SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 28 September 2023

doi: 10.2903/j.efsa.2023.8348

Safety and efficacy of feed additives consisting of essential oils from the fruit and stems of *Foeniculum vulgare* Mill. ssp. *vulgare*: Bitter fennel oil for use in all animal species and sweet fennel oil for use in dogs and cats (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, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Andrew Chesson, Josef Schlatter, Johannes Westendorf, Yvette Dirven, Paola Manini, Fabiola Pizzo 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 fruit and stems of *Foeniculum vulgare* Mill. (bitter fennel oil and sweet fennel oil), when used as sensory additives (flavourings). For long-living and reproductive animals, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considered of low concern the use of bitter fennel oil (with a content of estragole up to 6.1%) at the proposed use level in complete feed: 0.6 mg/kg for laying hens and rabbits, 1.0 mg/kg for sows and dairy cows, 1.5 mg/kg for sheep/goats, horses and cats, 1.9 mg/kg for dogs and 7.1 mg/kg for ornamental fish. For short-living animals (animals for fattening), the Panel had no safety concern when bitter fennel oil is used at the proposed use level in complete feed of 18.2 mg/kg for chickens for fattening, 24.3 mg/kg for turkeys for fattening and 25 mg/kg for piglets, pigs for fattening, veal calves, cattle for fattening, sheep/goats, horses, rabbits and salmon. These conclusions were extrapolated to other physiologically related species. The use of sweet fennel oil (with a content of estragole up to 5.0%) was considered of low concern at the proposed use level in complete feed of 2.3 mg/kg for dogs and 1.9 mg/kg cats. The use of bitter fennel oil in animal feed is expected to be of no concern for consumers and the environment. The additives under assessment should be considered as irritants to skin and eyes, and as dermal and respiratory sensitisers. Due to the high concentration of estragole (> 1%), fennel oils are classified as suspected of causing genetic defects and of causing cancer and should be handled accordingly. Since the fruit of *F. vulgare* Mill. ssp. *vulgare* and its preparations are recognised to flavour food, no further demonstration of efficacy was necessary.

Keywords: sensory additives, flavouring compounds, essential oil, *Foeniculum vulgare* Mill., sweet fennel oil, bitter fennel oil, safety, component-based approach

Requestor: European Commission

Question number: EFSA-Q-2010-01286 (new EFSA-Q-2023-00587)

Correspondence: feedap@efsa.europa.eu

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

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output (in alphabetical order of the last name): Jaume Galobart, Matteo Lorenzo Innocenti and Maria Vittoria Vettori.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Durjava, M., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Villa, R. E., Woutersen, R., Brantom, P., Chesson, A., … Dusemund, B. (2023). Safety and efficacy of feed additives consisting of essential oils from the fruit and stems of *Foeniculum vulgare* Mill. ssp. *vulgare*: Bitter fennel oil for use in all animal species and sweet fennel oil for use in dogs and cats (FEFANA asbl). *EFSA Journal*, *21*(10), 1–36. https://doi.org/10.2903/j.efsa.2023.8348

ISSN: 1831-4732

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

Table of contents

1. Introduction

1.1. Background and Terms of Reference

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

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation/re-evaluation of 29 preparations (namely dill herb oil, dill seed extract, dill tincture, dong quai tincture, celery seed oil, celery seed extract (oleoresin), celery tincture, hares ear tincture, caraway seed oil, caraway oleoresin/extract, coriander oil, cumin oil, taiga root extract (solvent-based, sb), taiga root tincture, fennel oil, fennel tincture, common ivy extract (sb), opoponax oil, ginseng tincture, parsley oil, parsley tincture, anise oil, anise tincture, ajowan oil, Ferula Assa-foetida oil, anise star oil, anise star tincture, anise star terpenes and omicha tincture) belonging to botanically defined group (BDG) 02 – *Apiales/ Austrobaileyales* when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). During the assessment, the applicant withdrew the application for nine preparations (dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract, opoponax oil,[3] parsley oil, hares ear tincture, taiga root extract (sb), ajowan oil[4] and parsley tincture[5]). During the course of the assessment, this application was split and the present opinion covers only one out of the 20 remaining preparations under application: fennel oil from the seeds and stems of *Foeniculum vulgare* Mill. During the assessment, the applicant clarified that two types of additives fall into the definition "fennel oil", i.e., bitter fennel oil and sweet fennel oil, obtained by steam distillation of the fruit (and stems) of two different varieties of *Foeniculum vulgare* ssp. *vulgare* Mill.. The two preparations will be assessed individually. During the assessment, the applicant requested a change in the species limiting the application for authorisation of sweet fennel oil to dogs and cats.[6]

The remaining 19 preparations belonging to botanically defined group (BDG) 2 – Apiales and Austrobaileyales 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 3 January 2011.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of fennel oils from the fruit (and stems) of *Foeniculum vulgare* Mill. when used under the proposed conditions of use (see Sections 3.2.1.3 and 3.3.1.3).

1.2. Additional information

Fennel oil bitter and fennel oil sweet from *Foeniculum vulgare* Mill. are currently authorised as feed additives according to the entry in the European Union Register of Feed Additives pursuant to

[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 dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract, and opoponax oil.
[4] On 2 April 2020, EFSA was informed by the applicant about the withdrawal of the applications on parsley oil, hares ear tincture, taiga root extract (sb), ajowan oil.
[5] On 9 December 2020, the applicant informed EFSA about the withdrawal of the application on celery tincture.
[6] On 20 September 2023, the applicant informed EFSA on the withdrawal of the application for sweet fennel oil for certain animal species.

Regulation (EC) No 1831/2003 (2b natural products – botanically defined). They have not been assessed as feed additives in the EU.

There is no specific EU authorisation for any *F. vulgare* preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008[7] flavouring preparations produced from food, may be used without an evaluation and approval as long as 'they do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer and their use does not mislead the consumer'.

'Sweet fennel' (Foeniculi dulcis fructus) is described in a monograph of the European Pharmacopoeia 11.0 (PhEue, 2022a). The fruit is defined as the dry cremocarps and mericarps of *F. vulgare* Mill. ssp. *vulgare* var. *dulce* (Mill.) Batt. & Trab. with a minimum content of 20 mL of essential oil/kg in the anhydrous drug and with a minimum content of 80% of anethole in the essential oil. The concentration of estragole in the oil of the fruit is limited to a maximum of 10%.

'Bitter fennel' (Foeniculi amari fructus) is described in a monograph of the European Pharmacopoeia 11.0 (PhEur, 2022b). The fruit is defined as the dry cremocarps and mericarps of *F. vulgare* Mill. ssp. v*ulgare* var. *vulgare* with a minimum content of 40 mL of essential oil/kg in the anhydrous drug and with a minimum content of 60% of anethole and a minimum content of 15% of fenchone in the essential oil. The concentration of estragole in the oil of the fruit is limited to a maximum of 5%.

'Bitter-fennel fruit oil' (Foeniculi amari fructus aetheroleum) is described in a monograph of the European Pharmacopoeia 11.0 (PhEur, 2022c). It is defined as the essential oil obtained by steam distillation from the ripe fruits of *F. vulgare* Miller, ssp. *vulgare* var. *vulgare*.

'Bitter-fennel herb oil' (Foeniculi amari herbae aetheroleum) is described in a monograph of the European Pharmacopoeia 11.0 (PhEur, 2022d). It is defined as the essential oil obtained by steam distillation from the aerial parts of *F. vulgare* Mill., ssp. *vulgare* var. *vulgare* collected during fruiting.

For *F. vulgare* Miller ssp. *vulgare* var. *dulce* (Miller) Thellung, fructus (sweet fennel), the European Medicines Agency (EMA) issued an individual monograph for human medicinal use (EMA, 2007a), an opinion (EMA, 2007b) and an assessment report common to both *F. vulgare* Miller ssp. *vulgare* var. *vulgare* (bitter fennel) and *F. vulgare* Miller ssp. *vulgare* var. *dulce* (Miller) Thellung (sweet fennel) (EMA, 2008).

In 2005, EMA issued a public statement on the use of herbal medicinal products containing estragole, which lists *F. vulgare* Miller, ssp. *vulgare* var. *vulgare* (bitter fennel) and *F. vulgare* Miller ssp. *vulgare* var. *dulce* (Miller) (sweet fennel) among the plants containing estragole in fruit and in the essential oil (EMA, 2005, revised in 2021, EMA, 2021).

Many of the individual components of the essential oils under assessment have been already evaluated 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 list of flavouring compounds currently authorised for food[8] and feed[9] uses together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000[10] and the corresponding EFSA opinion are given in Table 1.

[7] 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.

[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] 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

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

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

CG	Chemical Group	Product – EU register name (common name)	FLAVIS No	EFSA* opinion, Year
02	Branched-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing branched-chain alcohols and acetals containing branched-chain aldehydes	Isopentyl 2-methylbutyrate	09.419	2012a
06	Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers	Linalool	02.013	2012b
		α-Terpineol	02.014	
		4-Terpinenol	02.072	
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	Fenchyl alcohol	02.038	2016a
		d-Fenchone[(b)]	07.159	
		d-Camphor[(c)]	07.215	
		Fenchyl acetate	09.269	
16	Aliphatic and alicyclic ethers	1,8-Cineole	03.001	2012c, 2021a
18	Allylhydroxybenzenes	1-Methoxy-4-(prop-1(*trans*)-enyl) benzene (*trans*-anethole)	04.010	2011
21	Aromatic ketones, secondary alcohols and related esters	4-Methoxyphenylacetone[(a)]	07.087	EFSA (2008) (AFC)
23	Benzyl alcohols, aldehydes, acids, esters and acetals	4-Methoxybenzaldehyde (anisaldehyde)	05.015	2012d
25	Phenol derivatives containing ring-alkyl, ring-alkoxy and side-chains with an oxygenated functional group	Thymol	04.006	2012e
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	1-Isopropyl-4-methylbenzene (p-cymene)	01.002	2015
		Terpinolene	01.005	
		α-Phellandrene	01.006	
		α-Terpinene	01.019	
		γ-Terpinene	01.020	
		d-Limonene	01.045	
		Pin-2(10)-ene (β-pinene)	01.003	2016b
		Pin-2(3)-ene (α-pinene)	01.004	
		β-Caryophyllene	01.007	
		Myrcene	01.008	
		Camphene	01.009	
		δ-3-Carene	01.029	
		Germacra-1(10),4(14),5-triene[(a),(d)]	01.042	2011, CEF
		β-Phellandrene[(a),(d)]	01.055	
		Limonene[(a)]	01.001	2015a, CEF
		Sabinene (4(10)-thujene)[(a)]	01.059	
		cis-3,7-Dimethyl-1,3,6-octatriene *cis*-β-Ocimene[(a)]	01.064	

(*): FEEDAP opinion unless otherwise indicated.
(a): Evaluated for use in food. According to Regulation (EC) 1565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.
(b): Present in the additive as a mixture of enantiomers (*d,l*-fenchone or (±)-fenchone), the ratio between *d*- and *l*-stereoisomers not given. JECFA and EFSA evaluated the enantiomer *d*-fenchone [07.159] for use in food and in feed (EFSA FEEDAP Panel, 2016a).

(c): Present in the additive as a mixture of enantiomers (*d,l*-camphor), the ratio between *d*- and *l*-stereoisomers not given. JECFA and EFSA evaluated the enantiomer *d*-camphor [07.159] (name in the register (1R,4R)-1,7,7-trimethylbicyclo[2.2.1] heptan-2-one) for use in food (EFSA, 2008) and in feed (EFSA FEDAP Panel, 2016a).

(d): Evaluated applying the 'Procedure' described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). 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, 2015a,b).

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[11] in support of the authorisation request for the use of bitter fennel oil and sweet fennel oil from the fruit and stems of *F. vulgare* as feed additives. The dossier was received on 7/9/2023 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00587.[12]

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

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings (CDGs). The applicant submitted a written agreement to reuse the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 2.[13]

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 in BDG 02 (Apiales and Austrobaileyales). During the assessment, the EURL issued a partial report[14] and an addendum to the report.[15] In particular, the EURL recommended a method based on gas chromatography coupled with flame ionisation detection (GC-FID) for the determination of the phytochemical markers 1-*methoxy-4-(prop-1(trans)-enyl) benzene* (hereinafter referred to as *trans*-anethole) *and fenchone* in *fennel oil* obtained by steam distillation of the fruit and stems of *F. vulgare* Mill. ssp. *vulgare* var. *vulgare (fennel oil bitter)* or from the seeds of *F. vulgare* Mill. ssp. *vulgare* var. *dulce (fennel oil sweet)*.[16]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of bitter fennel oil and sweet fennel oil from the fruit and stems of *F. vulgare* Mill. ssp. *vulgare* is in line with the principles laid down in Regulation (EC) No 429/2008[17] and the relevant guidance documents: Opinion of the Scientific Committee on harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic (EFSA, 2005), Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 2009), Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012f), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012g), 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

[11] FEED dossier reference: FAD-2010-0221.
[12] The original application EFSA-Q-2010-01286 was split on 7/9/2023 and a new EFSA-Q-2023-00587 was generated.
[13] Technical dossier/Supplementary information/Letter dated 29/4/2021.
[14] Preparations included in the partial report: dill herb oil, dill tincture, dong quai tincture, cumin oil, fennel tincture, parsley tincture, anise tincture, star anise tincture and ferula assa-foetida oil.
[15] Preparations included in the addendum: celery seed oil, caraway seed oil, coriander oil, taiga root tincture, fennel oil, common ivy extract (sb), ginseng tincture, anise oil, anise star oil, anise star terpenes and omicha tincture.
[16] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0221_en
[17] 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.

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 Scientific Committee, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee, 2019c), General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021a).[18]

3. Assessment

The additives under assessment, 'bitter fennel oil' and 'sweet fennel oil', are essential oils obtained from two different varieties of *F. vulgare* ssp. *vulgare* Mill., the plant parts used being fruit and stems or fruit only, respectively.

'Bitter fennel oil' originates from the fruit and stems of *F. vulgare* Mill. ssp. v*ulgare* var. *vulgare* and is intended for use as sensory additive (functional group: flavouring compounds) in feed and water for drinking for all animal species.

'Sweet fennel oil' is derived from the fruit of *F. vulgare* Mill. ssp. *vulgare* var. *dulce* (Mill.) Batt. & Trab. and is intended for use as sensory additive (functional group: flavouring compounds) in feed and water for drinking for cats and dogs.

3.1. Origin and extraction

F. vulgare Mill. ssp. *vulgare* var. *dulce* (Mill.) Batt. & Trab is a herb belonging to the Apiaceae family and is commonly referred to as 'sweet fennel', a term used to describe both, the plant and the fruit, the latter containing a sweet tasting oil. A more bitter tasting oil is characteristic for the fruit of another variety, *F. vulgare* Mill. ssp. v*ulgare* var. *vulgare* (vernacular name: 'bitter fennel', also used to describe its fruit).

F. vulgare is a perennial herbaceous plant belonging to the Apiaceae family. It is native to Southern Europe but has become naturalised in many parts of the world. Multiple yellow flowers are produced in terminal compound umbels which give rise to fruit which, when dried, are misleadingly called fennel seed. The young leaves and stems may be consumed raw or cooked. In particular, one cultivar group of *F. vulgare* (Florence fennel) produces bulb-like structures at the base of the stem and is widely used as a vegetable.

The ground or whole dried sweet fennel fruit are used as a spice for culinary purposes or to flavour alcoholic drinks.

Sweet and bitter fennel fruit have a long history of herbal use and are referenced in many medical traditions, particularly in relation to digestive disorders.

Bitter fennel oil is extracted by steam distillation from the fruit and stems of *F. vulgare* Mill. ssp. v*ulgare* var. *vulgare*, sweet fennel oil from the fruit of *F. vulgare* Mill. ssp. *vulgare* var. *dulce* (Mill.) Batt. & Trab. The volatile constituents are condensed and then separated from the aqueous phase by decantation.

3.2. Bitter fennel oil

3.2.1. Characterisation of bitter fennel oil

The additive under assessment is a pale yellow to yellow, clear mobile liquid with a characteristic aroma. In five batches of the additive (originating from Spain), the density (20°C) ranged between 892 and 899 kg/m^3 (specification: 890–905 kg/m^3) and the refractive index (20°C) between 1.492 and 1.496 (specification: (1.480–1.510). Bitter fennel oil is identified with the single Chemical Abstracts Service (CAS) number 8006-84-6, the European Inventory of Existing Commercial Chemical Substances (EINECS) number 283-414-6, the Flavor Extract Manufacturers Association (FEMA) number 2483,[19] and the Council of Europe (CoE) number 201.

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

[19] The Flavour and Extract Manufactures Association (FEMA) reference numbers 2483 is associated to sweet fennel oil. However, certain literature does not distinguish between bitter and sweet fennel oil (Burdock, 2009).

The product specifications used by the applicant are based on those developed by the International Organisation for Standardization (ISO) 17412: 2007 for oil of bitter fennel (*F. vulgare* Mill. ssp. *vulgare* var. *vulgare*)[20] which were adapted to reflect the concentrations of the main components of the essential oil. Six components contribute to the specifications as shown in Table 2, with *trans*-anethole and fenchone selected as phytochemical markers. The analysis of five batches of the additive showed compliance with these specifications when analysed by GC-FID and expressed as % of the total gas chromatographic peak area (% GC area).[21]

Table 2: Major constituents of the essential oil from the fruit and stems of *Foeniculum vulgare* var. *vulgare* (bitter fennel oil) as defined by specifications: batch to batch variation based on the analysis of five batches by gas chromatography with flame ionisation detector (GC-FID). The content of each constituent is expressed as the area percent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent EU register name	CAS No	FLAVIS No	% GC area		
			Specifications	Mean	Range
trans-Anethole	4180-23-8	04.010	15–30	24.9	22.7–27.5
Limonene	138-86-3	01.001	8.0–30	22.3	19.9–24.4
Fenchone[(a)]	1195-79-5	–	7.0–16	13.2	11.5–14.5
α-Phellandrene	99-83-2	01.006	6.5–25	9.6	7.7–13.3
Estragole[(b)]	140-67-0	04.011	2.0–7.0	3.7	3.1–3.9
Myrcene	123-35-3	01.008	1.0–12	6.5	5.8–7.0

EU: European Union; CAS no: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.
(a): Present in the additive as a mixture of enantiomers (*d,l*-fenchone), the ratio between *d*- and *l*-stereoisomers not given.
(b): Substance which shall not be added as such to food (Annex III), maximum level in food is set by Regulation (EC) No 1334/2008, including dairy products (50 mg/kg), processed fruits, vegetables (incl. mushrooms, fungi, roots, tubers, pulses and legumes), nuts and seeds (50 mg/kg), fish products (50 mg/kg) and non-alcoholic beverages (10 mg/kg).

The applicant provided the full characterisation of the five batches obtained by gas chromatography coupled with mass spectrometry (GC–MS).[22] In total, up to 72 peaks were detected in the chromatogram, 38 of which were identified and accounted on average for 99.2% (99.0–99.3%) of the product (as % GC area). The six compounds indicated in the product specifications accounted for 79.3% on average (range 77.8–80.9%) of % GC area (Table 2). Besides the six compounds indicated in the product specifications, 17 other compounds were detected at individual levels > 0.1% and are listed in Table 3. These 23 compounds together account on average for 98.5% (98.2–98.7%) of the product. The remaining 15 compounds (ranging between 0.003% and 0.09%) and accounting for 0.71% of the % GC area are listed in the footnote.[23] Based on the available data on the characterisation, bitter fennel oil is considered a fully defined mixture (EFSA Scientific Committee, 2019a).

The FEEDAP Panel notes that the maximum level proposed for estragole in the specification (7.0%) exceeds the highest level measured in the five batches. Therefore, batches with higher concentrations of estragole (up to 7.0%), could occasionally reach the market.

[20] Technical dossier/Supplementary information September 2021/Annex_III_SIn_reply_fennel_oil_bitter_ISO_17412-2000(E).
[21] Technical dossier/Supplementary information September 2021/Annex_III_SIn_reply_fennel_oil. GC-FID analysis.
[22] Technical dossier/Supplementary information September 2021/Annex_II.
[23] Additional constituents: *trans*-3,7-dimethyl-1,3,6-octatriene, *(Z)*-sabinol, linalool, α-terpineol, germacra-1(10),4(14),5-triene, *(E,E)*-2,6-alloocimene, *cis*-carene, 4-methoxyphenylacetone, *trans*-sabinene hydrate, fenchyl alcohol, fenchyl acetate, terpinolene, α-copaene, isopentyl 2-methylbutyrate and *cis*-sabinene hydrate.

Table 3: Constituents of the essential oil from the fruit and stems of *Foeniculum vulgare* var. *vulgare* (bitter fennel oil), accounting for > 0.1% 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 EU register name	CAS No	FLAVIS No	% GC area	
			Mean	Range
trans-Anethole	4180-23-8	04.010	31.4	29.6–33.7
Limonene	138-86-3	01.001	16.0	13.6–18.2
Fenchone[(a)]	1195-79-5	–	12.2	10.7–13.9
α-Phellandrene	99-83-2	01.006	9.1	7.6–11.6
Estragole	140-67-0	04.011	5.4	4.0–6.1
Myrcene	123-35-3	01.008	5.1	4.5–5.4
α-Pinene (pin-2(3)-ene)	80-56-8	01.004	6.31	5.39–6.92
β-Pinene (pin-2(10)-ene)	127-91-3	01.003	3.22	2.27–3.82
p-Cymene (1-isopropyl-4-methylbenzene)	99-87-6	01.002	2.39	1.86–2.73
β-Phellandrene	555-10-2	01.055	2.05	1.41–2.40
cis-3,7-Dimethyl-1,3,6-octatriene	3338-55-4	01.064	1.93	1.66–2.24
γ-Terpinene	99-85-4	01.020	0.78	0.54–0.92
δ-3-Carene	13466-78-9	01.029	0.58	0.24–0.85
Camphor[(b)]	76-22-2	–	0.30	0.20–0.72
Sabinene (4(10)-thujene)	3387-41-5	01.059	0.27	0.18–0.36
Camphene	79-92-5	01.009	0.24	0.23–0.26
cis-Anethole	25679-28-1	–	0.18	0.14–0.22
1,8-Cineole	470-82-6	03.001	0.17	0.03–0.72
α-Terpinene	99-86-5	01.019	0.16	0.08–0.33
Anisaldehyde (4-methoxybenzaldehyde)	123-11-5	05.015	0.15	0.10–0.18
α-Thujene	2867-05-2	–	0.15	0.08–0.30
Thymol	89-83-8	04.006	0.10	0.08–0.13
4-Terpinenol	562-74-3	02.072	0.10	0.08–0.12
Total			98.46	98.22–98.68[(c)]

EU: European Union; CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.
(a): Present in the additive as a mixture of enantiomers (*d,l*-fenchone), the ratio between *d*- and *l*-stereoisomers not given.
(b): Present in the additive as a mixture of enantiomers (*d,l*-camphor), the ratio between *d*- and *l*-stereoisomers not given.
(c): The values given for the Total are the lowest and the highest values of the sum of the components in the five batches analysed.

3.2.1.1. Substances of concern

The applicant performed a literature search regarding substances of concern and chemical composition of the plant species of *F. vulgare* var. *vulgare* and its preparations.[24] The presence of estragole (3.5–12%) in the fruit of *F. vulgare* var. *vulgare* is reported in the EFSA Compendium of botanicals as a substance of concern (EFSA, 2012).[25] Besides estragole, anethole (methoxy-4-(prop-1 (*trans*)-enyl) benzene [04.088]) was also identified. *trans*-Anethole [04.010], which is known to be prevalent over *cis*-anethole in the fruit of *F. vulgare*,[26] is an authorised feed flavouring previously assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2011).

3.2.1.2. 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-

[24] Technical dossier/Supplementary information September 2021/Literature search_Fennel_oil.
[25] Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals
[26] Anethole [04.088] is defined as a mixture of *trans*- and *cis*-anethole (isomeric ratio not specified). Sweet fennel oil is described to contain 79.8–83.1% of *trans*-anethole and no *cis*-anethole, bitter fennel oil from the fruit is described to contain 55–75% of *trans*-anethole and < 0.5% of *cis*-anethole (EMA, 2008; PhEur, 2022a,b,c,d).

chloride pesticides, organo-phosphorous pesticides, aflatoxins (B1, B2, G1, G2) and ochratoxin A. However, no data have been provided. Since bitter fennel 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.

3.2.1.3. Shelf-life

The typical shelf-life of bitter fennel 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).[27] However, no data supporting this statement were provided.

3.2.1.4. Conditions of use

Bitter fennel 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 by the applicant for the animal species and categories listed in Table 4. No use level has been proposed for the other target species and for the use in water for drinking.

Table 4: Conditions of use for the essential oil from the fruit and stems of *Foeniculum vulgare* var. *vulgare* (bitter fennel oil): maximum proposed use levels in complete feed for certain target animal categories

Animal category	Maximum proposed use level (mg/kg complete feed)
Long-living and reproductive animals	
Laying hen	0.6
Sow lactating	1.0
Dairy cow	1.0
Sheep/goat	1.5
Horse	1.5
Rabbit	0.6
Dog	1.9
Cat	1.5
Ornamental fish	7.1
Short-living animals (species for fattening)	
Chicken for fattening	18.2
Turkey for fattening	24.3
Piglet	25
Pig for fattening	25
Veal calf (milk replacer)	25
Cattle for fattening	25
Sheep/goat	25
Horse	25
Rabbit	25
Salmon	25

3.2.2. Safety

The safety assessment of bitter fennel oil is based on the maximum use levels proposed by the applicant for the animal species listed above (see Table 4).

Many of the components of bitter fennel oil, accounting for about 86% of the total 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[9] 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).

[27] Technical dossier/Section II.

Fenchone and camphor (as mixtures of isomers) have not been evaluated for use as flavourings but are closely related to the flavouring compounds *d*-fenchone [07.159] and *d*-camphor [07.215] already assessed in CG 8. These compounds were evaluated based on the application of the threshold of toxicological concern (TTC) approach and considered safe at individual use levels of 0.5 mg/kg complete feed for cattle, salmonids and non-food producing animals, and 0.3 mg/kg complete feed for pigs and poultry (EFSA FEEDAP Panel, 2016a). The level of camphor resulting from the intended use of the essential oil is lower than the level considered safe in feed for *d*-camphor [07.215]. Conversely, for fenchone, the level in feed resulting from the intended use of the essential oil is 10-fold higher than the level considered safe for *d*-fenchone [07.159]. Recently, *d*-camphor was assessed in tolerance studies with a mixture of flavourings referred to as 'Herbal mixture' in chickens for fattening, piglets, cattle for fattening and salmons. The tolerance studies showed that *d*-camphor was safe up to 5 mg/kg complete feed (EFSA FEEDAP Panel, 2023b). The FEEDAP Panel considers that the conclusions reached for *d*-camphor can be extrapolated to *d*-fenchone, the mixture of isomers of camphor and that of fenchone by applying read-across.

Two compounds listed in Table 1, germacra-1(10),4(14),5-triene [01.042] and β-phellandrene [01.055] 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, 2015a). As a result, these compounds are not authorised for use as flavours in food. For these compounds, in the absence of toxicity data, the FEEDAP Panel applies 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 Scientific Committee, 2019a).

Eleven components of bitter fennel oil have not been previously assessed for use as flavourings. The FEEDAP Panel notes they are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 8, 18 and 31 and for which a similar metabolic and toxicological profile is expected.[28] These 11 lipophilic compounds, accounting together for about 13% 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, 2011, 2015, 2016a,b).

The genotoxic potential for *(Z)*-sabinol was predicted by the applicant using the Organisation for Economic Co-operation and Development (OECD) quantitative structure-activity relationship (QSAR) Toolbox and structural alerts due to the presence of the vinyl/allyl alcohol group were identified.[29] For this compound, prediction of mutagenicity by the Ames test was made by read-across analyses of data available for similar substances (i.e. analogues obtained by categorisation). Categories were defined using general mechanistic and endpoint profilers as well as empirical profilers. Mutagenicity read-across-based predictions were found consistently negative for all categories of analogues. On this basis, the alerts raised were discounted.

The oil under assessment contains estragole, up to 6.1% of the % GC area and up to 7% according to specification.

Information on the absorption, distribution, metabolism and excretion (ADME) and on the toxicology of estragole is summarised in the next sections.

3.2.2.1. Absorption, distribution, metabolism and excretion of estragole

Estragole

Estragole is a lipophilic compound and, as such, readily and completely absorbed from the gastrointestinal tract in laboratory animals. Phase I metabolism is catalysed by cytochrome P450 (CYP450) enzymes, mainly in the liver. Demethylation of the 4-methoxygroup with formation of 4-allylphenol is followed by conjugation with glucuronic acid or sulfate and renal excretion. Oxidation of the double bond of the allyl-side chain leads to estragole-2′,3′-epoxide, which is hydrolysed to the corresponding diol with subsequent glucuronidation and excretion. Both metabolic pathways result in

[28] Six components (fenchone, *cis*-sabinene hydrate, *trans*-sabinene hydrate, camphor, *(Z)*-sabinol) are allocated to CG 08; *cis*-anethole is allocated to CG 18; five components (*trans*-3,7-dimethyl-1,3,6-octatriene, (*E,E*)-2,6-alloocimene, α-thujene, *cis*-carene and α-copaene) are allocated to CG 31. These compounds, representing about 13% of the GC area, are structurally related to compounds already authorised for use in food and feed as flavourings.

[29] Technical dossier/Supplementary information September 2021/Annex_VII_SIn reply_fennel_QSAR.

the detoxification of estragole. The formation of genotoxic metabolites is initiated by oxidation of the side chain with formation of 1′-hydroxyestragole. Sulfate-conjugation of the hydroxyl group leads to 1′-sulfoxyestragole, which is unstable and breaks down to form a highly reactive carbonium ion, which can react covalently with DNA (as reviewed in EMA, 2021).

The metabolism of estragole was evaluated in experimental animals with special focus on the formation of its proximate metabolite, 1′-hydroxyestragole and the influence of the dose administered on the quantity excreted in urine (Zangouras et al., 1981; Anthony et al., 1987, as referenced in EMA, 2021). When ^{14}C-estragole (4-[^{14}C-methoxyl]-allylbenzene) was given in low doses to rodents, the radioactivity was mainly excreted as $^{14}CO_2$ in exhaled air as a result of demethylation, only a minor portion was excreted in urine in the form of several metabolites resulting from hydroxylation in 1′-C and epoxidation at 2′,3′-C followed by ring hydrolysis. In a single study performed in two volunteers orally given 100 μg of methoxy-^{14}C-estragole, 1′-hydroxyestragole quantified in urine of both individuals was 0.2% and 0.4% of the dose; the majority of the radioactivity was excreted in expired air as $^{14}CO_2$ in the first 8 h (Sangster et al., 1987, as referenced in EMA, 2021). Metabolites identified in urine indicate that estragole follows a similar biotransformation profile in rats, mice and humans. There are no studies in human volunteers with high doses of estragole, but in rats and in mice it is consistently shown that as doses increase the urinary levels of 1′-estragole as glucuronide significantly increase (Zangouras et al., 1981; Anthony et al., 1987, as referenced in EMA, 2021).

3.2.2.2. Genotoxicity and carcinogenicity

For fully defined mixtures, the EFSA Scientific Committee (EFSA SC) recommends applying a component-based approach, i.e. assessing all components individually for their genotoxic potential using all available information, including read-across and QSAR considerations about their genotoxic potential (EFSA Scientific Committee, 2019b). Therefore, the potential genotoxicity of identified constituents is first considered. Then, *in vitro* genotoxicity studies performed with bitter fennel oils similar to the additive under assessment are taken into account, if deemed relevant.

Bitter fennel oil contains estragole (up to 7% by specification), a compound with experimentally proven genotoxicity and carcinogenicity in rodents (as reviewed in EC, 2001; EMA, 2021).

The carcinogenicity of estragole and other structurally related *p*-allylalkoxybenzenes has been reviewed by the FEEDAP Panel in the opinion on olibanum extract (EFSA FEEDAP Panel, 2022).

For *p*-allylalkoxybenzenes, the FEEDAP Panel identified a reference point for neoplastic endpoints derived from a carcinogenicity study in rat with methyleugenol (NTP, 2000) by applying the benchmark dose (BMD) approach with model averaging. Dose–response modelling using hepatocellular carcinomas in male rats as a response yielded a BMD lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) of 22.2 mg/kg body weight (bw) per day (Suparmi et al., 2019). This $BMDL_{10}$ value was selected as reference point for the assessment group of *p*-allylalkoxybenzenes, including estragole, irrespective of their relative potency (EFSA FEEDAP Panel, 2022).

Genotoxicity studies with bitter fennel oil

The applicant provided a literature search on the genotoxicity of essential oils obtained from *F. vulgare*.[30] No studies made with bitter fennel oil were retrieved.

3.2.2.3. Subchronic toxicity studies

The FEEDAP Panel identified a no observed adverse effect level (NOAEL) of 10 mg/kg bw per day for non-neoplastic lesions (changes in organ weight[31] and function, including effects on liver[32] and the glandular stomach[33]) from a 90-day study in mice with methyleugenol (NTP, 2000). Considering the structural similarity and the similar mode of action of *p*-allylalkoxybenzenes, the FEEDAP Panel selected the NOAEL of 10 mg/kg bw per day as reference point for the assessment group *p*-allylalkoxybenzenes for non-neoplastic endpoints (EFSA FEEDAP Panel, 2023a).

[30] Technical dossier/Supplementary information September 2021/Literature search_fennel_oil.

[31] 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.

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

[33] 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.

3.2.3. Safety for the target species

Tolerance studies with 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. The individual components can be grouped into assessment groups, based on structural and metabolic similarity. The combined toxicity can be predicted using the dose addition assumption within an assessment group, taking into account the relative toxic potency of each component.

As the additive under assessment is a fully defined mixture (the identified components represent > 99% of the % GC area, see Section 3.2.1), the FEEDAP Panel applied a component-based approach to assess the safety for target species of the essential oil. Camphor, which was included in tolerance studies with a mixture of flavourings 'Herbal mixture' (EFSA FEEDAP Panel, 2023b), and the structurally-related compounds (fenchone and fenchyl derivatives) are assessed separately. Estragole, a substance for which a concern for genotoxicity has been identified, is also assessed separately.

Components other than camphor, fenchone and fenchyl derivatives, and estragole

Based on considerations related to structural and metabolic similarities, the components were allocated to nine assessment groups, corresponding to the chemical groups (CGs) 2, 6, 8, 16, 18, 21, 23, 25 and 31, as defined in Annex I of Regulation (EC) No 1565/2000. For chemical group 31 ('aliphatic and aromatic hydrocarbons'), sub-assessment groups as defined in Flavouring Group Evaluation 25 (FGE.25) and FGE.78 were established (EFSA CEF Panel, 2015a,b). The allocation of the components to the (sub-)assessment groups is shown in Table 5 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 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 5.

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 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 of sub-chronic studies, from which NOAEL values could be derived, were available for linalool [02.013] and terpineol [02.230][34] in CG 6 (EFSA FEEDAP Panel, 2012b), 1,8-cineole in CG 16 (EFSA FEEDAP Panel, 2021a), *trans*-anethole [04.010] in CG 18 (EFSA FEEDAP Panel, 2011), anisaldehyde [05.015] in CG 23 (EFSA FEEDAP Panel, 2012d), thymol [04.006] in CG 25 (EFSA FEEDAP Panel, 2012e) and myrcene [01.008], *d*-limonene [01.045], *p*-cymene [01.002] and β-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016b). For benzaldehyde [05.013] in CG 23, the FEEDAP Panel concluded that the maximum proposed concentration of 25 mg/kg complete feed is safe (EFSA FEEDAP Panel, 2012e). In addition, for benzyl alcohol [02.010] the FAF Panel 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 chronic study in rats (EFSA FAF Panel, 2019).

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

[34] Terpineol is a mixture of four isomers: α-terpineol [02.014], a mixture of *(R)*-(+)-α-terpineol and *(S)*-(−)-α-terpineol, β-terpineol, γ-terpineol and 4-terpinenol [02.072] (or δ-terpineol). The specification for terpineol [02.230] covers α-, β-, γ and δ-terpineol. Composition of mixture: 55–75% α-terpineol, 16–23% γ-terpineol, 1–10% *cis*-β-terpineol, 1–13% *trans*-β-terpineol and 0–1% δ-terpineol (EFSA CEF Panel, 2015c) FGE.18Rev 3.

For benzaldehyde [05.013] in CG 23, read-across was applied from benzyl alcohol [02.010] applying to the NOAEL of 400 mg/kg bw per day an additional uncertainty factor of 2 to take into account the higher reactivity of aldehyde compared to the alcohol.

The NOAELs of 44, 250 and 222 mg/kg bw per day for the representative compounds of CG 31, myrcene [01.008], limonene [01.001] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within sub-assessment groups II (*cis*-3,7-dimethyl-1,3,6-octatriene [01.064] and *trans*-3,7-dimethyl-1,3,6-octatriene), III (α-phellandrene [01.006], β-phellandrene [01.055], γ-terpinene [01.020] and α-terpinene [01.019]) and V (α-pinene [01.004], β-pinene [01.003], δ-3-carene [01.029], sabinene [01.059], α-thujene and camphene [01.009]),[35] respectively (EFSA CEF Panel, 2015a,b). The NOAEL of 222 mg/kg bw per day was also applied to *(Z)*-sabinol, *trans*-sabinene hydrate and *cis*-sabinene hydrate in CG 8.

For the remaining compounds,[36] NOAEL values were not available and read-across was not possible. Therefore, the TTC approach was applied (EFSA FEEDAP Panel, 2017b; EFSA Scientific Committee, 2019c). All these compounds belong to Cramer class I, except camphor and linalool oxide (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 5.

For risk characterisation, the margin of exposure (MOE) was calculated for each component as the ratio between the reference point and the exposure. For each assessment group, the combined (total) margin of exposure (MOET) was calculated as the reciprocal of the sum of the reciprocals of the MOE of the individual substances (EFSA Scientific Committee, 2019a). A MOET > 100 allowed for interspecies differences and intra-individual variability (as in the default 10 × 10 uncertainty factor). The compounds resulting individually in an MOE > 50,000 were not further considered in the assessment group as their contribution to the MOE(T) is negligible. They are listed in the footnote.[37]

The approach to the safety assessment of bitter fennel oil for the chickens for fattening is summarised in Table 5. The calculations were done for chickens for fattening, the species with the highest ratio of feed intake/bw and represent the worst-case scenario at the use level of 18.2 mg/kg in feed, without considering the presence of camphor, fenchone and fenchyl derivatives, and estragole.

Table 5: Compositional data, intake values, reference points, margin of exposure (MOE) for the individual components of bitter fennel oil classified according to assessment groups and combined margin of exposure (MOET) for each assessment group

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 16								
1,8-Cineole	03.001	0.72	0.131	0.0118	(II)	**100**	8,489	
CG 18								
trans-Anethole	04.010	33.78	6.148	0.5519	(I)	**300**	544	
CG 21								
4-Methoxyphenylacetone	07.087	0.06	0.012	0.0010	I	*3*	2,869	
CG 23								
Anisaldehyde	05.015	0.06	0.033	0.0030	(I)	**10[(d)]**	3,363	

[35] Some of these compounds are not listed in Table 5 because their individual margin of exposure (MOE) was >50,000.
[36] 4-Methoxyphenylacetone [07.087], *(E,E)*-2,6-alloocimene, *cis*-carene and germacra-1(10),4(14),5-triene [01.042].
[37] Compounds included in the assessment groups but not reported in the table: isopentyl 2-methylbutyrate (CG 2); linalool, 4-terpinenol and α-terpineol (CG 6); *(Z)*-sabinol, *trans*-sabinene hydrate and *cis*-sabinene hydrate (CG 8); *cis*-anethole (CG 18); terpinolene [01.005] (CG 31, III); α-cubebene (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**
CG 25								
Thymol	04.006	0.13	0.023	0.0021	(I)	36	17,080	
CG 31, II (Acyclic alkanes)								
Myrcene	01.008	5.46	0.993	0.0891	(I)	**44**	494	
cis-β-Ocimene	01.064	2.24	0.407	0.0365	(I)	44	1,204	
trans-3,7-Dimethyl-1,3,6-octatriene	–	0.12	0.021	0.0019	(I)	44	2,3017	
(E,E)-2,6-Alloocimene	–	0.06	0.011	0.0010	I	*3*	2,915	
MOET CG 31, II								308
CG 31, III (Cyclohexene hydrocarbons)								
Limonene	01.001	18.22	3.315	0.2976	(I)	**250**	840	
α-Phellandrene	01.006	11.60	2.111	0.1895	(I)	250	1,319	
β-Phellandrene	01.055	2.40	0.437	0.0392	(I)	250	6,375	
γ-Terpinene	01.020	0.92	0.168	0.0151	(I)	250	1,6578	
α-Terpinene	01.019	0.33	0.060	0.0054	(I)	250	46,227	
MOET CG 31, III								457
CG 31, IVe (Benzene hydrocarbons, alkyl)								
p-Cymene	01.002	2.73	0.497	0.0477	(I)	**154**	3,449	
CG 31, V (Bi-, tricyclic, non-aromatic hydrocarbons)								
α-Pinene	01.004	6.92	1.260	0.1131	(I)	222	1,963	
β-Pinene	01.003	3.82	0.696	0.0625	(I)	222	3,553	
δ-3-Carene	01.029	0.85	0.154	0.0138	(I)	222	16,061	
Sabinene	01.059	0.36	0.065	0.0058	(I)	222	38,060	
α-Thujene	–	0.30	0.054	0.0048	(I)	222	46,059	
Camphene	01.009	0.26	0.048	0.0043	(I)	222	52,059	
cis-Carene	–	0.09	0.015	0.0014	I	*3*	2,160	
MOET CG 31, V								723
CG 31, VI (macrocyclic non-aromatic hydrocarbons)								
Germacra-1(10),4(14),5-triene	01.042	0.15	0.027	0.0024	I	*3*	1,232	

(a): Intake calculations for the individual components are based on the use level of 18.2 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 anisaldehyde of 20 mg/kg bw per day (derived from a 42-day study in rat, where several effects, i.e. increase in body weight, reduced platelet count and hyperplasia of squamous epithelium, were observed starting from 100 mg/kg bw per day in females and at 500 mg/kg bw per day in males) was halved owing to the short duration of the study.

As shown in Table 5, for all assessment groups, the MOET was ≥ 308. Therefore, no safety concern was identified for the bitter fennel oil when used as a feed additive for chickens for fattening at the proposed use levels (18.2 mg/kg) without considering camphor, fenchone and fenchyl derivatives, and estragole.

From the lowest MOET of 308 for chickens for fattening, the MOET for CG 31, II (acyclic alkanes) was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 6.

Table 6: Combined margin of exposure (MOET) for the assessment group CG 31, II (acyclic alkanes) calculated for the different target animal categories at the proposed use levels of bitter fennel oil in complete feed

Animal category	Body weight (kg)	Daily feed intake (g DM/day)	Proposed use levels (mg/kg feed)[(a)]	Lowest MOE
Long-living and reproductive animals				
Laying hens	2	106	0.6	13,926
Sow lactating	175	5,280	1.0	14,761
Dairy cow	650	20,000	1.0	14,285
Sheep/goat	60	1,200	1.5	14,761
Horse	400	8,000	1.5	14,761
Rabbit	2	100	0.6	14,761
Dog	15	250	1.9	13,710
Cat	3	60	1.5	14,761
Ornamental fish	0.012	0.054	7.1	12,474
Short-living animals (species for fattening)				
Chicken for fattening	2	158	18.2	308
Turkey for fattening	3	176	24.3	309
Piglet	20	880	25	403
Pig for fattening	60	2,200	25	479
Veal calf (milk replacer)	100	1,890	25	932
Cattle for fattening	400	8,000	25	886
Sheep/goat	60	1,200	25	886
Horse	400	8,000	25	886
Rabbit	2	100	25	354
Salmon	0.12	2.1	25	984

DM, dry matter.
(a): Complete feed containing 88% DM, milk replacer 94.5% DM.

Table 6 shows that, when the additive was used at the proposed use levels in complete feed, the MOET is above the value of 100 for all species. 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 bitter fennel oil as additive in cat feed needs a wider margin of exposure. A MOET of 500 is considered adequate. Therefore, for all species, no safety concern (without considering the presence of estragole, camphor, fenchone and fenchyl derivatives) was identified for bitter fennel oil, when used as a feed additive at the proposed use levels.

Camphor, fenchone and fenchyl derivatives

The applicant included camphor in tolerance studies with a mixture of flavourings ('Herbal mixture') to demonstrate its safe use at higher levels than those currently evaluated as safe in feed (EFSA FEEDAP Panel, 2023b).

The applicant provided further evidence on the safety of camphor in the form of tolerance trials in chickens for fattening, piglets, cattle for fattening and salmons, which showed that *d*-camphor was safe up to 5 mg/kg complete feed (EFSA FEEDAP Panel, 2023b). In the current assessment the applicant proposed to read-across from *d*-camphor to its enantiomer and to *d*-fenchone and its enantiomer. The FEEDAP Panel considers that the proposal for read-across is justified by the structural similarity between the two compounds (Api et al., 2018) and that the conclusions of the tolerance studies with *d*-camphor are applicable to *l*-camphor, *d*- and *l*-fenchone and the fenchyl derivatives, fenchyl alcohol [02.038] and fenchyl acetate [09.269], present in the additive. For these compounds belonging to CG 8, a concentration in feed of 5 mg/kg complete feed is considered safe. At the

maximum proposed use level of 25 mg/kg complete feed for bitter fennel oil, the combined concentration of camphor, fenchone and fenchyl derivatives would be 3.68 mg/kg, which is lower than the concentration considered safe for all animal species and therefore not of concern.

Estragole

Estragole belongs to the assessment group *p*-allylalkoxybenzenes, a group of compounds which are genotoxic and carcinogenic. According to the General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021a), different reference points and a different magnitude of the MOE(T) 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(T) with a magnitude > 10,000, when comparing the estimated exposure to genotoxic and/or carcinogenic substances with a $BMDL_{10}$ from a rodent carcinogenicity study, is considered 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, 2023a). In the current assessment this reference point is applied to assess the exposure of long-living and reproductive animals to estragole.

For short-living animals, genotoxicity and carcinogenicity endpoints are not considered relevant, therefore a lower magnitude of the MOE(T) (> 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 from a 90-day study in mice with methyleugenol (NTP, 2000). In the current assessment this reference point is applied to assess the exposure of short-living animals to estragole.

Estragole (4.0–6.1%) was detected in all batches of the oil under assessment (see Section 3.2.1). The FEEDAP Panel notes that bitter fennel oil with higher concentrations of estragole may occasionally reach the market. In particular, the applicant set a specification for estragole up to 7.0%.

Thus, for the assessment of estragole for the target species the FEEDAP Panel considered two possible scenarios.

a) A bitter fennel oil with a content of estragole corresponding to the highest specification of 7.0%

The highest daily intake of estragole was calculated considering the maximum proposed use level of the additive in feed for the different animal categories and the concentration of estragole in the additive corresponding to the highest specification of 7.0%. The intake values are reported in Table 7, together with the corresponding MOE calculated considering the relevant reference point for long-living and reproductive animals and for short-living animals (species for fattening).

Table 7: Target animal intake of estragole and margin of exposure (MOE) calculated at the maximum proposed use level of bitter fennel oil in feed for an essential oil with a content of estragole corresponding to the highest specification of 7%

Animal category	Daily Feed intake	Body weight	Proposed use levels	Estragole intake[(a)]	MOE[(b),(c)]
	g DM/day	kg	mg/kg feed[(d)]	µg/kg bw per day	
Long-living and reproductive animals[(b)]					
Laying hens	106	2	0.6	2.5	8,697
Sow lactating	5,280	175	1.0	2.4	9,167
Dairy cow	20,000	650	1.0	2.4	8,989
Sheep/goat	1,200	60	1.5	2.4	9,219
Horse	8,000	400	1.5	2.4	9,219
Rabbit	100	2	0.6	2.4	9,219
Dog	250	15	1.9	2.5	8,734

Animal category	Daily Feed intake	Body weight	Proposed use levels	Estragole intake[a]	MOE[b],[c]
	g DM/day	kg	mg/kg feed[d]	µg/kg bw per day	
Cat	60	3	1.5	2.4	9,219
Ornamental fish	0.054	0.012	7.1	2.5	8,656
Short-living animals (species for fattening)[c]					
Chicken for fattening	158	2	18.2	114	87
Turkey for fattening	176	3	24.3	113	88
Piglet	880	20	25	88	114
Pig for fattening	2,200	60	25	73	137
Veal calf (milk replacer)	1,890	100	25	35	286
Cattle for fattening	8,000	400	25	40	251
Sheep/goat	1,200	60	25	40	251
Horse	8,000	400	25	40	251
Rabbit	100	2	25	99	101
Salmon	2.1	0.12	25	35	287

DM: dry matter.
(a): The intake value of estragole is calculated at the specification of 7%.
(b): The MOE for long-living and reproductive animals is calculated as the ratio of the reference point ($BMDL_{10}$ of 22.2 mg/kg bw per day) to the intake.
(c): The MOE for short-living animals (species for fattening) is calculated as the ratio of the reference point (no observed adverse effect level (NOAEL) of 10 mg/kg bw per day) to the intake.
(d): Complete feed containing 88% DM, milk replacer 94.5% DM.

When the estimated exposures of long-living animals to estragole are compared to the $BMDL_{10}$ of 22.2 mg methyleugenol/kg bw per day (Suparmi et al., 2019), a MOE < 10,000 which is considered of concern, is obtained (Table 7).

For short-living animals (except chickens for fattening and turkeys for fattening), the magnitude of the MOE is > 100, and is of no safety concern, when comparing the exposure to the reference point for non-neoplastic endpoints.

Considering that analysed values of estragole provided for the batches under assessment are below the highest specification, the FEEDAP Panel proposed a scenario based on the analytical values provided for the five batches described in Section 3.2.1.

b) A bitter fennel oil with a content of estragole, corresponding to the highest analysed concentration (6.1%)

The highest daily intake of estragole was calculated considering the maximum proposed use level of the additive in feed for the different animal categories and the highest analysed concentration of estragole (6.1%). The intake values are reported in Table 8, together with the corresponding MOE calculated considering the relevant reference point for long-living and reproductive animals and for species for fattening.

Table 8: Target animal intake of estragole and margin of exposure (MOE) calculated at the maximum proposed use level of bitter fennel oil in feed for an essential oil with a content of estragole corresponding to the highest analysed concentration (6.1%)

Animal category	Daily Feed intake	Body weight	Proposed use levels	Estragole intake[a]	MOE[b],[c]
	g DM/day	kg	mg/kg feed[d]	µg/kg bw per day	
Long-living and reproductive animals[b]					
Laying hens	106	2	0.6	2.2	9,980
Sow lactating	5,280	175	1.0	2.1	10,519
Dairy cow	20,000	650	1.0	2.1	10,315
Sheep/goat	1,200	60	1.5	2.1	10,579

Animal category	Daily Feed intake	Body weight	Proposed use levels	Estragole intake[a]	MOE[b],[c]
	g DM/day	kg	mg/kg feed[d]	µg/kg bw per day	
Horse	8,000	400	1.5	2.1	10,579
Rabbit	100	2	0.6	2.1	10,579
Dog	250	15	1.9	2.2	10,022
Cat	60	3	1.5	2.1	10,579
Ornamental fish	0.054	0.012	7.1	2.2	9,934
Short-living animals (species for fattening)[c]					
Chicken for fattening	158	2	18.2	100	100
Turkey for fattening	176	3	24.3	99	101
Piglet	880	20	25	76	131
Pig for fattening	2,200	60	25	64	157
Veal calf (milk replacer)	1,890	100	25	31	328
Cattle for fattening	8,000	400	25	35	289
Sheep/goat	1,200	60	25	35	289
Horse	8,000	400	25	35	289
Rabbit	100	2	25	87	115
Salmon	2.1	0.12	25	30	330

DM: dry matter; MOE: margin of exposure.
(a): The intake value of estragole is calculated considering the highest analysed value in the additive (6.1%).
(b): The MOE for long-living and reproductive animals is calculated as the ratio of the reference point ($BMDL_{10}$ of 22.2 mg/kg bw per day) to the intake.
(c): The MOE for short-living animals (species for fattening) is calculated as the ratio of the reference point (no observed adverse effect level (NOAEL) of 10 mg/kg bw per day) to the intake.
(d): Complete feed containing 88% DM, milk replacer 94.5% DM.

The estimated exposures for long-living animals to estragole are compared to the $BMDL_{10}$ of 22.2 mg methyleugenol/kg bw per day (Suparmi et al., 2019). A MOE of 10,000 or greater is considered of low concern (Table 8).

For short-living animals, the magnitude of the MOE is > 100, and is of no safety concern, when comparing the exposure to the reference point for non-neoplastic endpoints.

3.2.3.1. Conclusions on safety for the target species

Based on the MOE calculated considering the presence of estragole in bitter fennel oil and the conditions of use in the different species, the FEEDAP Panel concludes that:

a) *For bitter fennel oil with a content of estragole at the highest proposed specification of 7.0%*

- For long-living and reproductive animals (including those animals reared for laying/ breeding/reproduction) the use of the additive is considered of concern (MOE < 10,000) at the proposed level in complete feed.
- For short-living animals, the Panel has no safety concern when the additive is used at the maximum proposed use level in complete feed of 25 mg/kg for pigs for fattening, piglets and other Suidae species for meat production, veal calves (milk replacer), cattle for fattening and other ruminants for fattening and camelids at the same physiological stage, horses and other Equidae for meat production and rabbits for meat production, salmonids and minor fin fish. For turkeys for fattening, chickens for fattening and minor poultry for fattening, the respective proposed use levels are considered of concern.

b) *For bitter fennel oil with a content of estragole corresponding to the highest analysed concentration (6.1% estragole)*

- For long-living and reproductive animals (including those animals reared for laying/ breeding/reproduction) the use of the additive is considered of low concern (MOE > 10,000) at the proposed level in complete feed of 0.6 mg/kg for laying hens and other laying/reproductive birds including animals reared for laying/reproduction and

ornamental birds and rabbits, 1.0 mg/kg for sows and other Suidae species for reproduction including animals reared for reproduction, and dairy cows and other ruminants and camelids for milk production and reproduction including animals reared for milk production/reproduction, 1.5 mg/kg for sheep/goats, horses and other Equidae and cats, 1.9 mg/kg for dogs and 7.1 mg/kg for ornamental fish.

- For short-living animals, the Panel has no safety concern when the additive is used at the maximum proposed use level in complete feed of 25 mg/kg for pigs for fattening, piglets and other Suidae species for meat production, veal calves (milk replacer), cattle for fattening and other ruminants for fattening and camelids at the same physiological stage, horses and other Equidae for meat production and rabbits for meat production, salmonids and minor fin fish, 24.3 mg/kg for turkeys for fattening and 18.2 mg/kg chickens for fattening and minor poultry for fattening.
- For any other species, the additive is considered of low/no concern at 0.6 mg/kg complete feed.
- The conclusions are summarised in Table 9.

Table 9: Maximum feed concentrations of a bitter fennel oil (with a content of estragole corresponding to the highest analysed value of 6.1%) in complete feed (mg/kg) considered of low concern for long-living and reproductive animals and of no concern for target species for fattening

Animal category	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	0.6
Sows and other Suidae species for reproduction including animals reared for reproduction	1.0
Dairy cows and other ruminants (except for sheep/goat) and camelids for milk production and reproduction including animals reared for milk production/reproduction	1.0
Sheep/goat	1.5
Horses and other Equidae	1.5
Rabbits	0.6
Dogs	1.9
Cats	1.5
Ornamental fish	7.1
Short-living animals (species for fattening)[(b)]	
Chickens for fattening and minor poultry for fattening	18.2
Turkeys for fattening	24.3
Pigs for fattening	25.0
Piglets and other Suidae species for meat production	25.0
Veal calves (milk replacer)	25.0
Cattle for fattening and other ruminants for fattening (except for sheep/goat) and camelids at the same physiological stage	25.0
Sheep/goat for meat production	25.0
Horses and other Equidae for meat production	25.0
Rabbit for meat production	25.0
Salmonids and minor fin fish	25.0
Any other species	0.6

(a): Based on a MOE > 10,000 for long-living and reproductive animals, calculated as the ratio of the reference point ($BMDL_{10}$ of 22.2 mg/kg bw per day) to the intake.
(b): Based on a MOE > 100 for target species for fattening, calculated as the ratio of the reference point (no observed adverse effect level (NOAEL) of 10 mg/kg bw per day) to the 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 concern/no concern when consumed via feed alone.

3.2.4. Safety for the consumer

Bitter fennel oil, described in the Fenaroli as fennel oil (common) from *F. vulgare* Mill. var. *amara* is added to food for flavouring purposes. Although individual consumption figures are not available, the Fenaroli's handbook of flavor ingredients cites intake values of 0.031 mg/kg per day for sweet fennel (FEMA 2482) and for sweet fennel oil (FEMA 2483) of 0.006 mg/kg bw per day (Burdock, 2009).

Many of the individual constituents of the essential oil under assessment are currently authorised as food flavourings without limitations and have been already assessed for consumer safety when used as feed additives in animal production (see Table 1, 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 bitter fennel oil are expected to be extensively metabolised and excreted in the target species. Also, for estragole, the available data indicate that it is absorbed, metabolised and rapidly excreted and is not expected to accumulate in animal tissues and products. Consequently, residues in food products are unlikely (see Section 3.2.2.1).

The FEEDAP Panel considers it unlikely that the consumption of food products derived from animals given bitter fennel oil at the proposed maximum use levels would increase human background exposure of compounds present in the fennel oil. Thus, no concern would be expected for the consumer from the use of bitter fennel oil up to the highest use level in feed which is considered of no or low concern for target animals.

3.2.5. Safety for the user

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

The applicant provided their safety data sheet for bitter fennel oil,[38] where hazards for users have been identified.

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

Due to the high level of estragole (> 1%), the applicant also proposes to classify the additive according to Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP Regulation)[39] as suspected of causing genetic defects (category 2 mutagen) and of causing cancer (category 2 carcinogen).

For preparations with these classifications, precautionary statements as indicated in the Regulation (EC) No 1272/2008 have to be followed, and the additive should be handled accordingly.[40]

3.2.6. Safety for the environment

F. vulgare is a native species to Europe where it is widely grown both for commercial and decorative purposes. Therefore, the use of bitter fennel oil under the proposed conditions of use in animal feed is not expected to pose a risk for the environment.

3.3. Sweet fennel oil

3.3.1. Characterisation of sweet fennel oil

The essential oil obtained from the fruit of *F. vulgare* var. *dulce* is a pale yellow to yellow, clear mobile liquid with a characteristic aroma. In five batches of the additive (from Moldavia), the specific

[38] Technical dossier/Supplementary Information September 2021/Annex_IX_Sin_reply_fennel_oil_bitter_MSDS. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1A), serious eye irritation (H319, category 2), suspected of causing genetic defects (H341, Muta 2), suspected of causing cancer (H351, Carc. 2).

[39] Regulation (EC) No 1271/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

[40] Directive 2004/37/EC of the European Parliament and of the Council of 29 April 2004 on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (Sixth individual Directive within the meaning of Article 16(1) of Council Directive 89/391/EEC). OJ L 158, 30.4.2004, p. 50.

gravity (20°C) was 1.54 kg/m^3 in all batches (specification not reported), the refractive index (20°C) between 0.964 and 0.968 (specification: 1.52–1.55) and the specific optical rotation (at 20°C) between +12.92° and 15.00° (specifications: between +5° and + 22°).[41] Sweet fennel oil is identified with the single CAS number 84455-29-8, the EINECS number 282-892-3, the FEMA number 2483[19] and CoE number 200.

The product specifications are based on the Association Francaise de Normalisation NF T 75–257,[42] which were adapted to reflect the concentrations of the main components of the essential oil, as shown in Table 10, with *trans*-anethole and fenchone selected as phytochemical markers. The analysis of five batches of the additive showed compliance with these specifications when analysed by GC-FID and expressed as % GC area.[43]

Table 10: Major constituents of the essential oil from sweet fennel fruit (sweet fennel oil) as defined by specifications and batch to batch variation based on the analysis of five batches by gas chromatography with flame ionisation detector (GC-FID). 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	CAS No	FLAVIS No	% GC area		
EU register name			Specification	Mean	Range
trans-Anethole	4180-23-8	04.010	60–82	77.7	76.1–79.9
Fenchone[(a)]	1195-79-5	–	1.0–20	5.26	5.10–5.40
Limonene	138-86-3	01.001	1.0–8.0	5.85	5.70–6.10
Estragole[(b)]	140-67-0	04.011	2.0–6.0	3.76	3.60–4.10
α-Pinene	80-56-8	01.004	1.0–8.0	3.64	2.00–4.40
α-Phellandrene	99-83-2	01.006	0.2–5.0	1.43	1.10–1.70

EU: European Union; CAS no: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.
(a): Present in the additive as a mixture of enantiomers (*d,l*-fenchone), the ratio between *d*- and *l*-stereoisomers not given.
(b): Substance which shall not be added as such to food (Annex III), maximum level in food is set by Regulation (EC) No 1334/2008, including dairy products (50 mg/kg), processed fruits, vegetables (incl. mushrooms, fungi, roots, tubers, pulses and legumes), nuts and seeds (50 mg/kg), fish products (50 mg/kg) and non-alcoholic beverages (10 mg/kg).

The applicant provided the full characterisation of the five batches obtained by GC–MS.[44] In total, 29 chromatographic peaks were identified and accounted on average for 97.6% (97.3–98.1%) of the % GC area. The six compounds indicated in the product specifications accounted for 97.6% on average (range 94.3–100.7%) of % GC area. Besides the six compounds, eight other compounds were detected at individual levels > 0.1% and are listed in Table 11. These 14 compounds together account on average for 99.44% (99.38–99.51%) of the product. The remaining 15 compounds (ranging between 0.01% and 0.09%) and accounting for 0.50% are listed in the footnote.[45] Based on the available data on the characterisation, sweet fennel oil is considered a fully defined mixture (EFSA Scientific Committee, 2019a).

The FEEDAP Panel notes that the maximum level proposed for estragole in the specification (6.0%) exceeds the highest level measured in the five batches. Therefore, batches with higher concentrations of estragole (6.0%), could occasionally reach the market.

[41] Technical dossier/Supplementary information September 2021/Annex_II_ SIn_Reply_fennel_oil_COA_chrom.
[42] Technical dossier/Supplementary information September 2021/Annex_IV_ _SIn_reply_fennel_oil_bitter_NF T 75-257.pdf.
[43] Technical dossier/Supplementary information September 2021/Annex_III_SIn_reply_fennel_oil/Table 3.
[44] Technical dossier/Supplementary information September 2021/Annex_II_ SIn_Reply_fennel_oil_COA_chrom.
[45] Additional constituents: constituents < 0.1% and ≥ 0.01% (n = 12): (*Z*)-anethol, camphene, sabinene, (*E*)-α-bergamotene, germacra-1(10),4(14),5-triene, β-caryophyllene, *cis*-3,7-dimethyl-1,3,6-octatriene, terpinolene, (*Z*)-α-Bergamotene, 1,8-cineole, α-thujene and *trans*-sabinene hydrate. Constituents < 0.01% (n = 3): 4-terpinenol, α-terpinene and α-fenchene.

Table 11: Other constituents of the essential oil from the sweet fennel fruit (sweet fennel oil) accounting for > 0.1% of the composition (based on the analysis of five batches by gas chromatography–mass spectrometry (GC–MS)) not included in the specification. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent	CAS No	FLAVIS No	% GC area	
EU register name			Mean	Range
trans-Anethole	4180-23-8	04.010	78.0	77.2–79.2
Fenchone[(a)]	1195-79-5	–	5.30	5.18–5.42
Limonene	138-86-3	01.001	5.15	4.88–5.56
Estragole[(b)]	140-67-0	04.011	4.42	4.11–4.96
α-Pinene	80-56-8	01.004	3.34	1.85–3.95
Myrcene	123-35-3	01.008	0.33	0.23–0.41
4-Methoxyphenylacetone	122-84-9	07.087	0.29	0.22–0.35
Anisaldehyde (4-methoxybenzaldehyde)	123-11-5	05.015	0.28	0.25–0.30
β-Pinene	127-91-3	01.003	0.26	0.15–0.32
β-Phellandrene	555-10-2	01.055	0.19	0.14–0.22
γ-Terpinene	99-85-4	01.020	0.18	0.14–0.21
p-Cymene (1-isopropyl-4-methylbenzene)	99-87-6	01.002	0.18	0.15–0.20
Camphor[(c)]	76-22-2	–	0.11	0.07–0.13
Total			99.44	99.38–99.51[(d)]

EU: European Union; CAS no: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.
(a): Present in the additive as a mixture of enantiomers (*d,l*-fenchone), the ratio between *d*- and *l*-stereoisomers not given.
(b): Substance which shall not be added as such to food (Annex III), maximum level in food is set by Regulation (EC) No 1334/2008, including dairy products (50 mg/kg), processed fruits, vegetables (incl. mushrooms, fungi, roots, tubers, pulses and legumes), nuts and seeds (50 mg/kg), fish products (50 mg/kg) and non-alcoholic beverages (10 mg/kg).
(c): Present in the additive as a mixture of enantiomers (*d,l*-camphor), the ratio between *d*- and *l*-stereoisomers not given.
(d): The values given for the Total are the lowest and the highest values of the sum of the components in the five batches analysed.

3.3.1.1. Substances of concern

The applicant performed a literature search regarding substances of concern and chemical composition of the plant species of *F. vulgare* var. *dulce* and its preparations.[24] The presence of estragole (1.5–8.1%) in the essential oil of the fruit of *F. vulgare* var. *dulce* is reported in the EFSA Compendium of botanicals as a substance of concern (EFSA, 2012).[25] Besides estragole, anethole (methoxy-4-(prop-1(*trans*)-enyl) benzene [04.088]) has been also identified. *trans*-Anethole [04.010], which is known to be prevalent over *cis*-anethole in the fruit of *F. vulgare*,[46] is an authorised feed flavouring previously assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2011).

3.3.1.2. 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-phosphorous pesticides, aflatoxins (B1, B2, G1, G2) and ochratoxin A. However, no data were provided on the presence of these impurities. Since sweet fennel 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.

3.3.1.3. Shelf-life

The typical shelf-life of the additive is stated to be at least 12 months, when stored in tightly closed containers under standard conditions (in a cool, dry place protected from light).[27] No separate or additional stability studies for flavouring additives were performed.

[46] Anethole [04.088] is defined as a mixture of *trans*- and *cis*-anethole (isomeric ratio not specified). Sweet fennel oil is described to contain 79.8–83.1% of *trans*-anethole and no *cis*-anethole, bitter fennel oil is described to contain 55–75% of *trans*-anethole and < 0.5% of *cis*-anethole (EMA, 2008).

3.3.1.4. Conditions of use

Sweet fennel oil is intended to be added to feed for dogs and cats at the maximum proposed use level in complete feed of 2.3 and 1.9 mg/kg complete feed, respectively. No use level has been proposed by the applicant for the use in water for drinking.

3.3.2. Safety

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

Many of the major volatile components, accounting for about 94% of the GC area, have been previously assessed and considered safe for use as flavourings, and are currently authorised for food[8] and feed[9] uses. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Additional considerations on the volatile components not assessed by EFSA have been addressed in Section 3.2.2.

In addition to the compounds already considered in bitter fennel oil, two components not previously assessed for use as flavourings were identified in sweet fennel oil: *(Z)*-α-bergamotene and *(E)*-α-bergamotene, two compounds belonging to CG 31, which are expected to be rapidly absorbed from the gastrointestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2015, 2016b).

The ADME and the genotoxicity and carcinogenicity of estragole have been already addressed in Sections 3.2.2.1 and 3.2.2.2. The outcome of the literature search on genotoxicity of sweet fennel oil is reported in the next paragraph.

3.3.2.1. Genotoxicity studies with sweet fennel oils

The applicant provided a literature search on the genotoxicity of essential oils obtained from *F. vulgare*.[47] Only one study was retrieved in which fennel fruit essential oil obtained from sweet fennel was tested *in vitro* applying the Comet assay and the micronucleus test in human hepatoma (HepG2) cell line (Levorato et al., 2018). The test item, characterised by GC–MS analysis, presented a composition similar to the fennel oil sweet under assessment. No induction of DNA strand breaks and no increase in the frequency of micronuclei were observed after short and continuous treatments, while a concentration-related and statistically significant increase of apoptosis was induced. Cell cycle perturbation was also observed, consistent with cytotoxic effects at high concentrations (above 0.5 uL/mL).

Additional genotoxicity studies on (sweet) fennel oil preparations provided by the applicant were retrieved in WHO and EMA monographs/assessment reports. However, in all the studies submitted (Morimoto et al., 1982; Yamamoto et al., 1982; Ishidate Jr et al., 1984; Mahmoud et al., 1992) major shortcomings were identified (e.g. unknown composition of the test item; difficulties in retrieving results). Therefore, none of the recovered articles was further considered for the assessment of the genotoxicity of sweet fennel oil.

3.3.3. Safety for the target species

Tolerance studies and/or toxicological studies 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% 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. Fenchone, which is structurally related to camphor (included in tolerance studies with a mixture of flavourings, 'Herbal mixture', see Sections 3.2.2 and 3.2.3), and estragole, a substance for which a concern for genotoxicity has been identified, are assessed separately.

Components other than fenchone and estragole

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 3.2.3.

[47] Technical dossier/Supplementary information September 2021/Literature search_fennel_oil and Additional references.

For those compounds[48] for which NOAEL values derived from toxicity studies were not available and read-across was not possible, the TTC approach was applied (EFSA FEEDAP Panel, 2017b; EFSA Scientific Committee, 2019a).

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.[49]

The approach to the safety assessment of sweet fennel oil for dogs is summarised in Table 12.

Table 12: Compositional data, intake values, reference points and margin of exposure (MOE) for the individual components of sweet fennel oil for dogs 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**
Constituent	–	%	mg/kg	mg/kg bw per day	–	mg/kg bw per day	–
CG 18							
trans-Anethole	04.010	79.16	1.821	0.0345	(I)	**300**	8,700
CG 21							
4-Methoxyphenylacetone	07.087	0.35	0.017	0.0003	I	*3*	19,904

(a): Intake calculations for the individual components are based on the use level of 2.3 mg/kg in feed for dog. 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.

As shown in Table 12, the MOE was > 100 for all the assessment groups.

For cats, the corresponding calculations are shown in Table 13.[49]

Table 13: Compositional data, intake values, reference points and margin of exposure (MOE) for the individual components of sweet fennel oil for cats 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**
Constituent	–	%	mg/kg	mg/kg bw per day	–	mg/kg bw per day	–
CG 18							
trans-Anethole	04.010	79.16	1.504	0.0342	(I)	**300**	8,776
CG 21							
4-Methoxyphenylacetone	07.087	0.35	0.007	0.0001	I	*3*	20,079

[48] 4-methoxyphenylacetone [07.087], (*Z*)-α-bergamotene, (*E*)-α-bergamotene, and germacra-1(10),4(14),5-triene [01.042].

[49] Compounds included in the assessment groups but not reported in the table: 4-terpinenol [02.072] (CG 6); camphor and *trans*-sabinene hydrate (CG 8); 1,8-cineole [03.001] (CG 16); *cis*-anethole (CG 18); anisaldehyde (CG 23); myrcene [01.008] and *cis*-3,7-dimethyl-1,3,6-octatriene [01.064] (CG 31, II); limonene [01.001], α-phellandrene [01.006], β-phellandrene [01.055], γ-terpinene [01.020], terpinolene [01.005] and α-terpinene [01.019] (CG 31, III); p-cymene [01.002] (CG 31, IVe); α-pinene [01.004], β-pinene [01.003], camphene [01.009], sabinene [01.059], β-caryophyllene [01.007], (*E*)-α-bergamotene, (*Z*)-α-bergamotene, α-thujene and α-fenchene, (CG 31, V); germacra-1(10),4(14),5-triene [01.042] (CG 31, VI).

(a): Intake calculations for the individual components are based on the use level of 1.9 mg/kg in feed for cat. 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.

Generally, for cats, a MOET > 500 is considered adequate, knowing their unusually low capacity for glucuronidation of compounds (Court and Greenblatt, 1997; Lautz et al., 2021). As shown in Table 13, the MOET was > 500 for all the assessment groups.

Therefore, for dogs and cats, no safety concern (without considering the presence of fenchone and estragole) was identified for sweet fennel oil, when used as a feed additive at the proposed use levels.

No specific proposals have been made by the applicant for the use level in water for drinking. The FEEDAP Panel considers that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed (EFSA FEEDAP Panel, 2010).

Fenchone

Based on the outcome of tolerance trials in chickens for fattening, piglets, cattle for fattening and salmons with *d*-camphor and by applying read-across, the FEEDAP Panel concluded that a concentration of fenchone in feed of 5 mg/kg complete feed is considered safe for all animal species (EFSA FEEDAP Panel, 2023b). Therefore, the levels of fenchone of 0.125 and 0.103 mg/kg complete feed resulting from the use of sweet fennel oil at the maximum proposed use level of 2.3 and 1.9 mg/kg complete feed for dogs and cats are considered safe.

Estragole

Estragole (4.11–4.96%) was detected in all batches of the oil under assessment (see Section 3.3.1). The FEEDAP Panel notes that sweet fennel oil with higher concentrations of estragole may occasionally reach the market. In particular, the applicant set a specification for estragole up to 6.0%.

Thus, for the assessment of estragole for the target species the FEEDAP Panel considered two possible scenarios:

a) Sweet fennel oil with a content of estragole corresponding to the highest specification of 6.0%

The highest daily intake of estragole was calculated considering the maximum proposed use level of the additive in feed for dogs and cats and a content of estragole corresponding to the highest specification of 6.0%. The assessment of the exposure to estragole for dogs and cats is reported in Table 14.

Table 14: Estragole intake of dogs and cats calculated at the maximum proposed use level of the additive in feed for an essential oil with a content of estragole corresponding to the highest specification of 6.0% and margin of exposure (MOE) calculated based on $BMDL_{10}$ of 22.2 mg/kg bw per day derived from rodent carcinogenicity studies with methyleugenol

Animal category Long-living and reproductive animals	**Daily feed intake**	**Body weight**	**Use level in feed**	**Estragole intake[(a)]**	**MOE[(b)]**
	kg DM	**kg**	**mg/kg feed[(c)]**	**µg/kg bw per day**	
Dog	0.25	15	2.2	2.6	8,494
Cat	0.06	3	1.9	2.6	8,568

DM: dry matter.
(a): The intake value of estragole is calculated considering the highest specification (6.0%).
(b): The MOE for dogs and cats is calculated as the ratio of the reference point ($BMDL_{10}$ of 22.2 mg/kg bw per day) to the intake.
(c): Complete feed containing 88% DM, milk replacer 94.5% DM.

When the estimated estragole exposures of dogs and cats are compared to the $BMDL_{10}$ of 22.2 mg methyleugenol/kg bw per day (Suparmi et al., 2019), a MOET < 10,000 which is considered of concern, is obtained at the proposed use levels in feed (Table 13).

b) Sweet fennel oil with a content of estragole, corresponding to the highest analysed concentration (4.96% rounded to 5.0%)

The assessment of the exposure to estragole for dogs and cats is reported in Table 15.

Table 15: Estragole intake of dogs and cats calculated at the maximum proposed use level of the additive in feed for an essential oil with a content of estragole, corresponding to the highest analysed concentration (5.0%) and margin of exposure (MOE) calculated based on $BMDL_{10}$ of 22.2 mg/kg bw per day derived from rodent carcinogenicity studies with methyleugenol

Animal category Long-living and reproductive animals	Daily feed intake	Body weight	Use levelin feed	Estragole intake[a]	MOE[b]
	kg DM	kg	mg/kg feed[c]	µg/kg bw per day	
Dog	0.25	15	2.2	2.2	10,193
Cat	0.06	3	1.9	2.1	10,282

DM: dry matter.
(a): The intake value of estragole is calculated considering the highest analysed value in the additive (4.96%).
(b): The MOE for dogs and cats is calculated as the ratio of the reference point ($BMDL_{10}$ of 22.2 mg/kg bw per day) to the intake.
(c): Complete feed containing 88% DM, milk replacer 94.5% DM.

When the estimated estragole exposures of dogs and cats are compared to the $BMDL_{10}$ of 22.2 mg methyleugenol/kg bw per day (Suparmi et al., 2019), a MOE > 10,000 which is considered of low concern, is obtained at the proposed use levels in feed (Table 15).

3.3.3.1. Conclusions on the safety for the target species

Based on the MOE calculated considering the presence of estragole in sweet fennel oil and the conditions of use in the different species, the FEEDAP Panel concludes that:

a) *For sweet fennel oil with a content of estragole at the highest proposed specification of 6.0%*

- The use of the additive is considered of concern (MOE < 10,000) at the proposed levels in complete feed of 2.2 mg/kg for dogs and 1.9 mg/kg for cats.

b) *For sweet fennel oil with a content of estragole, corresponding to the highest analysed concentration (5.0%)*

- The use of the additive is considered of low concern (MOE > 10,000) at the proposed use levels in complete feed of 2.2 mg/kg for dogs and 1.9 mg/kg for cats.

3.3.4. 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 for sweet fennel oil,[50] where hazards for users have been identified.

The additive contains anisaldehyde and *trans*-anethole, two compounds for which hazards for skin and eye contact and respiratory exposure were recognised (EFSA FEEDAP Panel, 2011, 2012d).

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

Due to the high level of estragole (> 1%), the applicant also proposes to classify the additive according to Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP Regulation)[39] as suspected of causing genetic defects (category 2 mutagen) and of causing cancer (category 2 carcinogen).

[50] Technical dossier/Supplementary information September 2021/Annex_IX.

For preparations with these classifications, precautionary statements as indicated in Regulation (EC) No 1272/2008 have to be followed, and the additive should be handled accordingly.[51]

3.3.5. Safety for the environment

No environmental risk assessment is necessary for the use of essential oils in dogs and cats (EFSA FEEDAP Panel, 2019).

3.4. Efficacy

Bitter and sweet fennel oils are listed in Fenaroli's Handbook of Flavor Ingredients (Burdock, 2009).[52]

Since bitter and sweet fennel fruit and its preparations are recognised to flavour food and their function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

4. Conclusions

Fennel oils from *F. vulgare* ssp. may be produced from plants of different geographical origins and by various processes resulting in preparations with different composition and toxicological profiles, particularly concerning the presence of estragole.

Bitter fennel oil

Based on the MOE calculated considering the presence of estragole in bitter fennel oil and the conditions of use in the different species, the FEEDAP Panel concludes that:

a) *For bitter fennel oil with a content of estragole at the highest proposed specification of 7.0%*
   - For long-living and reproductive animals (including those animals reared for laying/ breeding/reproduction) the use of the additive is considered of concern (MOE < 10,000) at the proposed level in complete feed.
   - For short-living animals, the Panel has no safety concern when the additive is used at the maximum proposed use level in complete feed of 25 mg/kg for pigs for fattening, piglets and other Suidae species for meat production, veal calves (milk replacer), cattle for fattening and other ruminants for fattening and camelids at the same physiological stage, horses and other Equidae for meat production and rabbits for meat production, salmonids and minor fin fish. For turkeys for fattening, chickens for fattening and minor poultry for fattening, the respective proposed use levels are considered of concern.

b) *For bitter fennel oil with a content of estragole corresponding to the highest analysed concentration (6.1% estragole)*
   - For long-living and reproductive animals (including those animals reared for laying/ breeding/reproduction) the use of the additive is considered of low concern (MOE > 10,000) at the proposed use level in complete feed of 0.6 mg/kg for laying hens and other laying/reproductive birds including animals reared for laying/reproduction and ornamental birds and rabbits, 1.0 mg/kg for sows and other Suidae species for reproduction including animals reared for reproduction, and dairy cows and other ruminants and camelids for milk production and reproduction including animals reared for milk production/reproduction, 1.5 mg/kg for sheep/goats, horses and other Equidae and cats, 1.9 mg/kg for dogs and 7.1 mg/kg for ornamental fish.
   - For short-living animals, the Panel has no safety concern when the additive is used at the maximum proposed use level in complete feed of 25 mg/kg for pigs for fattening, piglets and other Suidae species for meat production, veal calves (milk replacer), cattle for fattening and other ruminants for fattening and camelids at the same physiological stage, horses and other Equidae for meat production and rabbits for meat production,

[51] Directive 2004/37/EC of the European Parliament and of the Council of 29 April 2004 on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (Sixth individual Directive within the meaning of Article 16(1) of Council Directive 89/391/EEC). OJ L 158, 30.4.2004, p. 50.

[52] The Flavour and Extract Manufactures Association (FEMA) reference numbers 2042 and 2483 are associated to fennel, sweet and sweet fennel oil. However, certain literature does not distinguish between bitter and sweet fennel oil (Burdock, 2009).

salmonids and minor fin fish, 24.3 mg/kg for turkeys for fattening and 18.2 mg/kg chickens for fattening and minor poultry for fattening.
- For any other species, the additive is considered of low/no concern at 0.6 mg/kg complete feed.
- The conclusions of the FEEDAP Panel are summarised as following:

Animal category	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	0.6
Sows and other Suidae species for reproduction including animals reared for reproduction	1.0
Dairy cows and other ruminants (except for sheep/goat) and camelids for milk production and reproduction including animals reared for milk production/reproduction	1.0
Sheep/goat	1.5
Horses and other Equidae	1.5
Rabbits	0.6
Dogs	1.9
Cats	1.5
Ornamental fish	7.1
Short-living animals (species for fattening)[(b)]	
Chickens for fattening and minor poultry for fattening	18.2
Turkeys for fattening	24.3
Pigs for fattening	25.0
Piglets and other Suidae species for meat production	25.0
Veal calves (milk replacer)	25.0
Cattle for fattening and other ruminants for fattening (except for sheep/goat) and camelids at the same physiological stage	25.0
Sheep/goat for meat production	25.0
Horses and other Equidae for meat production	25.0
Rabbit for meat production	25.0
Salmonids and minor fin fish	25.0
Any other species	0.6

(a): Based on a MOE > 10,000 for long-living and reproductive animals, calculated as the ratio of the reference point ($BMDL_{10}$ of 22.2 mg/kg bw per day) to the intake.
(b): Based on a MOE > 100 for target species for fattening, calculated as the ratio of the reference point (no observed adverse effect level (NOAEL) of 10 mg/kg bw per day) to the 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 concern/no concern when consumed via feed alone.

No concern would be expected for the consumer from the use of bitter fennel oil up to the highest use level in feed which is considered of no or low concern for target animals.

Bitter fennel oil under assessment should be considered as irritant to skin and eyes and the respiratory tract and as a dermal and respiratory sensitiser. Due to the high concentration of estragole (> 1%) fennel oils are classified as suspected of causing genetic defects and of causing cancer and should be handled accordingly.

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

Since the fruit of *F. vulgare* Mill. ssp. v*ulgare* var. *vulgare* and its preparations are recognised to flavour food and its function in feed would be essentially the same, no further demonstration of efficacy is considered necessary for bitter fennel oil.

Sweet fennel oil

Based on the MOE calculated considering the presence of estragole in sweet fennel oil and the conditions of use in cats and dogs, the FEEDAP Panel concludes that:

a) *For sweet fennel oil with a content of estragole at the highest proposed specification of 6.0%*
   - The use of the additive is considered of concern (MOE < 10,000) at the proposed levels in complete feed of 2.2 mg/kg for dogs and 1.9 mg/kg for cats 1.9 mg/kg.

b) *For sweet fennel oil with a content of estragole, corresponding to the highest analysed concentration (5.0%)*
   - The use of the additive is considered of low concern (MOE > 10,000) at the proposed use levels in complete feed of 2.2 mg/kg for dogs and 1.9 mg/kg for cats.

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 concern/no concern when consumed via feed alone.

Sweet fennel oil under assessment should be considered as irritant to skin and eyes and the respiratory tract and as a dermal and respiratory sensitiser. Due to the high concentration of estragole (> 1%) fennel oils are classified as suspected of causing genetic defects and of causing cancer and should be handled accordingly.

Since the fruit of *F. vulgare* Mill. ssp. *vulgare* var. *dulce* (Mill.) Batt. & Trab. and its preparations are recognised to flavour food and its function in feed would be essentially the same, no further demonstration of efficacy is considered necessary for sweet fennel oil.

5. Recommendations

Although the FEEDAP Panel is aware that bitter fennel oil with estragole of 7.0% or sweet fennel oil with estragole of 6.0% according to specification could be present on the market, the analytical data provided by the applicant demonstrate that fennel oils with reduced contents of this genotoxic and carcinogenic substance can be produced.

In line with the principles of the General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic when used as feed additives (EFSA FEEDAP Panel, 2021), that 'manufacturing processes of botanical feed additives should avoid selective extraction and enrichment of genotoxic and/or carcinogenic substances and should aim at the reduction of these substances' the FEEDAP Panel recommends that fennel oils intended to be used as feed additive should contain the lowest possible concentrations of estragole and in any case not more than 6.1% and 5.0% in bitter fennel oil and sweet fennel oil, respectively.

6. Documentation provided to EFSA/Chronology

Date	Event
28/10/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 02 – Apiales and Austrobaileyales 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
26/02/2013	EFSA informed the applicant (EFSA ref. 7150727) that, in view of the workload, the evaluation of applications on feed flavourings would be re-organised by giving priority to the assessment of the chemically defined feed flavourings, as agreed with the European Commission
24/06/2015	Technical hearing during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products": data requirement for the risk assessment of botanicals
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract, and opoponax oil
24/06/2019	Application validated by EFSA – Start of the scientific assessment
03/07/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization, safety for the target species, safety for the consumer, safety for the user, safety for the environment*

Date	Event
30/09/2019	Comments received from Member States
07/09/2021	Reception of supplementary information from the applicant (partial dataset on fennel oil) – Scientific assessment remains suspended
31/10/2022	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives – partial report related to nine additives (*dill herb oil, dill tincture, dong quai tincture, cumin oil, fennel tincture, parsley tincture, anise tincture, star anise tincture and ferula assa-foetida oil*)
16/12/2022	Reception of an addendum of the Evaluation report of the European Union Reference Laboratory for Feed Additives – final report related to 11 additives (*celery seed oil, caraway seed oil, coriander oil, taiga root tincture, fennel oil, common ivy extract (sb), ginseng tincture, anise oil, anise star oil, anise star terpenes and omicha tincture*)
10/03/2023	Reception of supplementary information from the applicant (partial dataset on fennel oil) – Scientific assessment remains suspended
07/09/2023	The application was split and a new EFSA-Q-2023-00587 was assigned to the preparations included in the present assessment
07/09/2023	Scientific assessment re-started
28/09/2023	Opinion adopted by the FEEDAP Panel on fennel oils (EFSA-Q-2023-00587). End of the Scientific assessment for the preparations included in the present assessment. The assessment of other preparations belonging to BDG 02 is still ongoing

References

Anthony A, Caldwell J, Hutt AJ and Smith RL, 1987. Metabolism of estragole in rat and mouse and influence of dose size on excretion of the proximate carcinogen 1′-hydroxyestragole. Food and Chemical Toxicology, 25, 799–806. https://doi.org/10.1016/0278-6915(87)90257-2

Api AM, Belsito D, Botelho D, Bruze M, Burton GA Jr, Buschmann J, Dagli ML, Date M, Dekant W, Deodhar C, Francis M, Fryer AD, Jones L, Joshi K, La Cava S, Lapczynski A, Liebler DC, O'Brien D, Patel A, Penning TM, Ritacco G, Romine J, Sadekar N, Salvito D, Schultz TW, Sipes IG, Sullivan G, Thakkar Y, Tokura Y and Tsang S, 2018. RIFM fragrance ingredient safety assessment, Fenchone, CAS Registry Number 1195-79-5. Food and Chemical Toxicology, 112, S209–S217. https://doi.org/10.1016/j.fct.2018.08.052

Burdock GA, 2009. Fenaroli's handbook of flavor ingredients. 6th Edition. CRC Press. Taylor & Francis Group. Boca Raton, FL, pp. 692–694. https://doi.org/10.1201/9781439847503

Court MH and Greenblatt DJ, 1997. Molecular basis for deficient acetaminophen glucuronidation in cats. An Interspecies Comparison of Enzyme Kinetics in Liver Microsomes. Biochemical Pharmacology, 53, 1041–1047. https://doi.org/10.1016/s0006-2952(97)00072-5

Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard–a decision tree approach. Food and Cosmetics Toxicology, 16, 255–276. https://doi.org/10.1016/s0015-6264(76)80522-6

EC (European Commission), 2001. Opinion of the Scientific Committee on Food on Estragole (1-allyl-4-methoxybenzene) 2001. 583 SCF/CS/FLAV/FLAVOUR/6 ADD 2 Final. Available online: http://ec.europa.eu/food/fs/sc/scf/out104_en.pd [Accessed: 26 September 2001].

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA Journal 2005;3(10):282, 31 pp. https://doi.org/10.2903/j.efsa.2005.282

EFSA (European Food Safety Authority), 2008. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from Commission on Flavouring Group Evaluation 69, (FGE.69) Aromatic substituted secondary alcohols, ketones and related esters. EFSA Journal 2008;869, 35 pp. https://doi.org/10.2903/j.efsa.2008.896

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

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2010. Guidance on the data required for the risk assessment of flavourings to be used in or on foods. EFSA Journal 2010;8(6):1623, 38 pp. https://doi.org/10.2093/j.efsa.2010.1623

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

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

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

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Gundert-Remy U, Husøy T, Moldeus P, Oskarsson A, Shah R, Waalkens-Berendsen I, Wölfle D, Benigni R, Bolognesi C, Chipman K, Cordelli E, Degen G, Marzin D, Svendsen C, Carfì M, Kovalkovicova N, Martino C, Vianello G and Mennes W, 2019. Scientific Opinion on Flavouring Group Evaluation 208 Revision 3 (FGE.208Rev3): consideration of genotoxicity data on alicyclic aldehydes with a,b-unsaturation in ring/ side-chain and precursors from chemical subgroup 2.2 of FGE.19. EFSA Journal 2019;17(1):5569, 50 pp. https://doi.org/10.2903/j.efsa.2019.5569

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

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

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

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Scientific opinion on the safety and efficacy of aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers (chemical group 6) when used as flavourings for all animal species. EFSA Journal 2012;10(11):2966, 25 pp. https://doi.org/10.2903/j.efsa.2012.2966

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

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

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

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

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

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

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

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

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

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

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

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

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

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Kouba M, Fašmon Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brantom P, Chesson A, Westendorf J, Galobart J, Manini P, Pizzo F and Dusemund B, 2021a. Scientific Opinion on the safety and efficacy of feed additives consisting of expressed lemon oil and its fractions from *Citrus limon* (L.) Osbeck and of lime oil from *Citrus aurantiifolia* (Christm.) Swingle for use in all animal species. EFSA Journal 2021;19(4):6548, 55 pp. https://doi.org/10.2903/j.efsa.2021.6548

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

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Fašmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brantom P, Chesson A, Westendorf J, Manini P, Pizzo F and Dusemund B, 2022. Scientific Opinion on the safety and efficacy of a feed additive consisting of an extract of olibanum from *Boswellia serrata* Roxb. ex Colebr. for use in dogs and horses (FEFANA asbl). EFSA Journal 2022;20(3):7158, 24 pp. https://doi.org/10.2903/j.efsa.2022.7158

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brantom P, Chesson A, Schlatter J, Schrenk D, Westendorf J, Manini P, Pizzo F and Dusemund B, 2023a. Scientific Opinion on the safety and efficacy of a feed additive consisting of an essential oil from the leaves of *Laurus nobilis* L. (laurel leaf oil) for all animal species (FEFANA asbl). EFSA Journal 2023;21(3):7875, 28 pp. https://doi.org/10.2903/j.efsa.2023.7875

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Villa RE, Woutersen R, Brantom P, Chesson A, Dierick N, Martelli G, Westendorf J, Ortuño Casanova J, Dirven Y, Firmino JP and Manini P, 2023b. Safety of 41 flavouring compounds providing an Herbal flavour and belonging to different chemical groups for use as feed additives in all animal species (FEFANA asbl). EFSA Journal 2023;21(X):8340, 25 pp. https://doi.org/10.2903/j.efsa.2023.8340

EFSA Scientific Committee, 2009. Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements, on request of EFSA. EFSA Journal 2009; 7(9):1249, 19 pp. https://doi.org/10.2093/j.efsa.2009.1249

EFSA Scientific Committee, More SJ, Hardy A, Bampidis V, Benford D, Bennekou SH, Bragard C, Boesten J, Halldorsson TI, Hernandez-Jerez AF, Jeger MJ, Knutsen HK, Koutsoumanis KP, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Nielsen SS, Schrenk D, Solecki R, Turck D, Younes M, Benfenati E, Castle L, Cedergreen N, Laskowski R, Leblanc JC, Kortenkamp A, Ragas A, Posthuma L, Svendsen C, Testai E, Dujardin B, Kass GEN, Manini P, Zare Jeddi M, Dorne J-LCM and Hogstrand C, 2019a. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal 2019;17(3):5634, 77 pp. https://doi.org/10.2903/j.efsa.2019.5634

EFSA Scientific Committee, More S, Bampidis V, Benford D, Boesten J, Bragard C, Halldorsson T, Hernandez-Jerez A, Hougaard-Bennekou S, Koutsoumanis K, Naegeli H, Nielsen SS, Schrenk D, Silano V, Turck D, Younes M, Aquilina G, Crebelli R, Gürtler R, Hirsch-Ernst KI, Mosesso P, Nielsen E, Solecki R, Carfi M, Martino C, Maurici D, Parra Morte J and Schlatter J, 2019b. Statement on the genotoxicity assessment of chemical mixtures. EFSA Journal 2019;17(1):5519, 11 pp. https://doi.org/10.2903/j.efsa.2019.5519

EFSA Scientific Committee, More SJ, Bampidis V, Benford D, Bragard C, Halldorsson TI, Hernandez-Jerez AF, Hougaard BS, Koutsoumanis KP, Machera K, Naegeli H, Nielsen SS, Schlatter JR, Schrenk D, Silano V, Turck D, Younes M, Gundert-Remy U, Kass GEN, Kleiner J, Rossi AM, Serafimova R, Reilly L and Wallace HM, 2019c. Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. EFSA Journal 2019;17(6):5708, 17 pp. https://doi.org/10.2903/j.efsa.2019.5708

EMA (European Medicines Agency), 2005. Committee on Herbal Medicinal Products (HMPC). Public statement on the use of herbal medicinal products containing estragole. EMEA/HMPC/137212/2005. Available online: https://www.ema.europa.eu/en/documents/scientific-guideline/public-statement-use-herbal-medicinal-products-containing-estragole_en.pdf

EMA (European Medicines Agency), 2007a. Committee on Herbal Medicinal Products (HMPC). Community herbal monograph on *Foeniculum vulgare* Miller subsp. *vulgare* var. *dulce* (Miller) Thellung, fructus. EMEA/HMPC/263293/2007. Available online: https://www.ema.europa.eu/en/documents/herbal-monograph/final-community-herbal-monograph-foeniculum-vulgare-miller-subsp-vulgare-var-dulce-miller-thellung_en.pdf

EMA (European Medicines Agency), 2007b. Opinion of the Committee on Herbal Medicinal Products (HMPC) on a Community herbal monograph on *Foeniculum vulgare* Miller subsp. *vulgare* var. *dulce* (Miller) Thellung, fructus. EMEA/HMPC/280074/2007, EMEA/HMPC/M/H/0012. Available online: https://www.ema.europa.eu/en/documents/herbal-opinion/opinion-committee-herbal-medicinal-products-community-herbal-monograph-foeniculum-vulgare-miller_en.pdf

EMA (European Medicines Agency), 2008. Committee on Herbal Medicinal Products (HMPC). Assessment report on *Foeniculum vulgare* Miller. EMEA/HMPC/137426/2006. Available online: https://www.ema.europa.eu/en/documents/herbal-report/assessment-report-foeniculum-vulgare-miller_en.pdf

EMA (European Medicines Agency), 2021. Committee on Herbal Medicinal Products (HMPC). Public statement on the use of herbal medicinal products containing estragole. EMA/HMPC/137212/2005 Rev 1. Available online: https://www.ema.europa.eu/en/documents/other/second-draft-revision-1-public-statement-use-herbal-medicinal-products-containing-estragole_en.pdf

Ishidate M Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M and Matsuoka A, 1984. Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology, 22, 623–636. https://doi.org/10.1016/0278-6915(84)90271-0

Lautz LS, Jeddi MZ, Girolami F, Nebbia C and Dorne JLCM, 2021. Metabolism and pharmacokinetics of pharmaceuticals in cats (Felix sylvestris catus) and implications for the risk assessment of feed additives and contaminants. Toxicology Letters, 338, 114–127. https://doi.org/10.1016/j.toxlet.2020.11.014

Levorato S, Dominici L, Fatigoni C, Zadra C, Pagiotti R, Moretti M and Villarini M, 2018. In vitro toxicity evaluation of estragole-containing preparations derived from Foeniculum vulgare Mill. (fennel) on HepG2 cells. Food and Chemical Toxicology, 111, 616–622. https://doi.org/10.1016/j.fct.2017.12.014

Mahmoud I, Alkofahi A and Abdelaziz A, 1992. Mutagenic and toxic activities of several spices and some Jordanian medicinal plants. International Journal of Pharmacognosy, 30, 81–85. https://doi.org/10.3109/13880209209053961

Morimoto I, Watanabe F, Osawa T, Okitsu T and Kada T, 1982. Mutagenicity screening of crude drugs with Bacillus subtilis rec-assay and Salmonella/microsome reversion assay. Mutation Research/Environmental Mutagenesis and Related Subjects, 97, 81–102. https://doi.org/10.1016/0165-1161(82)90007-3

Munro IC, Ford RA, Kennepohl E and Sprenger JG, 1996. Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern. Food and Chemical Toxicology, 34, 829–867. https://doi.org/10.1016/s0278-6915(96)00049-x

NTP (National Toxicology Program), 2000. NTP Technical Report on the Toxicology and carcinogenesis studies of methyleugenol (CAS NO. 93-15-2) in F344/N rats and B6C3F1 mice (gavage study). NTP, Technical Report Series, 491, 1–420. Available online: https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr491.pdf

PhEur (European Pharmacopoeia), 2022a. "Fennel, sweet" (*Foeniculi dulcis fructus*). European Pharmacopoeia, 11th Edition. Monograph 04/2011:0825. European Directorate for the Quality of Medicines and Health.

PhEur (European Pharmacopoeia), 2022b. "Fennel, bitter" (*Foeniculi amari fructus*). European Pharmacopoeia, 11th Edition. Monograph 04/2013:0824. European Directorate for the Quality of Medicines and Health.

PhEur (European Pharmacopoeia), 2022c. "Bitter-fennel fruit oil" (*Foeniculi amari fructus aetheroleum*). European Pharmacopoeia, 11th Edition. Monograph 01/2008:1826. European Directorate for the Quality of Medicines and Health.

PhEur (European Pharmacopoeia), 2022d. "Bitter-fennel berb oil" (*Foeniculi amari herbae aetheroleum*). European Pharmacopoeia, 11th Edition. Monograph 07/2009:2380. European Directorate for the Quality of Medicines and Health.

Sangster SA, Caldwell AJ, Hutt A, Anthony A and Smith RL, 1987. The metabolic disposition of [methoxy-^{14}C]-labelled *trans*-anethole, estragole and p-propylanisole in human volunteers. Xenobiotica, 17, 1223–1232. https://doi.org/10.3109/00498258709167414

Suparmi S, Ginting AJ, Mariyam S, Wesseling S and Rietjens IMCM, 2019. Levels onogof methyleugenol and eugenol in instant herbal beverages available on the Indonesian market and related risk assessment. Food and Chemical Toxicology, 125, 467–478. https://doi.org/10.1016/j.fct.2019.02.001

Yamamoto H, Mizutani T, Nomura H, 1982. [Studies on the mutagenicity of crude drug extracts. I]. Yakugaku Zasshi, 102, 596–601. Japanese. https://doi.org/10.1248/yakushi1947.102.6_596

Zangouras A, Caldwell J, Hutt AJ and Smith RL, 1981. Dose-dependent conversion of estragole in the rat and mouse to the carcinogenic metabolite 1′-hydroxyestragole. Biochemical Pharmacology, 30, 1383–1386. https://doi.org/10.1016/0006-2952(81)90329-4

Abbreviations

AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
BDG	botanically defined group
BMD	benchmark dose
$BMDL_{10}$	Benchmark dose (BMD) lower confidence limit for a benchmark response of 10%
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CDG	Chemically Defined Group
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	Chemical Group
CoE	Council of Europe
DM	dry matter
EEIG	European economic interest grouping
EINECS	European Inventory of Existing Chemical Substances
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
FL-No	FLAVIS number
GC	Gas Chromatography
GC-FID	Gas Chromatography with Flame Ionisation Detector
GC–MS	Gas Chromatography–Mass Spectrometry
ISO	International Standard Organization
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
NTP	National Toxicology Program
QSAR	quantitative structure–activity relationship
SC	EFSA Scientific Committee
TTC	threshold of toxicological concern