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Safety and efficacy of a feed additive consisting of a tincture derived from the fruit of *Foeniculum vulgare* Mill. ssp. *vulgare* var. *dulce* (sweet fennel tincture) for use in all animal species (FEFANA asbl)

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

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of a tincture from the fruit of *Foeniculum vulgare* Mill. ssp. v*ulgare* var. *dulce* (sweet fennel tincture) when used as a sensory additive in feed and water for drinking for all animal species. The product is a [redacted] solution, with a dry matter content of approximately 2.16%. The product contained 0.0586% polyphenols (of which 0.0052% were flavonoids), anethole (0.0006%), anisaldehyde (0.0035%) and estragole (0.0006%). The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that sweet fennel tincture is safe at the maximum proposed use levels of 200 mg/kg complete feed for horses and 50 mg/kg complete feed for all other animal species. The FEEDAP Panel considered that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount which is considered safe when consumed via feed. No safety concern would arise for the consumer from the use of sweet fennel tincture up to the maximum proposed use levels in feed. Sweet fennel tincture should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser. When handling the additive, exposure of unprotected users to estragole cannot be excluded. Therefore, to reduce the risk, the exposure of the users should be minimised. *F. vulgare* is native to Europe. The use of sweet fennel tincture as a flavour in animal feed was not expected to pose a risk for the environment. Since the fruit of *F. vulgare* and its preparations were recognised to flavour food and their function in feed would be essentially the same, no demonstration of efficacy was considered necessary.

Keywords: sensory additives, flavouring compounds, *Foeniculum vulgare* Mill. Ssp. *vulgare* var. *dulce*, sweet fennel tincture, anisaldehyde, anethole, estragole

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Correspondence: feedap@efsa.europa.eu

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

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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 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)[2,3] 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 (namely dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract, opoponax oil,[4] parsley oil, hares ear tincture, taiga root extract (sb), ajowan oil[5] and celery tincture[6]). These preparations were deleted from the register of feed additives.[7] 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: sweet fennel tincture (*Foeniculum vulgare* Mill. subsp. v*ulgare* var. *dulce*) for all animal species.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 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 the product sweet fennel tincture (*F. vulgare*), when used under the proposed conditions of use (see Section 3.2.2).

The remaining 19 preparations belonging to botanically defined group (BDG) 02 – *Apiales/ Austrobaileyales* under application are assessed in separate opinions.

1.2. Additional information

A tincture from *F. vulgare* Mill. (fennel tincture) is currently authorised as a feed additive according to the entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined). It has not been assessed as a feed additive in the EU.

[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, 1,050 Brussels, Belgium.
[3] On 27 February 2019, EFSA was informed by the applicant about the transfer of contact point for this application to Manghebati SAS, zone de la Basse Haye–BP 42133–35,221 Chateaubourg Cedex.
[4] 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.
[5] 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.
[6] On 9 December 2020, the applicant informed EFSA about the withdrawal of the application on celery tincture.
[7] Register of feed additives, Annex II, withdrawn by OJ L162, 10.05.2021, p. 5.

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[8] 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) are described in a monograph of the European Pharmacopoeia 10.0 (PhEur, 2020a). They are defined as the dry cremocarps and mericarps of *Foeniculum 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) are described in a monograph of the European Pharmacopoeia 10.0 (PhEur, 2020b). They are defined as the dry cremocarps and mericarps of *Foeniculum 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%.

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).

The main identified individual components of sweet fennel tincture are anisaldehyde, (4-methoxybenzaldehyde) a compound identified with the EU Flavour Information System (FLAVIS) number [05.015], anethole (1-methoxy-4-(1-propenyl) benzene [04.088]). Anisaldehyde and *trans*-anethole [04.010], which is known to be prevalent over *cis*-anethole in the fruit of *F. vulgare*,[9] have been assessed for use in feed and food by the FEEDAP Panel and the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), respectively. In its assessment of benzyl alcohols, aldehydes, acids, esters and acetals (chemical group 23) (EFSA FEEDAP Panel, 2012a), the FEEDAP Panel concluded that 4-methoxybenzaldehyde was safe at the use level of 1 mg/kg complete feed for all animal species. For *trans*-anethole [04.010], a compound belonging to chemical group 18, the FEEDAP Panel concluded that the additive was safe at the maximum proposed use level of 25 mg/kg for all animal species except fish for which the use was contra-indicated (EFSA FEEDAP Panel, 2011). Anisaldehyde and *trans*-anethole were considered safe for the consumer. However, for *trans*-anethole, the lack of data on metabolism and residues in poultry precluded an assessment of consumer exposure from this source. Both anisaldehyde and *trans*-anethole were also considered safe for the environment, whereas hazards for skin and eye contact and respiratory exposure were recognised for all the compounds belonging to chemical groups 23 and 18.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[10] in support of the authorisation request for the use of sweet fennel tincture from *F. vulgare* as a feed additive.

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

[8] 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.

[9] 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).

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

assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

Some of the components of the tincture 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 02, including the current one under assessment.[11]

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance/agent 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). In particular, for the characterisation of sweet fennel tincture the EURL recommended methods based on spectrophotometry (for the determination of total polyphenols in the feed additive) and high-performance thin-layer chromatography (HPTLC) (for the determination of the content of total flavonoids and of the phytochemical marker anisaldehyde in the feed additive).[12]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of sweet fennel tincture from *F. vulgare* is in line with the principles laid down in Regulation (EC) No 429/2008[13] 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), Statement on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed (EFSA SC, 2012), 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, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA SC, 2019b), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA SC, 2019c) and General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021).[14]

3. Assessment

The additive under assessment, sweet fennel tincture, is derived from the fruit from *F. vulgare* Mill. ssp. *vulgare* var. *dulce* (Mill.) Batt. & Trab. (sweet fennel) 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

F. vulgare Mill. ssp. *vulgare* var. *dulce* (Mill.) Batt. & Trab is an annual 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).

[11] Technical dossier FAD-2010-0335/Supplementary information February 2018/2018-01-30_SInReply_cardamom.
[12] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0221_en
[13] 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.
[14] https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf

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

The tincture is produced from the dried fruit of sweet fennel by extended extraction for 3 weeks under ambient conditions with a [redacted]. After this period the tincture is recovered by pressing to separate solid and liquid phases and the extracted solution is then clarified by filtration.

3.2. Characterisation

3.2.1. Characterisation of the tincture

The tincture is a brown liquid, with a characteristic odour, slightly aniseed. It has an average density of [redacted] and a pH of 5.97 (5.90–6.02).[15] It is soluble in water.

Table 1 summarises the results of proximate analysis of five batches of the additive.[16] The solvent represents about 97.8% of the additive leaving a dry matter (DM) content of about 2.2%. The dry matter consists of inorganic material measured as ash (24.6%) and a plant-derived organic fraction of 75.4%, which includes protein, lipids and 'carbohydrates'.

Table 1: Proximate analysis of a tincture derived from sweet fennel fruit (sweet fennel tincture) based on the analysis of five batches (mean and range in %, w/w)

Constituent	Mean	Range
	% (w/w)	% (w/w)
Dry matter	2.16	1.79–2.75
Ash	0.53	0.46–0.60
Organic fraction	1.63	1.33–2.15
Proteins	0.27	0.13–0.29
Lipids	0.017	0.012–0.023
'Carbohydrates'(1)	1.34	1.19–1.75
Solvent	97.84	97.25–98.21

(1): 'Carbohydrates' (by difference) include secondary plant metabolites, such as phenolic compounds.

The constituent defined as 'carbohydrates' in Table 1 describes the fraction of organic matter remaining after subtraction of the values for protein and lipids. It contains a variety of compounds including phenolic compounds, in addition to any carbohydrate present.

The fraction of secondary metabolites was characterised in the same batches of the tincture and the results are summarised in Table 2. The tincture was shown to contain polyphenols (0.0586%) determined by spectrophotometry (at 760 nm) and expressed as gallic acid equivalents, several unidentified flavonoids (0.0052%) separately determined by HPTLC and expressed as chlorogenic acid equivalents[17] and anisaldehyde (0.0035%) determined by HPTLC at 254 nm using a reference standard.[18]

From published literature, it is known that, apart from the components specified in Table 2, phenolic acids, such as tannic acid, gallic acid and chlorogenic acid, have been identified in the fruit of *F. vulgare* Mill. (subspecies and variety not indicated) (Singh et al., 2004). Moreover, according to literature, in sweet fennel fruit, the flavonoids kaempferol-3-*O*-α-L-rhamnoside, isoquercitrin and quercetin-3-*O*-β-D-glucuronide have been detected (Soliman et al., 2002).

[15] Technical dossier/Supplementary information October 2020/Annex_II_3_Results of analysis.
[16] Technical dossier/Supplementary information October 2020.
[17] Technical dossier/Supplementary information October 2020/Section_II_Identity and Annex II_3.
[18] Technical dossier/Supplementary information October 2020/ Annex II_8_Detailed report of anisaldehyde quantification.

The applicant performed a literature search to identify substances of concern in *F. vulgare* and its botanical preparations, essential oils and aqueous and hydroalcoholic extracts.[19] Among the compounds identified, the presence of estragole (1.5–8.1%) in the essential oil from the fruit of *F. vulgare* Mill. ssp. v*ulgare* var. *dulce* (Mill.) Batt. & Trab is reported in the EFSA Compendium of botanicals as substance of concern for *F. vulgare* (EFSA, 2012).[20] Estragole was reported in samples of an essential oil obtained from fresh fruit of *F. vulgare* by hydrodistillation (2.5–8.3%) by Aprotosoaie et al. (2010). No information on substances of concern in hydroalcoholic extracts was retrieved.

The content of anethole (5.25–6.57 mg/kg) and estragole (5.17–6.40 mg/kg) was determined in five batches of the additive by gas chromatography–mass spectrometry (GC–MS).[21] There is no specification defining limit values for undesirable compounds in the tincture.

The identified secondary metabolites account only on average for 3.12% of the dry matter content of the tincture (range: 3.01–3.24%).

Table 2: Characterisation of the fraction of secondary metabolites of a tincture derived from sweet fennel fruit (sweet fennel tincture) based on the analysis of five batches (mean and range, results are expressed as % of the tincture, w/w)

Constituent	Method	Mean	Range
		% (w/w)	% (w/w)
Total polyphenols	Folin–Ciocalteu	0.0586	0.0460–0.0725
Flavonoids	HPTLC	0.0052	0.0036–0.0065
Anisaldehyde	HPTLC	0.0035	0.0030–0.0045
Anethole	GC–MS	0.0006	0.0005–0.0007
Estragole	GC–MS	0.0006	0.0005–0.0006

HPTLC: high-performance thin-layer chromatography; GC–MS: gas chromatography–mass spectrometry.

The applicant controls contamination at the level of the raw material, including knowledge of the cultivation conditions and pesticides applied. Specifications are set with suppliers covering cadmium < 1 mg/kg, mercury < 0.1 mg/kg and lead < 5 mg/kg, pesticides, polycyclic aromatic hydrocarbons (< 10 μg/kg benzo(a)pyrene, < 50 μg/kg for the sum of benzo(a)pyrene, benzo(a)anthracene, benzo (b)fluorantene and chrysene) and microbial contamination.[22] Two certificates of analysis of the raw material (fennel fruit) showing compliance were provided.[23] Estragole (0.11%) and fenchone (0.04%) were determined in one batch. Analysis of impurities in the tincture is made on irregular basis and does not form part of the Hazard Analysis and Critical Control Points (HACCP) Plan.

3.2.2. Stability

The shelf-life of the tincture is declared by the applicant to be at least 36 months when stored in tightly closed containers under standard conditions. No evidence was provided to support this claim.

3.2.3. Conditions of use

The additive is intended for use in feed and in water for drinking for all animal species. The applicant proposes a maximum concentration of 50 mg sweet fennel tincture/kg complete feed for all animal species, except for horses, for which the proposed use is 200 mg/kg complete feed. No use level has been proposed by the applicant for the use in water for drinking.

3.3. Safety

The safety assessment is based on the highest proposed use levels in feed, which are 200 mg/kg complete feed for horses and 50 mg/kg complete feed for all other species.

No studies to support the safety for target animals, consumers or users were performed with the additive under assessment.

[19] Technical dossier/Supplementary information October 2020/Annex II_4_Bibliographic search on the composition of sweet fennel and sweet fennel extracts.
[20] Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals.
[21] Technical dossier/Supplementary information March 2021.
[22] Technical dossier/Supplementary information October 2020/Annex II_6_Fennel seeds (raw material)_COA.
[23] Technical dossier/Supplementary information October 2020/Annex_II_5_ Fennel seeds (raw material)_TDS.

The additive under assessment, sweet fennel tincture, is a mixture consisting of 98.84% (w/w) of a water/ethanol mixture. The concentration of plant derived compounds is about 2.16% (w/w) of the tincture. The dry matter included ash, protein, lipids and carbohydrates, which are not of concern, and are not further considered.

Among the secondary plant metabolites, total phenolic compounds including flavonoids were quantified but not identified. They will be assessed based on considerations at the level of the assessment group (see Section 3.3.3.3). These compounds are readily metabolised and excreted and are not expected to accumulate in animal tissues and products.

The additive contains anisaldehyde [05.015] and anethole with the *trans*-isomer being expected to be prevalent over the *cis*-isomer. Anisaldehyde and *trans*-anethole [04.010] have been evaluated by EFSA for use as a flavour in food and feed (EFSA FEEDAP Panel, 2011, 2012a) and are currently authorised for use in food[24] without limitations and for use in feed (except for *trans*-anethole in fish and poultry)[25] at individual use levels higher than those resulting from the intended use of the tincture in feed.

Anisaldehyde is rapidly absorbed, distributed, metabolised and excreted. From a 42-day study in rat, a no observed adverse effect level (NOAEL) of 20 mg/kg body weight (bw) per day was identified (EFSA FEEDAP Panel, 2012a). *trans*-Anethole has been evaluated as flavouring for food and feed use (WHO, 1999, 2000; EFSA FEEDAP Panel, 2011). It is rapidly absorbed and is mainly metabolised via *O*-demethylation and epoxidation of the side chain, followed by formation of diols in rodents and humans. Some metabolites of *trans*-anethole have given rise to safety concern. The epoxide has been shown to be cytotoxic, hepatotoxic and genotoxic in some studies. However, after reviewing the available data, the Joint FAO/WHO Expert Committee of Food Additives (JECFA) finally concluded in 2000 that *trans*-anethole was unlikely to be genotoxic *in vivo* and set an acceptable daily intake (ADI) of 2 mg/kg bw based on a NOAEL of 300 mg/kg bw in a 90-day study in rats by applying an uncertainty factor (UF) of 200, with the value rounded to one significant figure (WHO, 1999, 2000). The FEEDAP Panel considered that the conclusions for *trans*-anethole can be applied to *cis*-anethole.

Trace concentrations (5.17–6.40 mg/kg) of estragole, a compound with experimentally proven genotoxicity and carcinogenicity in rodents (as reviewed in EMA, 2021), were detected in all batches of the additive. Information on the absorption, distribution, metabolism and excretion and on the toxicology of estragole is summarised in the next sections.

3.3.1. Absorption, distribution, metabolism and excretion of estragole

Estragole is a lipophilic compound and, as such, readily and completely absorbed from the gastrointestinal tract. Phase I metabolism is catalysed by 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 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′-hydroxyestragole. 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 and only a minor portion in urine in the form of several metabolites resulting from hydroxylation in 1′-C and epoxidation at 2′,3′-C followed by ring hydrolysis. In a single study conducted 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 given dose; the majority of the radioactivity was excreted in expired air as

[24] 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.

[25] 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

$^{14}CO_2$ 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 (Zangouras et al., 1981; Anthony et al., 1987, as referenced in EMA, 2021) it is consistently shown that as doses increase the urinary levels of 1′-hydroxyestragole as glucuronide significantly increases.

3.3.2. Toxicology of estragole

Estragole was included in the diet of female CD-1 mice at 0, 2.3 and 4.6 g/kg diet for 12 months. At least 50% of the animals in the exposed groups developed hepatic tumours by 18 months,[26] which were diagnosed as hepatomas types A (hepatocellular adenomas) or B (hepatocellular adenocarcinomas) or mixed types A and B. The animals fed the control diet did not show any hepatic tumour (Miller et al., 1983).

The FEEDAP Panel notes that there is high uncertainty in derivation of a benchmark dose (BMD) lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) for estragole from a carcinogenicity study in CD-1 mice.[27]

Since estragole shares the same mode of action as methyleugenol, both being representatives of the group of *p*-allylalkoxybenzenes, the FEEDAP Panel applies to estragole a $BMDL_{10}$ of 22.2 mg/kg bw per day, derived from a carcinogenicity study in rat with methyleugenol (NTP, 2000) by applying model averaging (Suparmi et al., 2019) (for details, see EFSA FEEDAP Panel, 2022).

3.3.3. Safety for the target species

In the absence of tolerance studies and/or toxicity data from repeated dose studies in laboratory animals performed with the additive under assessment, the approach to the safety assessment of the mixture is based on its individual components or groups of components. For anisaldehyde and anethole, subchronic studies are available, from which a no observed adverse effect level (NOAEL) can be derived. For *p*-allylalkoxybenzenes rodent carcinogenicity studies with methyleugenol are available from which a $BMDL_{10}$ can be derived. For the group assessment of phenolic compounds and flavonoids, in the absence of data, the threshold of toxicological concern (TTC) is applied to derive maximum safe feed concentrations for the whole groups in the tincture (EFSA FEEDAP Panel, 2017b).

3.3.3.1. Anisaldehyde

At the maximum proposed use level of 50 mg sweet fennel tincture/kg complete feed, the highest concentration of anisaldehyde (≤ 0.0045% of the tincture) would be 0.002 mg/kg feed, resulting in an intake of less than 1 μg/kg bw per day for the target species (ranging from 0.012 in ornamental fish to 0.20 μg/kg bw per day in chickens for fattening). For horses, at the maximum proposed use level of 200 mg/kg complete feed, the highest concentration in feed would be 0.009 mg anisaldehyde/kg and the highest intake would be 0.21 μg anisaldehyde/kg bw per day. These concentrations are several orders of magnitude below the concentrations in feed which were considered safe by the FEEDAP Panel in its opinion on chemical group 23, i.e. 1 mg/kg complete feed for all animal species (EFSA FEEDAP Panel, 2012a) based on a NOAEL of 20 mg/kg bw per day derived from a 42-day study in rat, where several effects (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. Therefore, no concern for the target species is expected.

3.3.3.2. Anethole

At the maximum proposed use level of 50 mg sweet fennel tincture/kg complete feed, the highest concentration of anethole (≤ 0.0007% of the tincture) would be 0.0003 mg/kg feed, resulting in an intake of less than 1 μg/kg bw per day for the target species (ranging from 0.002 μg/kg bw per day in ornamental fish to 0.03 μg/kg bw per day in chickens for fattening). For horses, at the maximum proposed use level of 200 mg/kg complete feed, the highest concentration in feed would be 0.001 mg anethole/kg and the highest intake would be 0.03 μg anethole/kg bw per day. These concentrations are several orders of magnitude below the concentrations in feed which were considered safe by the

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

[27] This strain of mice spontaneously develops a high incidence of hepatocellular adenomas and carcinomas, and the relevance of these tumours for human risk assessment is questionable. In addition, BMD modelling with only two dose levels is adding extra uncertainty in the derivation of the $BMDL_{10}$ value.

FEEDAP Panel in its opinion on chemical group 18, i.e. 25 mg/kg complete feed for all animal species except fish, owing to the structural similarity of *trans*-anethole with eugenol, which is used in water as an anaesthetic for fish (EFSA FEEDAP Panel, 2011). The conclusions were based on a NOAEL of 300 mg/kg bw per day derived from a 90-day study in rat with *trans*-anethole (WHO, 2000, Minnema, 1997, unpublished report) based on elevated serum γ-glutamyl transferases. For fish, the available data indicate that *trans*-anethole would be well below (5,000-fold) the maximum acceptable concentration in feed for Cramer Class I (1.5 mg/kg feed for salmonids, EFSA FEEDAP Panel, 2017b). At the concentration of 0.0003 mg/kg complete feed in fish, anaesthetic effects of *trans*-anethole are not expected. Therefore, no concern for the target species is expected.

3.3.3.3. Phenolic compounds including flavonoids

Among the secondary metabolites, 0.0586% are polyphenols including 0.0053% flavonoids.

At the maximum proposed use level of 50 mg sweet fennel tincture/kg complete feed, the highest concentration of the fraction of polyphenols after subtraction of values for flavonoids (≤ 0.066% of the tincture, measured by the Folin–Ciocalteu method) would be 0.033 mg/kg feed. Although the individual compounds were not identified, the occurrence of phenolic acids, such as tannic acid, gallic acid and chlorogenic acid, have to be expected in sweet fennel fruit according to literature (see Section 3.2.1). These compounds are assigned to Cramer Class I. The available data indicate that their concentrations would be well below the maximum acceptable concentration in feed for Cramer Class I (ranging from 0.3 mg/kg feed for poultry to 1.5 mg/kg feed for salmonids and dogs). For horses, at the maximum proposed use level of 200 mg/kg complete feed, the highest concentration of polyphenols would be 0.132 mg/kg feed, which is 10-fold lower than the maximum acceptable concentration of 1.3 mg/kg for Cramer Class I compounds in feed for horses (EFSA FEEDAP Panel, 2017b). Therefore, no concern for the target species arises from polyphenols other than flavonoids in sweet fennel tincture.

At least five flavonoids were detected but not identified and quantified (as chlorogenic acid equivalents) accounting together for ≤ 0.0066% of the tincture. At the maximum proposed use level of 50 mg sweet fennel tincture/kg complete feed this would correspond to 0.0033 mg/kg feed. Although the individual compounds were not identified, flavonoids are assigned to Cramer Class III. The available data indicate that flavonoids would be below the maximum acceptable concentration in feed for Cramer Class III (ranging from 0.02 mg/kg feed for poultry to 0.08 mg/kg feed for salmonids and dogs). For horses, at the maximum proposed use level of 200 mg/kg complete feed the highest concentration of flavonoids would be 0.013 mg/kg feed, which is below the maximum acceptable concentration of 0.07 mg/kg for Cramer Class III compounds in feed for horses. Therefore, the presence of flavonoids is not considered of concern for the target species.

Overall, no concern for the target species arises from the phenolic fraction and the presence of flavonoids.

3.3.3.4. Estragole

Estragole was detected in all batches of the additive (5.17–6.40 mg/kg).

At the maximum proposed use level of 50 mg sweet fennel tincture/kg complete feed, the highest concentration of estragole (0.00064%, measured by GC–MS method) would be 0.3 μg/kg feed, resulting in an intake of less than 0.03 μg/kg bw per day for the target species (ranging from 0.002 in ornamental fish to 0.029 μg/kg bw per day in chickens for fattening). For horses, at the maximum proposed use level of 200 mg/kg feed, the highest concentration would be 1 μg estragole/kg feed and the highest intake would be 0.029 μg estragole/kg bw per day (Table 3).

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 also applied to estragole.

Table 3: Target animal intake of estragole (as μg/kg bw per day) and margin of exposure (MOE) calculated at the maximum proposed use level of the additive in feed for each target animal category

Target species	Daily feed intake	Body weight	Use level	Estragole intake[(a)]	MOE[(b)]
	kg DM/day	kg	mg/kg	μg/kg bw per day	
Chickens for fattening	0.158	2	50	0.029	772,785
Laying hens	0.106	2	50	0.019	1,151,887
Turkey for fattening	0.176	3	50	0.021	1,034,746
Piglet	0.88	20	50	0.016	1,387,500
Pig for fattening	2.2	60	50	0.013	1,650,000
Sow lactating	5.28	175	50	0.011	2,035,001
Veal calf (milk replacer)	1.89	100	50	0.006	3,213,159
Cattle for fattening	8	400	50	0.007	3,052,501
Dairy cows	20	650	50	0.011	1,969,355
Sheep/goat	1.2	60	50	0.007	3,052,501
Horse	8	400	200	0.029	763,125
Rabbit	0.1	2	50	0.018	1,221,000
Salmon	0.0021	0.12	50	0.006	33,916,68
Dog	0.25	15	50	0.006	3,591,177
Cat	0.06	3	50	0.007	3,052,501
Ornamental fish	0.00054	0.012	50	0.002	12,210,003

DM: dry matter; bw: body weight.
(a): The values of estragole in feed is calculated considering that estragole is present at a concentration corresponding to the maximum analysed value in the additive (6.4 mg estragole/kg tincture).
(b): The MOE for estragole is calculated as the ratio of the reference point ($BMDL_{10}$) to the intake.

When the estimated exposures for the different animal categories to estragole are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day, a MOE of at least 760,000 is calculated (Table 3). The magnitude of this MOE is indicative of a low concern for the target species.

3.3.3.5. Conclusions on safety for the target species

The additive under assessment, sweet fennel tincture, is safe up to maximum proposed use levels of 200 mg/kg complete feed for horses and 50 mg/kg complete feed for all other animal species.

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

3.3.4. Safety for the consumer

Sweet fennel fruit and their preparations including ethanolic extracts are added to a wide range of food categories as spice or for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites values of 0.032 mg/kg bw per day for 'fennel sweet' and 0.006 mg/kg bw per day for fennel sweet oil.

No data on residues in products of animal origin were made available for any of the constituents of the tincture. When considering the ADME of the individual components, the phenolic compounds, including flavonoids, present in the additive at concentrations below the thresholds for Cramer Class I compounds or Cramer Class III compounds, respectively, will be readily metabolised and excreted and are not expected to accumulate in animal tissues and products. Similarly, for anisaldehyde and anethole, the available data indicate that they are absorbed, metabolised and rapidly excreted and are not expected to accumulate in animal tissues and products. Although the FEEDAP Panel could not conclude on the safety for the consumer of *trans*-anethole when used as a feed additive in poultry species at the proposed use level of 25 mg/kg complete feed (EFSA FEEDAP Panel, 2011), the administration of 0.0003 mg/kg of anethole to poultry species is considered of no concern for the consumer. For estragole, not detected but possibly occurring at a concentration between the limit of

detection (LOD) and the limit of quantification (LOQ), the available data indicate that it is absorbed, metabolised and rapidly excreted and is not expected to accumulate in animal tissues and products (see Section 3.3.1).

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

Consequently, no safety concern would be expected for the consumer from the use of sweet fennel tincture up to the maximum proposed use levels in feed.

3.3.5. Safety for the user

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

The applicant provided information according to Classification, Labelling and Packaging (CLP) Regulation (EC) 1272/2008[28] concerning the presence of ethanol in the tincture.[29]

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

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

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

3.3.6. Safety for the environment

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

3.4. Efficacy

F. vulgare Mill. var *dulce* and its preparations are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009), by the Flavour and Extract Manufactures Association (FEMA) with the reference numbers 2,482 (fennel, sweet) and 2,483 (fennel sweet oil).

Since 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

Sweet fennel tincture from *F. vulgare* Mill. ssp. v*ulgare* var. *dulce* (Mill.) Batt. & Trab. may be produced from plants of different origins and by various processes resulting in preparations with different composition and toxicological profiles. Thus, the following conclusions apply only to sweet fennel tincture which contains ≤ 6.4 mg/kg estragole and is produced from the fruit of *F. vulgare* Mill. ssp. v*ulgare* var. *dulce* (Mill.) Batt. & Trab.

The additive is safe at the maximum proposed use levels of 200 mg/kg complete feed for horses and 50 mg/kg complete feed for all other animal species. The FEEDAP Panel considers that the use of the additive in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount which is considered safe when consumed via feed.

No safety concern would arise for the consumer from the use of sweet fennel tincture up to the maximum proposed use levels in feed.

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

The use of sweet fennel tincture as a flavour in animal feed is not expected to pose a risk for the environment.

[28] Regulation (EC) No 1272/2008 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. 1–1355.

[29] H319: causes serious eye irritation (relevant for dermal exposure).

Since 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 for the tincture under assessment.

5. Recommendation

The specification should ensure that the estragole concentrations should be as low as possible and should not exceed 6.4 mg/kg sweet fennel tincture.

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. 7,150,727) 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) - Scientific assessment remains suspended
22/06/2022	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 and safety for target species*
29/08/2022	Reception of supplementary information from the applicant (partial submission) - Scientific assessment remains suspended
16/09/2022	The application was split and a new EFSA-Q-2022-00569 was assigned to the preparation included in the present assessment
31/10/2022	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives - Scientific assessment re-started
22/11/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment for the preparation included in the present assessment. The assessment of other preparations is still ongoing

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Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
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
CDG	chemically defined group
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CLP	Classification, Labelling and Packaging
CYP450	cytochrome P450
DM	dry matter
EEIG	European economic interest grouping
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavour and Extract Manufactures Association
FFAC	Feed Flavourings authorisation Consortium of FEFANA (EU Association of Specialty Feed Ingredients and their Mixtures)
FLAVIS	The EU Flavour Information System
GC–MS	gas chromatography–mass spectrometry
HACCP	Hazard Analysis and Critical Control Points
HPTLC	high-performance thin-layer chromatography
JECFA	Joint FAO/WHO Expert Committee of Food Additives
LOD	limit of detection
LOQ	limit of quantification
MOE	margin of exposure
NOAEL	no observed adverse effect level
NTP	national toxicology program
sb	solvent-based
SC	EFSA Scientific Committee
TTC	threshold of toxicological concern
UF	uncertainty factor
WHO	World Health Organization