methods* |
| **08/01/2013** | Reception of supplementary information from the applicant - Scientific assessment remains suspended |
| **26/02/2013** | EFSA informed the applicant (EFSA ref. 7150727) that, in view of the workload, the evaluation of applications on feed flavourings would be re-organised by giving priority to the assessment of the chemically defined feed flavourings, as agreed with the European Commission |
| **20/01/2014** | Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives |
| **24/06/2015** | Technical hearing during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products": data requirement for the risk assessment of botanicals |
| **17/12/2019** | EFSA informed the applicant that the evaluation process restarted |
| **18/12/2019** | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for target species, safety for the consumer, safety for the user and environment* |
| **21/03/2023** | Reception of supplementary information from the applicant (partial dataset: clove oil) - Scientific assessment remains suspended |
| **06/06/2023** | Reception of an amendment of the Evaluation report of the European Union Reference Laboratory for Feed Additives related to geranium rose oil, eucalyptus oil, lemongrass oil and clove oil |
| **07/06/2023** | The application was split and a new EFSA-Q-2023-00397 was assigned to the preparation included in the present assessment |
| **08/06/2023** | Scientific assessment re-started for the preparation included in the present assessment |
| **05/07/2023** | Opinion adopted by the FEEDAP Panel on eucalyptus oil (EFSA-Q-2023-00397). End of the Scientific assessment for the preparation included in the present assessment. The assessment of other preparations in BGD 07 is still ongoing |

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## Abbreviations

| | |
|---|---|
| AFC | EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food |
| BDG | Botanically defined group |
| bw | body weight |
| CAS | Chemical Abstracts Service |
| CDG | Chemically defined group |
| CEF | EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| CG | chemical group |
| CLP | Classification, Labelling and Packaging |
| CoE | Council of Europe |
| EEIG | European economic interest grouping |
| EINECS | European Inventory of Existing Chemical Substances |
| EMA | European Medicines Agency |
| 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 |
| FLAVIS No | FLAVIS number |
| GC | gas chromatography |
| GC–FID | gas chromatography with flame ionisation detector |
| GC–MS | gas chromatography–mass spectrometry |
| ISO | International standard organisation |
| LOQ | limit of quantification |
| JECFA | The Joint FAO/WHO Expert Committee on Food Additives |
| MOE | margin of exposure |
| MOET | combined margin of exposure (total) |
| NOAEL | no observed adverse effect level |
| OECD | Organisation for Economic Co-operation and Development |
| PhEur | European Pharmacopoeia |
| QSAR | Quantitative Structure–Activity Relationship |
| SC | EFSA Scientific Committee |
| TTC | threshold of toxicological concern |
| WHO | World Health Organization |

## Appendix A – Methyleugenol in clove bud oil: Maximum daily intake and margin of exposure for the different target species

The maximum daily intake of methyleugenol 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/safe use level (see Table 5) of the additive in feed for the different target animal categories (ranging from 28.8 to 50 mg/kg complete feed) and
- assuming that methyeugenol is present at a concentration corresponding to the highest analysed value in the additive (0.128%).

The margin of exposure (MOE) for each animal category is calculated as the ratio of the reference point to the intake: the BMDL10 of 22.2 mg methyeugenol/kg bw per day for long-living and reproductive animals; the NOAEL of 10 mg methyeugenol/kg bw per day for target species for fattening (EFSA FEEDAP Panel, 2023).

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, 2021b),[15] '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 margin of exposure (MOE) with a magnitude of $\geq$ 10,000, when comparing estimated exposure to genotoxic and/or carcinogenic substances with a $BMDL_{10}$ from a rodent carcinogenicity study, would be indicative of a low concern for the target species (EFSA SC, 2019a)'. For short-living animals, genotoxicity and carcinogenicity endpoints are not considered relevant, therefore a lower magnitude of the MOET (> 100) when comparing estimated exposure with a reference point based on non-neoplastic endpoints is considered adequate (EFSA FEEDAP Panel, 2021b).

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

**Table A.1:** Target animal intake of methyleugenol (as μg/kg bw per day) and margin of exposure (MOE) at the maximum proposed use level of clove bud oil in feed for target animal category

| Animal category | Daily Feed intake | Body weight | Max safe use levels | Intake[a] | Lowest MOE[b] |
|---|---|---|---|---|---|
| | g DM/day | kg | mg/kg[1] | μg/kg bw/day | |
| **Long-living and reproductive animals** | | | | | |
| Laying hens | 0.106 | 2 | 28.5 | 2.197 | 10,104 |
| Sow lactating | 5.28 | 175 | 50 | 2.194 | 10,117 |
| Dairy cow | 20 | 650 | 50 | 2.238 | 9,921 |
| Sheep/goat | 1.2 | 60 | 50 | 1.455 | 15,263 |
| Horse | 8 | 400 | 50 | 1.455 | 15,263 |
| Rabbit | 0.1 | 2 | 30.5 | 2.218 | 10,008 |
| Dog | 0.25 | 15 | 50 | 1.212 | 18,315 |
| Cat | 0.06 | 3 | 25 | 0.727 | 30,525 |
| Ornamental fish | 0.00054 | 0.012 | 50 | 0.327 | 67,833 |
| **Target species for fattening** | | | | | |
| Chicken for fattening | 0.158 | 2 | 32 | 3.677 | 2,720 |
| Turkey for fattening | 0.176 | 3 | 43 | 3.669 | 2,725 |
| Piglet | 0.88 | 20 | 50 | 3.200 | 3,125 |
| Pig for fattening | 2.2 | 60 | 50 | 2.667 | 3,750 |
| Veal calf (milk replacer) | 1.89 | 100 | 50 | 1.280 | 7,239 |
| Cattle for fattening | 8 | 400 | 50 | 1.455 | 6,875 |
| Sheep/goat | 1.2 | 60 | 50 | 1.455 | 6,875 |

| Animal category | Daily Feed intake | Body weight | Max safe use levels | Intake[(a)] | Lowest MOE[(b)] |
|---|---|---|---|---|---|
| | g DM/day | kg | mg/kg[(1)] | µg/kg bw/day | |
| Horse | 8 | 400 | 50 | 1.455 | 6,875 |
| Rabbit | 0.1 | 2 | 50 | 3.636 | 2,750 |
| Salmon | 0.0021 | 0.12 | 50 | 1.273 | 7,857 |

(a): The values of methyleugenol in feed is calculated considering the highest analysed value in the additive.
(b): The MOE for methyleugenol is calculated as the ratio of the reference point to the intake: for long-living and reproductive animals is based on $BMDL_{10}$ of 22.2 mg/kg bw per day derived from rodent carcinogenicity studies with methyleugenol; for target species for fattening based on a NOAEL of 10 mg/kg bw per day derived from a 90-day study with methyleugenol (NTP, 2000).

## Appendix B – Methyleugenol in clove leaf oil: Maximum daily intake and margin of exposure for the different target species

The maximum daily intake of methyleugenol 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/safe use level (see Table 8) of the additive in feed for the different target animal categories (ranging from 36 to 100 mg/kg complete feed) and
- assuming that methyeugenol is present at a concentration corresponding to the highest analysed value in the additive (0.03%).

The margin of exposure (MOE) for each animal category is calculated as the ratio of the reference point to the intake: the $BMDL_{10}$ of 22.2 mg methyeugenol/kg bw per day for long-living and reproductive animals; the NOAEL of 10 mg methyeugenol/kg bw per day for target species for fattening (EFSA FEEDAP Panel, 2023).

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),[15] '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 margin of exposure (MOE) with a magnitude of $\geq$ 10,000, when comparing estimated exposure to genotoxic and/or carcinogenic substances with a $BMDL_{10}$ from a rodent carcinogenicity study, would be indicative of a low concern for the target species (EFSA SC, 2019a)'. For short-living animals, genotoxicity and carcinogenicity endpoints are not considered relevant, therefore a lower magnitude of the MOET (> 100) when comparing estimated exposure with a reference point based on non-neoplastic endpoints is considered adequate (EFSA FEEDAP Panel, 2021b).

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

**Table B.1:** Target animal intake of methyleugenol (as μg/kg bw per day) and margin of exposure (MOE) at the maximum proposed use level of clove leaf oil in feed for target animal category

| Animal category | Daily Feed intake | Body weight | Max safe use levels | Intake[a] | Lowest MOE[b] |
|---|---|---|---|---|---|
| | g DM/day | kg | mg/kg[1] | μg/kg bw/day | |
| **Long-living and reproductive animals** | | | | | |
| Laying hens | 0.106 | 2 | 54 | 0.976 | 22,753 |
| Sow lactating | 5.28 | 175 | 95 | 0.977 | 22,719 |
| Dairy cow | 20 | 650 | 92 | 0.965 | 23,004 |
| Sheep/goat | 1.2 | 60 | 100 | 0.682 | 32,560 |
| Horse | 8 | 400 | 100 | 0.682 | 32,560 |
| Rabbit | 0.1 | 2 | 57 | 0.972 | 22,849 |
| Dog | 0.25 | 15 | 100 | 0.568 | 39,072 |
| Cat | 0.06 | 3 | 28 | 0.191 | 116,286 |
| Ornamental fish | 0.00054 | 0.012 | 100 | 0.153 | 144,711 |
| **Target species for fattening** | | | | | |
| Chicken for fattening | 0.158 | 2 | 36 | 0.970 | 10,314 |
| Turkey for fattening | 0.176 | 3 | 48 | 0.960 | 10,417 |
| Piglet | 0.88 | 20 | 65 | 0.975 | 10,256 |
| Pig for fattening | 2.2 | 60 | 77 | 0.963 | 10,390 |
| Veal calf (milk replacer) | 1.89 | 100 | 100 | 0.600 | 16,667 |
| Cattle for fattening | 8 | 400 | 100 | 0.682 | 14,667 |
| Sheep/goat | 1.2 | 60 | 100 | 0.682 | 14,667 |

| Animal category | Daily Feed intake | Body weight | Max safe use levels | Intake[(a)] | Lowest MOE[(b)] |
|---|---|---|---|---|---|
| | g DM/day | kg | mg/kg[(1)] | µg/kg bw/day | |
| Horse | 8 | 400 | 100 | 0.682 | 14,667 |
| Rabbit | 0.1 | 2 | 57 | 0.972 | 10,292 |
| Salmon | 0.0021 | 0.12 | 100 | 0.597 | 16,762 |

(a): The values of methyleugenol in feed is calculated considering the highest analysed value in the additive.
(b): The MOE for methyleugenol is calculated as the ratio of the reference point to the intake: for long-living and reproductive animals is based on $BMDL_{10}$ of 22.2 mg/kg bw per day derived from rodent carcinogenicity studies with methyleugenol; for target species for fattening based on a NOAEL of 10 mg/kg bw per day derived from a 90-day study with methyleugenol (NTP, 2000).

# Appendix C – Methyleugenol in a β-caryophyllene-rich clove leaf oil (CCL oil): Maximum daily intake and margin of exposure for the different target species

The maximum daily intake of methyleugenol 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/safe use level (see Table 12) of the additive in feed for the different target animal categories (ranging from 26 to 112 mg/kg complete feed) and
- assuming that methyeugenol is present at a concentration corresponding to the maximum analysed value in the additive (0.062%).

The margin of exposure (MOE) for each animal category is calculated as the ratio of the reference point to the intake: the $BMDL_{10}$ of 22.2 mg methyleugenol/kg bw per day for long-living and reproductive animals; the NOAEL of 10 mg methyelugenol/kg bw per day for target species for fattening (EFSA FEEDAP Panel, 2023).

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),[15] '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 margin of exposure (MOE) with a magnitude of $\geq$ 10,000, when comparing estimated exposure to genotoxic and/or carcinogenic substances with a $BMDL_{10}$ from a rodent carcinogenicity study, would be indicative of a low concern for the target species (EFSA SC, 2019a)'. For short-living animals, genotoxicity and carcinogenicity endpoints are not considered relevant, therefore a lower magnitude of the MOET (> 100) when comparing estimated exposure with a reference point based on non-neoplastic endpoints is considered adequate (EFSA FEEDAP Panel, 2021b).

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

**Table C.1:** Target animal intake of methyleugenol (as μg/kg bw per day) and margin of exposure (MOE) at the maximum proposed use level of a β-caryophyllene-rich fraction of clove leaf oil (CCL oil) in feed for target animal category

| Animal category | Daily Feed intake | Body weight | Max safe use levels | Intake[a] | Lowest MOE[b] |
|---|---|---|---|---|---|
| | g DM/day | kg | mg/kg[(1)] | μg/kg bw/day | |
| **Long-living and reproductive animals** | | | | | |
| Laying hens | 0.106 | 2 | 38 | 1.419 | 15,645 |
| Sow lactating | 5.28 | 175 | 67 | 1.424 | 15,587 |
| Dairy cow | 20 | 650 | 65 | 1.409 | 15,755 |
| Sheep/goat | 1.2 | 60 | 101 | 1.423 | 15,599 |
| Horse | 8 | 400 | 101 | 1.423 | 15,599 |
| Rabbit | 0.1 | 2 | 40 | 1.409 | 15,755 |
| Dog | 0.25 | 15 | 50 | 0.587 | 37,812 |
| Cat | 0.06 | 3 | 20 | 0.282 | 78,774 |
| Ornamental fish | 0.00054 | 0.012 | 50 | 0.159 | 140,043 |
| **Target species for fattening** | | | | | |
| Chicken for fattening | 0.158 | 2 | 26 | 1.447 | 6,910 |
| Turkey for fattening | 0.176 | 3 | 34 | 1.405 | 7,116 |
| Piglet | 0.88 | 20 | 46 | 1.426 | 7,013 |
| Pig for fattening | 2.2 | 60 | 54 | 1.395 | 7,168 |
| Veal calf (milk replacer) | 1.89 | 100 | 114 | 1.414 | 7,024 |
| Cattle for fattening | 8 | 400 | 101 | 1.423 | 7,027 |

| Animal category | Daily Feed intake | Body weight | Max safe use levels | Intake[a] | Lowest MOE[b] |
|---|---|---|---|---|---|
| | g DM/day | kg | mg/kg[(1)] | µg/kg bw/day | |
| Sheep/goat | 1.2 | 60 | 101 | 1.423 | 7,027 |
| Horse | 8 | 400 | 101 | 1.423 | 7,027 |
| Rabbit | 0.1 | 2 | 40 | 1.409 | 7,097 |
| Salmon | 0.0021 | 0.12 | 112 | 1.381 | 7,242 |

(a): The values of methyleugenol in feed is calculated considering the highest analysed value in the additive.
(b): The MOE for methyleugenol is calculated as the ratio of the reference point to the intake: for long-living and reproductive animals is based on $BMDL_{10}$ of 22.2 mg/kg bw per day derived from rodent carcinogenicity studies with methyleugenol; for target species for fattening based on a NOAEL of 10 mg/kg bw per day derived from a 90-day study with methyleugenol (NTP, 2000).