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Safety and efficacy of a feed additive consisting of an essential oil derived from the fruit of *Illicium verum* Hook.f. (star anise oil) for use in all animal species (FEFANA asbl)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of star anise oil from the fruit (without or with the presence of plant leaves) of *Illicium verum* Hook.f., when used as a sensory additive in feed and water for drinking for all animal species. 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 the additive in complete feed at 0.6 mg/kg for laying hens and rabbits, 1.0 mg/kg for sows and dairy cows, 1.6 mg/kg for sheep/goats, horses and cats, 1.9 mg/kg for dogs and 6.5 mg/kg for ornamental fish. For short-living animals, the Panel had no safety concern when the additive is used at 83.3 mg/kg for veal calves, 73.3 mg/kg for sheep/goats, cattle for fattening and horses for meat production, 83.8 mg/kg for salmonids, 24.8 mg/kg for turkeys for fattening, 18.5 mg/kg chickens for fattening, 33.3 mg/kg for piglets, 40 mg/kg for pigs for fattening and 29.3 mg/kg for rabbits for meat production. These conclusions were extrapolated to other physiologically related species. For any other species, the additive was considered of low concern at 0.6 mg/kg. The use of star anise oil in animal feed is expected to be of no concern for consumers and for the environment. The additive under assessment should be considered as an irritant to skin and eyes, and as a dermal and respiratory sensitiser. Due to the high concentration of estragole (≥ 1%), the additive is classified as suspected of causing genetic defects and of causing cancer and should be handled accordingly. Since the fruit of *I. verum* and its preparations are recognised to flavour food and their function in feed would be the same, no further demonstration of efficacy was necessary.

Keywords: sensory additives, flavouring compounds, *Illicium verum* Hook.f., star anise oil, estragole

Requestor: European Commission

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1. Introduction

1.1. Background and Terms of Reference

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

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation/re-evaluation of 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]). These preparations were deleted from the register of feed additives.[6] During the course of the assessment, this application was split and the present opinion covers one out of the 20 preparations under application: an essential oil derived from the fruit (or seeds) of *Illicium verum* Hook.f. (star anise oil) for all animal species.

The remaining 19 preparations belonging to botanically defined group (BDG) 02 – *Apiales/ 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 24 June 2019.

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 products star anise oil and star anise terpenes (*I. verum*), when used under the proposed conditions of use (see Sections 3.2.1.4).

1.2. Additional information

Star anise oil from *Illicium verum* Hook.f. is currently authorised as a feed additive according to the entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined) and foreseen for re-evaluation. It has not been assessed as a feed additive in the EU.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] On 13/03/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1050 Brussels, Belgium.
[3] On 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 parsey 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] Register of feed additives, Annex II, withdrawn by OJ L162, 10.05.2021, p. 5.

There is no specific EU authorisation for any *I. verum* 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'.

'Star anise (Anisi stellati fructus)' is described in a monograph of the European Pharmacopoeia 11.0 (PhEur, 2022a). It is defined as the dried composite fruit of *Illicium verum* Hook.f. with a minimum content of 70 mL/kg of essential oil in the anhydrous drug and with a minimum content of 86.0% of *trans*-anethole in the essential oil.

'Star anise oil (Anisi stellati aetheroleum)' is described in a monograph of the European Pharmacopoeia 11.0 (PhEur, 2022b). It is defined as the essential oil obtained by steam distillation from the dry ripe fruits of *Illicium verum* Hook.f.

A Summary Report on 'Anisi stellati fructus' has been published by the European Medicines Agency (EMA) Committee of Veterinary Medicinal Products (EMA, 2000).

In 2005, EMA issued a Public statement on the use of herbal medicinal products containing estragole, which lists *Illicium verum* Hook.f. among the plants containing estragole in the fruit and in the essential oil (EMA, 2005, revised in 2021 EMA, 2021).

Many of the individual components of the star anise oil and star anise terpenes have been already assessed as chemically defined flavourings for use in feed and food by the FEEDAP Panel, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) and the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). The list of flavouring compounds currently authorised for feed and/or food use, 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 listed in Table 1.

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

CG	Chemical Group	Product (EU register name)	FLAVIS No	EFSA opinion,* Year
01	Straight-chain primary aliphatic alcohols/ aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes	Hexanal	05.008	2013a
03	α, β-Unsaturated (alkene or alkyne) straight-chain and branched chain aliphatic primary alcohols/aldehydes/acids, acetals and ester	*(Z)*-Nerol	02.058	2016a
		Hex-2(*trans*)-enal	05.073	2019a
06	Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers	Linalool	02.013	2012a
		α-Terpineol	02.014	
		4-Terpinenol	02.072	
		Nerolidol[(b)]	02.232	
		(*E*)-3,7-Dimethylocta-1,5,7-trien-3-ol[(a)]	02.146	2011a, CEF 2015a, CEF

[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] 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

[9] 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.

[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.

CG	Chemical Group	Product (EU register name)	FLAVIS No	EFSA opinion,* Year
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	*d,l*-Borneol	02.016	2016b
13	Furanones and tetrahydrofurfuryl derivatives	Linalool oxide[(c)]	13.140	2012b
14	Furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms	Furfural	13.018	2016c
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)] (anisyl methyl ketone)	07.087	2008, EFSA (AFC)
23	Benzyl alcohols, aldehydes, acids, esters and acetals	Benzaldehyde	05.013	2012d
		4-Methoxybenzaldehyde (anisaldehyde)	05.015	
		Methyl 4-methoxybenzoate	09.713	
		Methyl benzoate	09.725	
26	Aromatic ethers including anisole derivatives	1,2-Dimethoxy-4-(prop-1-enyl)benzene[(d)] (methyl isoeugenol)	04.013	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	
		Pin-2(10)-ene (β-pinene)	01.003	2016d
		Pin-2(3)-ene (α-pinene)	01.004	
		β-Caryophyllene	01.007	
		Myrcene	01.008	
		Camphene	01.009	
		β-Ocimene[(e)]	01.018	
		δ-Carene	01.029	
		δ-Cadinene[(a),(f)]	01.021	2011b, CEF
		β-Phellandrene[(a),(f)]	01.055	
		3,7,10-Humulatriene[(a),(f)]	01.043	
		Limonene	01.001	2015b, CEF
		β-Bisabolene[(a)]	01.028	
		4(10)-Thujene (sabinene)[(a)]	01.059	
		α-Farnesene[(a)]	01.040	2015c, CEF

*: FEEDAP opinion unless otherwise indicated.
(a): Evaluated for use in food. According to Regulation (EC) 1565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.
(b): Nerolidol [02.232]: a mixture of (*E*) and (*Z*)-nerolidol was evaluated (EFSA FEEDAP Panel, 2012a).
(c): Linalool oxide [13.140]: A mixture of *cis*- and *trans*-linalool oxide (5-ring) was evaluated (EFSA FEEDAP Panel, 2012b).
(d): EFSA evaluated 1,2-dimethoxy-4-(prop-1-enyl)benzene [04.013] or methyl isoeugenol, a mixture of *(Z)*- and *(E)*-isomers (EFSA FEEDAP Panel, 2012e).
(e): β-Ocimene [01.018]: as a mixture of (*E*)- and (*Z*)-isomers, containing 50–70% (*E*)-isomer and 17–17% (*Z*)-isomer, was evaluated (EFSA FEEDAP Panel, 2016d).
(f): 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, 2011b) were not submitted and the CEF Panel was unable to complete its assessment (EFSA CEF Panel, 2015b).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[11] in support of the authorisation request for the use of star anise oil and star anise terpenes from *I. verum* as a feed additive. The dossier was received on 6 July 2023 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00398.[12]

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

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings. The applicant submitted a written agreement to use the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 2, including the current one under assessment.[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 animal feed. The evaluation report is related to the methods of analysis for each feed additive included the group BDG 02 (Apiales and Austrobaileyales). During the assessment, the EURL issued a partial report[14] and an addendum of the report.[15] In particular, for the characterisation of *anise star oil* the EURL recommended methods based on gas chromatography with flame ionisation detection (GC-FID) for the quantification of the phytochemical marker 1-methoxy-4-(prop-1(*trans*)-enyl)benzene (hereinafter referred as to *trans*-anethole) in *anise star oil*.[16]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of star anise oil from *I. verum* is in line with the principles laid down in Regulation (EC) No 429/2008[17] and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA SC, 2009), Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 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 efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019b), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA SC, 2019b), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA SC, 2019c), General approach to assess the safety for the target species of

[11] FEED dossier reference: FAD-2010-0221.

[12] The original application EFSA-Q-2010-01286 was split on 6 July 2023 and a new EFSA-Q-2023-00398 was generated.

[13] Technical dossier/Supplementary information/Letter dated 29/04/2021.

[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.

botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021b).[18]

3. Assessment

The additive under assessment, star anise oil, is derived from the fruit of *Illicium verum* Hook.f. (without or with the presence of plant leaves) and is intended for use as a sensory additive (functional group: flavouring compounds) in feed and water for drinking for all animal species.

3.1. Origin and extraction

Illicium verum Hook.f. is an evergreen tree belonging to the Schisandraceae family, native to Vietnam and China. It is widely grown in the region for its characteristic fruits which have a long history of culinary and traditional medical use. The individual fruit are star-shaped, consisting of a ring of reddish-brown carpels each containing a single seed, and are generally harvested just before ripening. The harvested fruit are commonly referred to as 'star anise', reflecting both their shape and their sensory profile, which closely resembles that of true anise fruit (*Pimpinella anisum* L.). For this reason, star anise fruit is often used as a cheaper substitute for anise fruit in food, alcoholic beverages and household products. The term 'star anise' is used to describe both the plant, *Illicium verum* (also called Chinese star anise), and its fruit. The fruit of Chinese star anise should not be confused with the highly poisonous fruit of Japanese star anise (*Illicum anisatum* L.) which resemble each other closely (PhEur Commentary, 2020).

There are no references to the use of star anise leaves as a spice or for traditional medical purposes in the standard literature.

Star anise oil is extracted from the dry fruit (without or with the presence of plant leaves) by steam distillation. The volatile constituents are condensed and then separated from the aqueous phase by decantation.

3.2. Characterisation

3.2.1. Characterisation of star anise oil

Star anise oil is a pale yellow to yellow clear mobile liquid, with a characteristic aroma. In seven recent batches of the additive (all originating from China), the refractive index (20°C) ranged between 1.553 and 1.558 (specification: 1.551–1.561), the density (20°C) between 978 and 987 kg/m^3 (specification: 978–994 kg/m^3), the optical rotation (20°C) between 0.2° and 1.0° (specification: ≤ 1.0).[19] Star anise oil is identified with the Chemical Abstracts Service (CAS) numbers 84650-59-9 and 68952-43-2, the European Inventory of Existing Commercial Chemical Substances (EINECS) number 283-518-1, the Flavor Extract Manufacturers Association (FEMA) number 2096 and the Council of Europe (CoE) number 238.

For star anise oil, the product specifications used by the applicant are based on those developed by the International Organization for Standardization (ISO) 11016:1999 for oil of star anise (*Illicium verum* Hook.f.),[20] adapted to reflect the concentrations of the main volatile components of the essential oil. Four components contribute to the specifications as shown in Table 2, with *trans*-anethole selected as the phytochemical marker. Analysis of four batches of the additive (two obtained from fruit and leaves and two from the fruit without leaves) showed compliance with these specifications when analysed by GC-FID and expressed as percentage of gas chromatographic peak area (% GC area).[21]

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

[19] Technical dossier/Supplementary information April 2023/Conf_Annex_IIa_SIn reply_anise star oil_CoA_chrom_batches_1–3 and Annex_IIb_ SIn reply_anise star oil_CoA_chrom_batches_4–7.

[20] Technical dossier/Supplementary information April 2022/Annex_III_SIn_reply_anise_star_oil_ISO.

[21] Technical dossier/Supplementary information April 2023/Annex_VIII_SIn_reply_anise_star_oil_raw_data.

Table 2: Major constituents of the essential oil from the fruit of *Illicium verum* Hook.f. (without or with the presence of plant leaves) as defined by specifications: batch to batch variation based on the analysis of four 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[(a)]	Mean	Range
trans-Anethole	4180-23-8	04.010	85–93	88.6	88.3–88.9
Estragole[(b)]	140-67-0	04.011	0.5–6.0	3.41	3.14–3.68
Limonene	5989-27-5	01.001	0.2–6.0	0.65	0.51–0.80
Linalool	78–70-6	02.013	0.2–2.5	1.24	1.17–1.34

EU: European Union; CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.
(a): Specifications defined based on GC-FID analysis.
(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 a full characterisation of the volatile constituents in seven batches, (five obtained from fruit and leaves and two from the fruit without leaves) by gas chromatography coupled with mass spectrometry (GC–MS).[22] In total, up to 85 constituents were detected, 58 of which were identified and accounted on average for 99.9% (99.6–100%) of the % GC area. The four compounds indicated in the product specifications accounted for about 95.6% on average (range 94.2–96.7%) of % GC area. Besides the four compounds, 23 other compounds were detected at individual levels > 0.05% and are listed in Table 3. These 27 compounds, together accounted on average for 99.5% (99.1–99.7%) of the GC area. The remaining 31 compounds (ranging between 0.003% and 0.05%) and accounting for 0.3% of the total GC area are listed in the footnote.[23] Based on the available data on the characterisation, star anise oil is considered a fully defined mixture (EFSA SC, 2019a).

The FEEDAP Panel notes that from the comparison of the analytical data of the seven batches, no relevant differences were observed in the composition of star anise oils obtained from fruit and leaves (five batches) and oils obtained from the fruit without leaves (two batches).

Table 3: Constituents of the essential oil from the fruit of *Illicium verum* Hook.f. (without or with the presence of plant leaves) accounting for > 0.05% of the composition (based on the analysis of seven batches by gas chromatography–mass spectrometry). The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent	CAS No	FLAVIS No	% GC area	
EU register name			Mean	Range
trans-Anethole	4180-23-8	04.010	90.56	89.33–91.75
Estragole	140-67-0	04.011	3.49	2.67–4.09
Limonene	5989-27-5	01.001	0.61	0.21–1.91
Linalool	78-70-6	02.013	0.95	0.79–1.15
Foeniculin	78259-41-3	–	0.67	0.18–1.34
α-Pinene (pin-2(3)-ene)	80-56-8	01.004	0.48	0.29–0.88

[22] Technical dossier/Supplementary information April 2023/ Conf_Annex_IIa_SIn reply_anise star oil_CoA_chrom_batches_1–3 and Annex_IIb_ SIn reply_anise star oil_CoA_chrom_batches_4–7.

[23] Additional constituents: (33 components < 0.05% and > 0.003%): α-terpinene, β-ocimene, 4-methoxyphenylacetone, β-pinene, epoxyanethole, (*E*)-methyl isoeugenol, (*E*)-nerolidol, T-cadinol, α-cadinol, viridiflorene, sabinene, bicycloelemene, (*Z*)-β-farnesene, α-Farnesene, β-elemene, 1H-indene-3-carboxaldehyde, 2,6,7,7a-tetrahydro-1,5-dimethyl-, methyl 4-methoxybenzoate, γ-cadinene, *cis*-linalool oxide (5-ring), alloaromadendrene, epi-β-caryophyllene, α-thujene, furfural, borneol, methyl benzoate, camphene, benzaldehyde, hexanal, (*E*)-3,7-dimethylocta-1,5,7-trien-3-ol, (*Z*)-nerol and hex-2(*trans*)-enal.

Constituent EU register name	CAS No	FLAVIS No	% GC area Mean	Range
(E)-α-Bergamotene	13474-59-4	–	0.48	0.19–0.78
α-Phellandrene	99-83-2	01.006	0.36	0.27–0.49
Anisaldehyde (4-methoxybenzaldehyde)	123-11-5	05.015	0.35	0.25–0.53
β-Caryophyllene	87-44-5	01.007	0.32	0.30–0.34
cis-Anethole	25679-28-1	–	0.27	0.18–0.34
β-Phellandrene	555-10-2	01.055	0.22	0.08–0.44
1,8-Cineole	470-82-6	03.001	0.17	0.15–0.22
Myrcene	123-35-3	01.008	0.15	0.05–0.28
δ-3-Carene	13466-78-9	01.029	0.14	0.12–0.18
4-Terpinenol	562-74-3	02.072	0.10	0.04–0.17
p-Cymene (1-isopropyl-4-methylbenzene)	99-87-6	01.002	0.08	0.06–0.16
β-Bisabolene	495-61-4	01.028	0.08	0.06–0.12
α-Copaene	3856-25-5	–	0.07	0.02–0.12
γ-Terpinene	99-85-4	01.020	0.07	0.05–0.07
Terpinolene	586-62-9	01.005	0.06	0.06–0.09
(Z)-α-Bergamotene	18252-46-5	–	0.06	0.05–0.11
α-Terpineol	98-55-5	02.014	0.06	0.03–0.09
Bicyclogermacrene	67650-90-2	–	0.06	0.02–0.08
δ-Cadinene	29350-73-0	1.021	0.05	0.02–0.07
3,7,10-Humulatriene	6753-98-6	01.043	0.05	0.04–0.06
Aromadendrene	72747-25-2	–	0.05	0.02–0.08
Total			99.5	99.1–99.71[(a)]

EU: European Union; CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.
(a): The values given for the total (range) are the lowest and the highest values of the sum of the components in the seven 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 *I. verum* and its preparations.[24] Phenylpropanoids e.g. *trans*-anethole (75–90%), estragole (methylchavicol, 0.34–5.04%) and safrole (0.14%) are reported in the EFSA Compendium of botanicals as substances of concern for the essential oil from the fruit of *I. verum* (EFSA, 2012).[25] The presence of safrole in star anise oil reported in the EFSA compendium of botanicals is based on two references, Council of Europe (2000) and Tisserand and Young (2014). However, the potential presence of safrole in unadulterated anise star oil from the fruit is waived in the PhEur Commentary (2020): '*Previous research suggested the presence of safrole in star anise oil, but this proved to be confounding due to the fact that safrole has approximately the same Rf value as foeniculin on the TLC plate. Safrole is a typical component of the oil from shikimi fruit, the fruit of Illicium anisatum L., also known as an adulterant of star anise fruit; the oil of these fruits could also get into the star anise oil if they were mixed up*'.

Several publications retrieved by the applicant consistently reported the occurrence of estragole (2–6%) in essential oils from the fruit of *I. verum* (e.g. Li et al., 2020; Dwivedy et al., 2018; EMA, 2021). Two publications also reported the presence of methyleugenol (0.12%) and safrole (0.21%) in essential oils from star anise fruit obtained by different manufacturing process and containing, respectively, 10% and 14.4% estragole (Nie et al., 2021; Sabry et al., 2021).

No literature was made available describing the composition/substances of concern in oil obtained from the leaves of *I. verum*.

[24] Technical dossier/Supplementary information April 2023/Literature search_anise_star_oil.
[25] Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals.

An analysis of the seven batches of star anise oil under assessment confirmed the presence of estragole in all batches (2.67–4.09%). Safrole was not detected in the oil under assessment (LOD, 0.01%).

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), organochloride pesticides, organophosphorous pesticides, aflatoxins (B1, B2, G1, G2) and ochratoxin A. However, no data have been provided on the presence of these impurities. Since star anise 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 star anise 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).[26] However, no data supporting this statement were provided.

3.2.1.4. Conditions of use

Star anise oil is intended to be added to feed and water for drinking for all animal species without a withdrawal period. Maximum use levels in complete feed were proposed for the animal species and categories listed in Table 4. No use level has been proposed by the applicant 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 of *Illicium verum* Hook.f. (without or with the presence of plant leaves): maximum proposed use levels in complete feed for certain animal categories

Animal category	Maximum 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	1.6
Horse	1.6
Rabbit	0.6
Dog	1.9
Cat	1.6
Ornamental fish	6.5
Short-living animals (species for fattening)	
Chicken for fattening	18.5
Turkey for fattening	24.8
Piglet	33.3
Pig for fattening	40.0
Veal calf (milk replacer)	83.3
Cattle for fattening	73.3
Sheep/goat	73.3
Horse	73.3
Rabbit	29.3
Salmon	83.8

3.2.2. Safety

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

[26] Technical dossier/Section II.

Many of the components of star anise oil, accounting for about 95% of the % GC peak areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for use in food[9] without limitations and for use in feed[8] 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). The FEEDAP Panel notes that for the major component of star anise oil, *trans*-anethole [04.010], the lack of data on metabolism and residues in poultry precluded an assessment of consumer exposure from this source (EFSA FEEDAP Panel, 2011).

Three compounds listed in Table 1, δ-cadinene [01.021], β-phellandrene [01.055] and 3,7,10-humulatriene [01.043], were evaluated in FGE25.Rev2 (EFSA CEF Panel, 2011b) 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 toxicity data (EFSA CEF Panel, 2011b). In the absence of such toxicological data, the EFSA CEF Panel was unable to complete its assessment (EFSA CEF Panel, 2015b). As a result, these compounds are not authorised for use as flavours in food. For these compounds, in the absence of toxicity data, the FEEDAP Panel applies the threshold of toxicological concern (TTC) approach or read-across from structurally related substances, as recommended in the Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a).

Foeniculin (0.67%) and 20 additional compounds, each accounting for < 0.5% of the % GC area, have not been previously assessed for use as flavourings. The FEEDAP Panel notes that 13 of them[27] are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 31 and a similar metabolic and toxicological profile is expected. These lipophilic compounds are expected to be rapidly absorbed from the gastrointestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2015, 2016d). Two compounds (T-cadinol and α-cadinol) are structurally related to flavourings assessed in CG 6 (EFSA FEEDAP Panel, 2012a). Four additional components, i.e. *(E)*-nerolidol, *cis*-linalool oxide, *cis*-anethole and *(E)*-methyl isoeugenol are structurally related to compounds that have been evaluated for use in food and/or feed and one component (epoxyanethole) is a metabolite of the major compound *trans*-anethole.

The oil under assessment contains estragole, up to 4.09% of the % GC area and up to 6% according to specification.

The following sections focus on estragole and on the other two compounds, 1H-indene-3-carboxaldehyde, 2,6,7,7a-tetrahydro-1,5-dimethyl- and foeniculin, not previously assessed or not structurally related to flavourings previously assessed, based on the evidence provided by the applicant in the form of literature searches and quantitative structure–activity relationship (QSAR) analysis. An update of the absorption, distribution, metabolism and excretion (ADME) of *trans*-anethole in poultry is also presented in the next section.

3.2.2.1. Absorption, distribution, metabolism and excretion

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 the detoxification of estragole. The formation of genotoxic metabolites is initiated by oxidation of the side chain with formation of 1′-hydroxy-estragole. Sulfate conjugation of the hydroxyl group leads to 1′-sulfooxyestragole, which is 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

[27] *(Z)*-β-Farnesene, β-elemene, α-copaene, *(E)*-α-bergamotene, *(Z)*-α-bergamotene, viridiflorene, α-thujene, aromadendrene, alloaromadendrene, bicyclogermacrene, epi-beta-caryophyllene, bicycloelemene and γ-cadinene.

portion was excreted in urine in the form of several metabolites resulting from hydroxylation at 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).

trans-Anethole in poultry

In laboratory animals, *trans*-anethole is rapidly absorbed, metabolised and excreted, the main route of excretion (> 90%) being the urine. *trans*-Anethole is mainly metabolised by three primary oxidation pathways: *O*-demethylation, omega side-chain oxidation and epoxidation of the side chain, followed by subsequent oxidation and hydration. The resulting products are extensively conjugated with sulfate, glucuronic acid, glycine and glutathione (WHO, 2000a,b). In its former assessment on CG 18 (EFSA FEEDAP Panel, 2011), the Panel noted that the metabolic pathways involved in the biotransformation of *trans*-anethole are common to mammalian species, but no data were available concerning its metabolic fate in poultry. Therefore, the FEEDAP Panel concluded that the efficient metabolism of *trans*-anethole in mammals and the subsequent rapid excretion of the metabolites preclude their accumulation in tissues and transfer to products. However, the lack of data on metabolism and residues in poultry precluded an assessment of consumer exposure from this source (EFSA FEEDAP Panel, 2011). However, phase I (CYP450 monooxygenase families, epoxide hydrolases) and phase II (glucuronide-, sulfate- and glutathione-transferases) enzymes involved in the biotransformation of several classes of compounds including CG 18 compounds are also expressed in birds as already described (EFSA FEEDAP Panel, 2013b, 2016d,e). Therefore, birds can also be assumed to have the ability to metabolise and excrete *trans*-anethole as mammals and there is no evidence that they or their metabolites would accumulate in tissues and cause a concern for consumer safety.

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 SC, 2019b). Therefore, the potential genotoxicity of identified constituents is first considered. Then, *in vitro* genotoxicity studies performed with star anise oils similar to the additive under assessment are taken into account, if deemed relevant.

The genotoxic potential for two substances, 1H-indene-3-carboxaldehyde, 2,6,7,7a-tetrahydro-1,5-dimethyl- and foeniculin, was predicted by the applicant using the Organization for Economic Co-operation and Development (OECD) QSAR Toolbox. Structural alerts for mutagenicity for 1H-indene-3-carboxaldehyde, 2,6,7,7a-tetrahydro-1,5-dimethyl- were due to the presence of the aldehyde group and for foeniculin due to the presence of the vinyl/allyl ethers group. For both substances, predictions of Ames mutagenicity (with and without S9) were made by 'read-across' analyses of data available for similar substances to the target compounds (i.e. analogues obtained by categorisation). Read-across-based predictions were found consistently negative for all categories of analogues. On this basis, the alerts raised were discounted.[28]

Estragole

Star anise oil contains estragole (up to 6% by specification), a compound with experimentally proven genotoxicity and carcinogenicity in rodents (as reviewed in EC, 2001; EMA, 2021).

The carcinogenicity of estragole, methyleugenol 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%

[28] Technical dossier/Supplementary information April 2023/Annex_VI_Sin_reply anise_star_oil_QSAR.

($BMDL_{10}$) of 22.2 mg/kg bw per day (Suparmi et al., 2019). This $BMDL_{10}$ value was selected as reference point for the assessment group of *p*-allylalkoxybenzenes irrespective of their relative potency (EFSA FEEDAP Panel, 2022).

Genotoxicity studies with star anise oils

The applicant provided a literature search on the genotoxicity of preparations obtained from the fruit of *I. verum*. The genotoxicity of star anise oil was reviewed in the FEMA GRAS assessment of natural flavour complexes (Rietjens et al., 2023). The FEEDAP Panel noted that the assessment was based on unpublished reports, which were not made available by the applicant. Therefore, none of the recovered articles was further considered for the assessment of the genotoxicity of star anise oil.

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[29] and function, including effects on liver[30] and the glandular stomach[31]) 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, 2023).

3.2.2.4. Safety for the target species

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

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

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. Estragole, a substance for which a concern for genotoxicity has been identified, is assessed separately.

Components other than estragole

Based on considerations related to structural and metabolic similarities, the components were allocated to nine assessment groups, corresponding to the chemical groups (CGs) 1, 3, 6, 7, 8, 13, 14, 16, 18, 21, 23, 25, 26 and 31, as defined in Annex I of Regulation (EC) No 1565/2000. For CG 31 ('aliphatic and aromatic hydrocarbons'), subassessment 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 body weight (bw) are used to express exposure in terms of mg/kg bw per day. The intake levels of the individual components calculated for chickens for fattening, the species with the highest ratio of feed intake/body weight per day, are shown in Table 5.

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification (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 allowing extrapolation from a known NOAEL of a component of an

[29] 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.

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

[31] 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.

assessment group to the other components of the group with no available NOAEL or, if sufficient evidence were available, for members of a (sub-)assessment group, to derive a (sub-)assessment group NOAEL.

Toxicological data of subchronic studies, from which NOAEL values could be derived, were available for acetaldehyde [05.001] the representative compound in CG 1 (EFSA FEEDAP Panel, 2013a), citral [05.020] and hex-2(*trans*)-enal [05.073] in CG 3 (EFSA FEEDAP Panel, 2016a, 2019a), linalool [02.013] and terpineol[32] [02.230] in CG 6 (EFSA FEEDAP Panel, 2012a), furfural [13.018] in CG 14 (EFSA FEEDAP Panel, 2016c), 1,8-cineole [03.001] in CG 16 (EFSA FEEDAP Panel, 2012c, 2021a), *trans*-anethole [04.010] in CG 18 (EFSA FEEDAP Panel, 2011), anisaldehyde [05.015] in CG 23 (EFSA FEEDAP Panel, 2012d), methyl isoeugenol [04.013] in CG 26 (EFSA FEEDAP Panel, 2012e), myrcene [01.008], d-limonene [01.045], p-cymene [01.002] and β-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016d).

The NOAEL of 120 mg/kg bw per day for acetaldehyde [05.001] was selected as reference point for CG 1 compounds and the NOAEL of 345 mg/kg bw per day for citral [05.020] was applied to *(Z)*-nerol in CG 3.

Considering the structural and metabolic similarities, for the subgroup of terpinyl derivatives in CG 6, i.e. α-terpineol [02.014], 4-terpinenol [02.072] and for the cadinyl derivatives (T-cadinol and α-cadinol), the reference point was selected based on the NOAEL of 250 mg/kg bw per day available for terpineol [02.230] and d-limonene [01.045]. The NOAEL of 117 mg/kg bw per day for linalool [02.013] was applied to *(E)*-nerolidol [02.232] in CG 6.

The NOAEL of 300 mg/kg bw per day for *trans*-anethole [04.010] was applied to *cis*-anethole in CG 18 and the NOAEL 100 mg/kg bw per day for methyl isoeugenol [04.013] was applied to *(E)*-methyl isoeugenol in CG 26.

Similarly, the NOAELs of 44, 250 and 222 mg/kg bw per day for the representative compounds of CG 31, myrcene [01.008], d-limonene [01.045] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within subassessment group II (β-ocimene [01.018], *(Z)*-β-farnesene and α-farnesene [01.040]), group III (α-phellandrene [01.006], β-phellandrene [01.055], β-bisabolene [01.028], terpinolene [01.005], γ-terpinene [01.020], α-terpinene [01.019] and β-elemene), and group V (α-pinene, α-thujene, *(E)*-α-bergamotene, δ-3-carene [01.029], *(Z)*-α-bergamotene, aromadendrene, bicyclogermacrene, α-copaene, δ-cadinene [01.021], β-pinene [01.003], viridoflorene, sabinene [01.059], alloroaromadendrene, γ-cadinene, α-thujene, camphene [01.009] and epi-β-caryophyllene)[33] (EFSA CEF Panel, 2015b,c).

For the remaining compounds,[34] toxicity studies were not available and read-across was not possible. Therefore, the TTC approach was applied (EFSA FEEDAP Panel, 2017b).

As the result of the hazard characterisation, a reference point was identified for each component in the assessment group based on the toxicity data available (NOAEL from *in vivo* toxicity study or read across) or from the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class (i.e. 3, 0.91 and 0.15 mg/kg bw per day, respectively, for Cramer Class I, II and III compounds, 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 SC, 2019a). An MOET > 100 allowed for interspecies and intra-individual variability (as in the default 10 × 10 uncertainty factor). The compounds resulting

[32] 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.

[33] Some of these compounds are not listed in Table 5 because their individual margin of exposure (MOE) was > 50,000.

[34] 1H-Indene-3-carboxaldehyde, 2,6,7,7a-tetrahydro-1,5-dimethyl-, *cis*-linalool oxide, 4-methoxyphenylacetone, methyl 4-methoxybenzoate, foeniculin, bicycloelemene, 3,7,10-humulatriene and epoxyanethole.

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.[35]

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

Essential oil composition			**Exposure**		**Hazard characterisation**		**Risk characterisation**	
Assessment group	**FLAVIS-No**	**Highest conc. in the oil**	**Highest feed conc.**	**Daily intake**	**Cramer class**[(b)]	**NOAEL**[(c)]	**MOE**	**MOET**
Constituent	–	**%**	**mg/kg**	**mg/kg bw/day**	–	**mg/kg bw/day**	–	–
CG 6								
Linalool	02.013	1.15	0.2128	0.01910	(I)	**117**	6,126	
CG 7								
1H-Indene-3-carboxaldehyde, 2,6,7,7a-tetrahydro-1,5-dimethyl-	–	0.02	0.0037	0.00033	III	*0.15*	452	
CG 13								
cis-Linalool oxide	–	0.01	0.0024	0.00022	II	*0.91*	4,215	
CG 16								
1,8-Cineole	03.001	0.22	0.0407	0.00365	(II)	**100**	27,369	
CG 18								
trans-Anethole	04.010	91.8	16.974	1.52378	(I)	**300**	197	
CG 21								
4-Methoxyphenylacetone	07.087	0.06	0.0111	0.00100	I	*3*	3,011	
CG 23								
Anisaldehyde	05.015	0.53	0.0981	0.00880	(I)	**10**[(d)]	1,136	
Methyl 4-methoxybenzoate	09.713	0.02	0.0031	0.00028	I	*3*	10,626	
MOET CG 23								1,026
CG 26								
Foeniculin	–	1.43	0.2475	0.02222	I	*3*	135	
CG 31, II								
Myrcene	01.008	0.28	0.0518	0.00465	(I)	44	9,462	
β-Ocimene	01.018	0.14	0.0259	0.00233	(I)	44	18,924	
MOET CG 31, II								6,308
CG 31, III								
Limonene	01.001	1.91	0.3534	0.03172	(I)	**250**	7,881	
α-Phellandrene	01.006	0.49	0.0907	0.00814	(I)	250	30,720	
β-Phellandrene	01.055	0.44	0.0814	0.00731	(I)	250	34,211	
MOET CG 31, III								5,300
CG 31, V (Bi-, tricyclic, non-aromatic hydrocarbons)								

[35] Compounds included in the assessment groups but not reported in the table: hexanal (CG 1); hex-2(*trans*)enal and *(Z)*-nerol (CG 3); 4-terpinenol, α-terpineol, *(E)*-nerolidol, T-cadinol, α-cadinol and *(E)*-3,7-dimethylocta-1,5,7-trien-3-ol (CG 6); *d,l*-borneol (CG 8); furfural (CG 14); *cis*-anethole (CG 18); benzaldehyde and methyl benzoate (CG 23); *(E)*-methyl isoeugenol (CG 26); *(Z)*-β-farnesene and α-farnesene (CG 31, II); β-bisabolene, terpinolene, α-phellandrene, γ-terpinene, α-terpinen and β-elemene (CG 31, III); p-cymene (CG 31, IVe); δ-3-carene, *(Z)*-α-bergamotene, aromadendrene, bicyclogermacrene, α-copaene, δ-cadinene, β-pinene, viridoflorene, sabinene, alloroaromadendrene, γ-cadinene, α-thujene, camphene and epi-β-caryophyllene (CG 31, VI).

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS-No	Highest conc. in the oil	Highest feed conc.	Daily intake	Cramer class[(b)]	NOAEL[(c)]	MOE	MOET
Constituent	–	%	mg/kg	mg/kg bw/day	–	mg/kg bw/day	–	–
α-Pinene	01.004	0.88	0.1628	0.01462	(I)	222	15,190	
(*E*)-α-Bergamotene	–	0.78	0.1443	0.01295	(I)	222	17,137	
β-Caryophyllene	01.007	0.32	0.0629	0.00565	(I)	**222**	39,315	
Bicycloelemene	–	0.03	0.0056	0.00050	(I)	222	6,021	
CG 31, V								3,168
CG31, VI								
3,7,10-Humulatriene	01.043	0.06	0.0105	0.00095	I	*3*	3,169	
CG 32								
Epoxyanethole	–	0.06	0.0111	0.00100	I	*3*	3,011	

(a): Intake calculations for the individual components are based on the use level of 18.5 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 20 mg/kg bw per day was halved to take into account of the short duration of the study (EFSA FEEDAP Panel, 2012d).

The approach to the safety assessment of star anise oil for the target species is summarised in Table 5. The calculations were done for chickens for fattening, the species with the highest ratio of feed intake/body weight and represent the worst-case scenario at the use level of 18.5 mg/kg.

As shown in Table 5, for all the assessment groups, the MOE(T) was higher than 100 (≥ 135). Therefore, no safety concern was identified for the star anise oil when used as a feed additive for chickens for fattening at the proposed use level (18.5 mg/kg) without considering the presence of estragole.

Table 6: Combined margin of exposure (MOET) for the assessment group 'aliphatic and acyclic ethers' (CG 16) calculated for the other target species at the proposed use level of star anise oil in complete feed

Animal category	Body weight (kg)	Feed intake (g DM/day)	Proposed use levels (mg/kg feed)[(a)]	Lowest MOET
Long-living and reproductive animals				
Laying hens	2	106	0.6	15,810
Sow lactating	175	5,280	1.0	16,759
Dairy cow	650	20,000	1.0	16,218
Sheep/goat	60	1,200	1.6	15,711
Horse	400	8,000	1.6	15,711
Rabbit	2	100	0.6	16,759
Dog	15	250	1.9	15,565
Cat	3	60	1.6	15,711
Ornamental fish	0.012	0.054	6.5	15,469
Short-living animals (species for fattening)				
Chicken for fattening	2	158	18.5	344
Turkey for fattening	3	176	24.8	343
Piglet	20	880	33.3	340

Animal category	Body weight (kg)	Feed intake (g DM/day)	Proposed use levels (mg/kg feed)[(a)]	Lowest MOET
Pig for fattening	60	2,200	40	318
Veal calf (milk replacer)	100	1,890	83.3	341
Cattle for fattening	400	8,000	73.3	343
Sheep/goat	60	1,200	73.3	343
Horse	400	8,000	73.3	343
Rabbit	2	100	29.3	333
Salmon	0.12	2.1	83.8	344

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

From the lowest MOET of 135 for chickens for fattening, the MOET for the assessment group 'aliphatic and acyclic ethers' (CG 16) was calculated for the target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 6.

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 star anise oil as additive in cat feed needs a wider margin of exposure. An MOET of 500 is considered adequate. Therefore, for all species, no safety concern (without considering the presence of estragole) was identified for star anise oil, when used as a feed additive at the proposed use levels.

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, 2021b), 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, an 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, 2022). 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 (2.67–4.09%) was detected in all batches of the oil under assessment (see Section 3.2.1). The FEEDAP Panel notes that the applicant proposed a higher specification (up to 6%) for the content of estragole in star anise oils.

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 maximum expected concentration in the additive (6%). The intake values are reported in Table 7, together with the corresponding MOE for the combined intake calculated considering the relevant reference point for long-living and reproductive animals and for species for fattening.

Table 7: Target animal intake of estragole and margin of exposure (MOE) calculated at the maximum proposed use level of star anise oil in feed for an essential oil with a content of estragole of 6%

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	2.17	10,239[(b)]
Sow lactating	5,280	175	1.0	2.06	10,792[(b)]
Dairy cow	20,000	650	1.0	2.10	10,582[(b)]
Sheep/goat	1,200	60	1.6	2.18	10,175[(b)]
Horse	8,000	400	1.6	2.18	10,175[(b)]
Rabbit	100	2	0.6	2.05	10,853[(b)]
Dog	250	15	1.9	2.16	10,282[(b)]
Cat	60	3	1.6	2.18	10,175[(b)]
Ornamental fish	0.054	0.012	6.5	1.99	11,132[(b)]
Short-living animals (species for fattening)[(c)]					
Chicken for fattening	158	2	18.5	100	100[(c)]
Turkey for fattening	176	3	24.8	99	101[(c)]
Piglet	880	20	33.3	100	100[(c)]
Pig for fattening	2,200	60	40	100	100[(c)]
Veal calf (milk replacer)	1,890	100	83.3	100	100[(c)]
Cattle for fattening	8,000	400	73.3	100	100[(c)]
Sheep/goat	1,200	60	73.3	100	100[(c)]
Horse	8,000	400	73.3	100	100[(c)]
Rabbit	100	2	29.3	100	100[(c)]
Salmon	2.1	0.12	83.8	100	100[(c)]

DM, dry matter.
(a): The intake value of estragole is calculated at the specification of 6%.
(b): The MOE for long-living and reproductive animals is calculated as the ratio of the reference point (BMDL10 of 22.2 mg/kg bw per day) to the combined intake.
(c): The MOE for short living animals (species for fattening) is calculated as the ratio of the reference point (NOAEL of 10 mg/kg bw per day) to the combined intake.
(d): Complete feed containing 88% DM, milk replacer 94.5% DM.

When the estimated exposures of long-living animals are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day derived for methyleugenol by Suparmi et al. (2019) from a rodent carcinogenicity study (NTP, 2000), an MOE > 10,000, which is indicative of low concern, is obtained for long-living and reproductive animals (Table 7).

For species 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.

Conclusions on safety for the target species

Based on the magnitude of the MOE, calculated considering the presence of estragole in star anise oil corresponding to the highest specification of 6% and the conditions of use in the different target species, the FEEDAP Panel concludes that the maximum proposed concentrations of the star anise oil under assessment in complete feed are considered of low concern for long-living and reproductive animals and of no concern for target species for fattening. The conclusions of the FEEDAP Panel are summarised in Table 8.

Table 8: Maximum feed concentrations of star anise oil 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 and camelids for milk production and reproduction including animals reared for milk production/reproduction	1.0
Sheep/goat	1.6
Horses and other Equidae	1.6
Rabbits	0.6
Dogs	1.9
Cats	1.6
Ornamental fish	6.5
Short-living animals (species for fattening)[(b)]	
Chickens for fattening and minor poultry for fattening	18.5
Turkeys for fattening	24.8
Pigs for fattening	40.0
Piglets and other Suidae species for meat production	33.3
Veal calves (milk replacer)	83.3
Cattle for fattening and other ruminants for fattening and camelids at the same physiological stage	73.3
Sheep/goat for meat production	73.3
Horses and other Equidae for meat production	73.3
Rabbit for meat production	29.3
Salmonids and minor fin fish	83.8
Any other species	0.6

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

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

3.2.2.5. Safety for the consumer

The fruit of *I. verum* and its preparations including its oil are added to a wide range of food categories as spice or for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites values of 0.001 mg/kg bw per day for star anise fruit and 0.006 mg/kg bw per day for star anise oil.

The majority of the individual constituents of the essential oil under assessment are currently authorised as food flavourings without limitations and have been already assessed for consumer safety when used as feed additives in animal production (see Table 1, 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 star anise oil are expected to be extensively metabolised and excreted by 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 at the proposed use levels in feed (see Section 3.2.2.1).

Considering the above and the reported human exposure due to direct use of star anise fruit and star anise oil in food (Burdock, 2009) it is unlikely that consumption of products from animals given star anise oil at the proposed maximum use level would increase human background exposure.

No safety concern would be expected for the consumer from the use of star anise oil up to the maximum proposed use level in feed for the target animals.

3.2.2.6. Safety for the user

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

The applicant made a literature search aimed at retrieving studies related to the safety of preparations obtained from *I. verum* for the users.[36] Sensitising properties of star anise oil have been reported in one publication (Rudzki and Grzywa, 1976).

The applicant produced a safety data sheet[37] for star anise oil, where hazards for users have been identified.

The essential oil under assessment should be considered as irritant to skin and eyes, and as a skin 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)[38] 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.[39]

3.2.2.7. Safety for the environment

Although *I. verum* is not a native species to Europe, the most abundant components of the essential oil (*trans*-anethole, estragole, limonene and linalool) are naturally occurring in European plants. The use of the essential oil under the proposed conditions of use in animal production is not expected to pose a risk to the environment.

3.3. Efficacy of star anise oil

The fruit of *I. verum* and its oil are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009), by the Flavour and Extract Manufactures Association (FEMA) with the reference numbers 2095 (star anise) and 2096 (star anise oil).

Since star anise 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

Star anise oil obtained by steam distillation of the fruit (without or with the presence of plant leaves) of *Illicium verum* Hook.f. 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.

Based on the magnitude of the MOE, calculated considering the presence of estragole in star anise oil corresponding to the highest specification of 6% and the conditions of use in the different target species, the FEEDAP Panel concludes that the maximum proposed concentrations of the star anise oil under assessment in complete feed are considered of low concern for long-living and reproductive

[36] Technical dossier/Supplementary information April 2023/Literature_search_Anise_star_oil.

[37] Technical dossier/Supplementary Information April 2023/Annex_VII_SIn reply_anise_star_oil_MSDS. For the oil: May cause allergic skin reactions (H317B), suspected to cause genetic defects (H341), suspect of causing cancer (H351). For the main component *trans*-anethole: hazards for skin sensitisation (H317, category 1B). For estragole: hazards for skin irritation (H315, category 2), skin sensitisation (H317, category 1B), suspected to cause genetic defects (H341), suspect of causing cancer (H351).

[38] 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, pp. 1–1,355.

[39] 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.

animals and of no concern for target species for fattening. 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 and camelids for milk production and reproduction including animals reared for milk production/reproduction	1.0
Sheep/goat	1.6
Horses and other Equidae	1.6
Rabbits	0.6
Dogs	1.9
Cats	1.6
Ornamental fish	6.5
Short-living animals (species for fattening)[(b)]	
Chickens for fattening and minor poultry for fattening	18.5
Turkeys for fattening	24.8
Pigs for fattening	40.0
Piglets and other Suidae species for meat production	33.3
Veal calves (milk replacer)	83.3
Cattle for fattening and other ruminants for fattening and camelids at the same physiological stage	73.3
Sheep/goat for meat production	73.3
Horses and other Equidae for meat production	73.3
Rabbit for meat production	29.3
Salmonids and minor fin fish	83.8
Any other species	0.6

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

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

The use of star anise oil up to the maximum proposed use level in feed is also expected to be of no concern for consumers.

Star anise oil should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser. Due to the high concentration of estragole (≥ 1%), the additive is classified as suspected of causing genetic defects and of causing cancer and should be handled accordingly.

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

Since the fruit of *I. verum* 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 for the essential oils under assessment.

Recommendation

Although the FEEDAP Panel is aware that star anise oil with 6% estragole could be present on the market, the analytical data provided by the applicant demonstrate that star anise oil with reduced contents of genotoxic and carcinogenic substances 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, 2021b), 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 star anise oil intended to be used as feed additive should contain the lowest possible concentrations of estragole.

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*
30/09/2019	Comments received from Member States
28/10/2020	Reception of supplementary information from the applicant (partial submission: anise tincture included in another assessment) - 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*)
11/04/2023	Reception of supplementary information from the applicant (partial submission: star anise oil and star anise terpenes)
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*)
07/06/2023	The application was split and a new EFSA-Q-2023-00398 was assigned to the preparations included in the present assessment
08/06/2023	Scientific assessment re-started
05/07/2023	Opinion adopted by the FEEDAP Panel on anise oil and anise tincture (EFSA-Q-2023-00398). 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

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
AFC	EFSA 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
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
CLP	Classification, Labelling and Packaging
CoE	Council of Europe
CYP450	cytochrome P450
DAD	diode array detection
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
FLAVIS-No	FLAVIS number
GC	gas chromatography
GC-FID	gas chromatography with flame ionisation detector
GC–MS	gas chromatography–mass spectrometry
ISO	International standard organisation
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
OECD	Organization for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PhEur	European Pharmacopoeia
sb	Solvent-based
SC	EFSA Scientific Committee
TTC	threshold of toxicological concern
UV	ultraviolet
WHO	World Health Organization