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Safety and efficacy of a feed additive consisting of a tincture derived from the fruit of *Illicium verum* Hook f. (star anise tincture) 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 a tincture from the fruit (pericarps and seeds) of *Illicium verum* Hook f. (star anise tincture) when used as a sensory feed additive for all animal species. The product is a [redacted] solution, with a dry matter content of approximately 1.86%. The product contained on average 0.2588% polyphenols (of which 0.0229% were flavonoids, including 0.0036% rutin), anethole (0.018%) and estragole (0.00039%). The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the star anise 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 star anise tincture up to the maximum proposed use levels in feed. Star anise tincture should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser. When handling the tincture, 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 star anise tincture as a flavour in animal feed was not expected to pose a risk for the environment. Since the fruit of *I. verum* and its preparations are recognised to provide flavour in food and their function in feed would be essentially the same, no demonstration of efficacy was considered necessary.

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

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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)[2,3] 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: star anise tincture (*Illicium verum* Hill f.) 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 star anise tincture (*I. verum*), when used under the proposed conditions of use (see Section 3.2.2).

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

1.2. Additional information

A tincture from *Illicium verum* Hook (anise star 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.

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[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 – 35221 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 *I. verum* 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.'

''Star anise' (Anisi stellati fructus) are described in a monograph of the European Pharmacopoeia 10.0 (PhEur, 2020). They are defined as the dried composite fruit of *Illicium verum* Hooker fil. 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.

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

In 2005, the European Medicines Agency (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).

The main identified individual component of star anise tincture is anethole (1-methoxy-4-(1-propenyl)benzene, a compound identified with the EU Flavour Information System (FLAVIS) number [04.088]). *trans*-Anethole [04.010], which is known to be prevalent over *cis*-anethole in the fruit of *Illicium verum*,[9] has 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 chemical group 18, the FEEDAP Panel concluded that *trans*-anethole 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. Although *trans*-anethole was considered safe for the consumer, the lack of data on metabolism and residues in poultry precluded an assessment of consumer exposure from this source. *trans*-Anethole was 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 group 18 (EFSA FEEDAP Panel, 2011).

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 star anise tincture from *I. verum* 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 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.[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 star anise 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 anethole in the feed additive).[12]

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[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). Star anise oil, as described in the European Pharmacopoeia 10.0 (PhEur, 2020), is specified to contain 86–93% of *trans*-anethole and 0.1%–0.5% of *cis*-anethole.

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

[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.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of star anise tincture from *I. verum* 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, 2012a), guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), 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, star anise tincture, is derived from the fruit of *Illicium verum* Hook f. 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 fruit 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 is commonly referred to as star anise, reflecting both their shape and their sensory profile, which closely resembles that of true anise (*Pimpinella anisum* L.). For this reason, star anise is often used as a cheaper substitute for anise 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 tincture is produced from the fruit (pericarp and seeds) of *I. verum* by extended extraction for 3 weeks under ambient conditions with a [redacted] and a plant to solvent ratio of [redacted]. The tincture is then 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 strong anise odour. It has an average density of [redacted] and a pH of 4.38 (4.33–4.46).[15] It is soluble in water.

[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.

[15] Technical dossier/Supplementary information October 2020/Annex_II_3_Results of analysis.

Table 1 summarises the results of proximate analysis of five batches of the additive.[16] The solvent represents about 98.1% of the additive leaving a dry matter (DM) content of about 1.86%. The dry matter consists of inorganic material measured as ash (10.2%) and a plant-derived organic fraction of 89.8%, which includes protein, lipids and 'carbohydrates'.

Table 1: Proximate analysis of a tincture derived from the fruit of *Illicium verum* Hook f. based on the analysis of five batches (mean and range in %, w/w)

Constituent	Mean % (w/w)	Range % (w/w)
Dry matter	1.85	1.57–2.17
Ash	0.19	0.18–0.20
Organic fraction	1.67	1.38–1.97
Proteins	0.04	0.03–0.05
Lipids	0.012	0.009–0.018
'Carbohydrates'	1.62	1.34–1.91
Solvent	98.14	97.83–98.43

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 plant-derived 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 (0.26%) determined by spectrophotometry (at 760 nm) and expressed as gallic acid equivalents, and at least three unidentified flavonoids (0.023%) separately determined by high-performance thin layer chromatography (HPTLC) expressed as rutin equivalents, including rutin (0.0036%).[17] The concentrations of anethole (0.0015%–0.0127%, *trans*-anethole being expected to be prevalent) were also determined by HPTLC (after extraction with hexane) of the same five batches of the tincture.[18]

From published literature, it is known that, apart from the components specified in Table 2, phenolic acids, such as ferulic acid, caffeic acid and gallic acid, have been identified in the fruit of *I. verum* (e.g. Aly et al., 2016).

The applicant performed a literature search to identify substances of concern in *I. verum* and its botanical preparations, essential oils and aqueous and hydroalcoholic extracts.[19] 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).[20] The literature search performed by the applicant confirmed that *trans*-anethole is the major component of the essential oil. The presence of estragole in essential oils from the fruit of *I. verum* (1%–2%) was reported by two references (Padmashree et al., 2007; Aly et al., 2016), whereas that of safrole was not reported. No information on substances of concern in ethanol–water extracts was retrieved.

The content of anethole (172–181 mg/kg) and estragole (3.9 mg/kg) was determined in five batches of the additive by gas-chromatography mass spectrometry (GC–MS). In the same batches, safrole was not detected (limit of detection, 10 mg/kg).[21] There is no specification defining limit values for undesirable compounds in the tincture.

The identified secondary metabolites account only on average for 3.9% of the dry matter content of the tincture (range: 3.43%–4.18%).

[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 anethole quantification.
[19] Technical dossier/Supplementary information October 2020/Annex II_4_Bibliographic data concerning chemical composition of star anise and star anise extracts.
[20] Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals.
[21] Technical dossier/Supplemnetary information March 2021.

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

Constituent	Method	Mean % (w/w)	Range % (w/w)
Total polyphenols	Folin–Ciocalteu	0.259	0.211–0.313
Flavonoids	HPTLC	0.023	0.022–0.024
Rutin	HPTLC	0.0036	0.0024–0.0044
Anethole(*)	GC–MS	0.0177	0.0172–0.0182
Estragole	GC–MS	0.00039	0.00039
Safrole(#)	GC–MS	–	n.d.

(*): A mixture of *trans*-anethole and *cis*-anethole, the trans-isomer being expected to be prevalent.
(#): n.d.; Limit of detection (LOD) 10 mg/kg (0.001%).

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 and microbial contamination.[22] Three certificates of analysis of the raw material (star anise seeds) showing compliance with these requirements were provided.[23] Analysis of impurities in the tincture is made on irregular basis and does not form part of the Hazard Analysis and Critical Control Points Plan (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 star anise 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 and users were performed with the additive under assessment.

The additive under assessment, star anise tincture, is a mixture consisting of 98.15% (w/w) of a water/ethanol mixture. The concentration of plant-derived compounds is about 1.85% (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 3.3.3.2). These compounds are readily metabolised and excreted and are not expected to accumulate in animal tissues and products.

The additive contains anethole, with the *trans*-isomer being expected to be prevalent. *trans*-Anethole [04.010] has been evaluated by EFSA for use as a flavour in food and feed (EFSA FEEDAP Panel, 2011) and is currently authorised for use in food[24] without limitations and for use in feed

[22] Technical dossier/Supplementary information October 2020/Annex II_6_Star anise (raw material)_COA.
[23] Technical dossier/Supplementary information October 2020/Annex_II_5_ Star anise (raw material)_TDS.
[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.

(except for fish and poultry)[25] at individual use levels higher than those resulting from the intended use of the tincture in feed.

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 the 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 and 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 body weight (bw) based on an 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.

The additive contains estragole (3.9 mg/kg), a compound with experimentally proven genotoxicity and carcinogenicity in rodents (as reviewed in EMA, 2021), that was detected in all batches of the additive. Although not detected in samples provided by the applicant, there is evidence from the literature that safrole is present in essential oils from the fruit of *I. verum*. Information on the absorption, distribution, metabolism and excretion (ADME) and on the toxicology of estragole and safrole is summarised in the next sections.

3.3.1. Absorption, distribution, metabolism and excretion of estragole and safrole

Estragole is a lipophilic compound and, as such, readily and completely absorbed from the gastrointestinal tract. Phase I metabolism is catalysed by Cytochromes P450 (CYP450) enzymes mainly in the liver. Demethylation of the 4-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 $^{14}CO_2$ 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.

Similar metabolic pathways have been described for safrole (EC, 2002; WHO, 2009) and other structurally related p-allylalkoxybenzenes.

3.3.2. Toxicology of estragole and safrole

Estragole and safrole are compounds with experimentally proven genotoxicity and carcinogenicity in rodents (EC, 2002; WHO, 2009; as reviewed in EMA, 2021).

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

[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.

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

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).

Similarly, safrole was included in the diet of female CD-1 mice at 0, 2.5 and 5.0 g/kg diet (corresponding to 58 and 117 mg/kg bw per day) for 12 months. At least 70% of the animals in the exposed groups developed hepatic tumours by 18 months,[27] which were diagnosed as hepatomas types A (hepatocellular adenomas) or B (hepatocellular adenocarcinomas) or mixed types A and B. The animals which were fed with the control diet did not show any hepatic tumour (Miller et al., 1983).

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 and safrole from a carcinogenicity study in CD-1 mice.[28]

Since estragole and safrole share 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 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. Anethole

At the maximum proposed use level of 50 mg star anise tincture/kg complete feed, the highest concentration of anethole ($\leq$ 0.018% of the tincture, measured by GC–MS method) would be up to 0.009 mg/kg feed, resulting in an intake of less than 1 μg/kg bw per day for the target species (ranging from 0.06 μg/kg bw per day in ornamental fish to 0.8 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 0.036 mg anethole/kg and the highest intake would be 0.8 μg anethole/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 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 an NOAEL of 300 mg/kg bw per day derived from a 90-day study in rat with *trans*-anethole (Minnema, 1997, unpublished report; WHO, 2000) based on elevated serum γ-glutamyl transferases. For fish, the available data indicate that *trans*-anethole would be well below (> 160-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 0.009 mg/kg complete feed in fish, anaesthetic effects of *trans*-anethole are not expected. Therefore, no concern for the target species is expected.

3.3.3.2. Phenolic compounds including flavonoids

Among the secondary metabolites, 0.259% are polyphenols including 0.0036% flavonoids.

At the maximum proposed use level of 50 mg star anise tincture/kg complete feed, the highest concentration of the fraction of polyphenols after subtraction of values for flavonoids ($\leq$ 0.290% of the tincture, measured by the Folin–Ciocalteu method) would be 0.145 mg/kg feed. Although the individual compounds were not identified, the occurrence of phenolic acids, such as ferulic acid, caffeic acid and gallic acid, has been described in literature for fruit of *I. verum* (see Section 3.2.1). These compounds are assigned to Cramer Class I and the available data indicate that their concentration

[27] Incidence of hepatomas in female mice (0/50, 34/50, 39/50).

[28] 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.

would be 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 0.579 mg/kg feed, which is below the maximum acceptable concentration of 1.3 mg/kg for Cramer Class I compounds in feed for horses. Therefore, no concern for the target species arises from polyphenols other than flavonoids in star anise tincture.

At least three flavonoids (two unidentified and rutin) were detected and quantified (as rutin equivalents) accounting together for ≤ 0.0044% of the tincture. At the proposed use level of 50 mg star anise tincture/kg complete feed, this would correspond to 0.0022 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 concentrations 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 concentrations of flavonoids would be 0.009 mg/kg feed, which is eightfold lower than 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.

3.3.3.3. Estragole and safrole

Estragole was detected in all batches of the additive (3.9 mg/kg) and safrole was below the LOD of 10 mg/kg.

At the maximum proposed use level of 50 mg star anise tincture/kg complete feed, the highest concentration of estragole (0.00039%, measured by GC–MS method) would be 0.2 μg/kg feed, resulting in an intake of less than 0.02 μg/kg bw per day for the target species (ranging from 0.001 in ornamental fish to 0.018 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 0.8 μg estragole/kg feed and the highest intake would be 0.018 μg estragole/kg bw per day.

At the maximum proposed use level of 50 mg tincture/kg in feed, a concentration of safrole in the additive corresponding to the LOD would lead to a concentration of 0.5 μg/kg complete feed (2 μg/kg for horses at the use level of 200 mg tincture/kg complete feed). The intake of the target animals (as μg/kg bw per day) at the maximum proposed use level of 50 mg/kg complete feed would range between 0.0026 and 0.045 μg safrole/kg bw per day (0.046 μg safrole/kg bw per day for horses).

Estragole and safrole belong to the same structural group (p-allylalkoxybenzenes) and share the same metabolic pathways, particularly the formation of the reactive 1′-sulfoxymetabolite (see Section 3.3.1) and the same mode of action.

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 and safrole. When the estimated combined exposures to estragole and safrole for the different animal categories are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day, a combined margin of exposure (MOET) is calculated for the different target species.

The highest daily intake of estragole and safrole for the different target animal categories and the corresponding MOET are reported in Table 3.

Table 3: Compositional data, intake values (calculated for chickens for fattening at 50 mg/kg complete feed), reference points and margin of exposure (MOE) for estragole and safrole (if present in the additive at the corresponding limit of detection) and combined margin of exposure (MOET) for the assessment group p-allylalkoxybenzenes

Composition		**Exposure**		**Hazard characterisation**	**Risk characterisation**	
Assessment group	**Highest conc. in the tincture**	**Highest feed conc.**	**Highest intake[(a)]**	**$BMDL_{10}$**	**MOE**	**MOET**
Constituent	**mg/kg**	**µg/kg**	**µg/kg bw per day**	**mg/kg bw per day**	–	–
p-Allylalkoxybenzenes						
Estragole	3.9	0.20	0.018	22.2	1,268,100	
Safrole	10.0	0.05	0.045	22.2	494,582	
MOET						355,815

(a): Intake calculations for the individual components are based on the use level of 50 mg/kg in feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point ($BMDL_{10}$) 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.

From the MOET for chickens for fattening, the MOET for p-allylalkoxybenzenes was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 4.

Table 4: Combined exposure and combined margin of exposure (MOET) for the assessment group p-allylalkoxybenzenes calculated at the maximum proposed use level of the additive in feed for target animal category

Animal category	**Daily feed intake**	**Body weight**	**Use level**	**Combined intake**	**MOET**
	kg DM/day	**kg**	**mg/kg**	**µg/kg bw per day**	–
Chicken for fattening	0.158	2	50	0.062	355,815
Laying hen	0.106	2	50	0.042	530,366
Turkey for fattening	0.176	3	50	0.046	476,430
Piglet	0.88	20	50	0.035	638,850
Pig for fattening	2.2	60	50	0.029	759,713
Sow lactating	5.28	175	50	0.024	936,980
Veal calf (milk replacer)	1.89	100	50	0.015	1,479,441
Cattle for fattening	8	400	50	0.016	1,405,469
Dairy cow	20	650	50	0.024	906,754
Sheep/goat	1.2	60	50	0.016	1,405,469
Horse	8	400	200	0.063	351,367
Rabbit	0.1	2	50	0.039	562,188
Salmon	0.0021	0.12	50	0.014	1,561,633
Dog	0.25	15	50	0.013	1,653,493
Cat	0.06	3	50	0.016	1,405,469
Ornamental fish	0.00054	0.012	50	0.004	5,621,877

When the estimated exposures for the different animal categories to the p-allylalkoxybenzenes 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, see Section 3.2.2), an MOET of at least 350,000 is calculated (see Table 4). The magnitude of this MOET is indicative of a low concern for the target species.

3.3.3.4. Conclusions on safety for the target species

The additive under assessment, star anise 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

The fruit of *I. verum* and its 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 0.001 mg/kg bw per day for star anise fruit and 0.006 mg/kg bw per day for star anise 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 anethole, the available data indicate that it is 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 0.009 mg/kg of anethole to poultry species is considered of no concern for the consumer. For estragole and safrole (not detected but possibly occurring at a concentration below the LOD), the available data indicate that they are absorbed, metabolised and rapidly excreted and are 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 star anise fruit and its preparations in food (Burdock, 2009), it is unlikely that the consumption of products from animals given anise 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 star anise 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[29] concerning the presence of ethanol in the tincture.[30]

The additive contains anethole, a compound for which hazards for skin and eye contact and respiratory exposure were recognised (EFSA FEEDAP Panel, 2011).

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

When handling the tincture, 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

Although *I. verum* is a not native species to Europe, flavonoids and polyphenols, the most abundant components in the tincture are naturally occurring in European plants. 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

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).

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[29] 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, p. 1–1355.

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

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 tincture from *Illicium verum* Hook f. 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 star anise tincture which contains ≤ 3.9 mg/kg estragole and ≤ 10 mg/kg safrole and is produced from the fruit (or seeds) of *I. verum*.

The additive is safe at the maximum proposed use level 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 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 star anise 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 tincture, 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 star anise tincture as a flavour in animal feed is not expected to pose a risk for 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 tincture under assessment.

5. Recommendation

The specification should ensure that the concentrations of estragole and safrole should be as low as possible and should not exceed 3.9 and 10 mg/kg star anise tincture, respectively.

6. Documentation provided to EFSA/Chronology

Date	Event
28/10/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 02 – Apiales and Austrobaileyales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)
09/11/2010	Reception mandate from the European Commission
26/02/2013	EFSA informed the applicant (EFSA ref. 7150727) that, in view of the workload, the evaluation of applications on feed flavourings would be re-organised by giving priority to the assessment of the chemically defined feed flavourings, as agreed with the European Commission
24/06/2015	Technical hearing during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products": data requirement for the risk assessment of botanicals
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract, and opoponax oil
24/06/2019	Application validated by EFSA – Start of the scientific assessment
03/07/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization, safety for the target species, safety for the consumer, safety for the user, safety for the environment*
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

Date	Event
16/09/2022	The application was split and a new EFSA-Q-2022-00571 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

References

Aly SE, Sabry BA, Shaheen MS and Hathout AS, 2016. Assessment of antimycotoxigenic and antioxidant activity of star anise (*Illicium verum*) *in vitro*. Journal of the Saudi Society of Agricultural Sciences, 15, 20–27. https://doi.org/10.1016/j.jssas.2014.05.003

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

Burdock GA, 2009. Fenaroli's handbook of flavor ingredients. 6th Edition. CRC Press, Taylor & Francis Group, Boca Raton, FL. pp. 1851–1852. https://doi.org/10.1201/9781439847503

EC (European Commission), 2002. Opinion of the Scientific Committee on Food on the safety of the presence of safrole (1-allyl-3,4-methylene dioxy benzene) in flavourings and other food ingredients with flavouring properties. Adopted on 12 December 2001. SCF/CS/FLAV/FLAVOUR/6 ADD3 Final 9 January 2002. Available online: https://ec.europa.eu/food/system/files/2016-10/fs_food-improvement-agents_flavourings-out116.pdf

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

EMA (European Medicines Agency), 2000. Committee for Veterinary Medicinal Products. Anisi stellati fructus. Summary report. EMEA/MRL/710/99_FINAL. Available online: https://www.ema.europa.eu/en/documents/mrl-report/anisi-stellati-fructus-summary-report-committee-veterinary-medicinal-products_en.pdf

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

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

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

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

Padmashree A, Roopa N, Semwal AD, Sharma GK, Agathian G and Bawa AS, 2007. Star anise (*Illicium verum*) and black caraway (*Carum nigrum*) as natural antioxidants. Food Chemistry, 104, 59–66. https://doi.org/10.1016/j.foodchem.2006.10.074

PhEur (European Pharmacopoeia), 2020. Star anise (Anisi stellati fructus). 10th Edition. European Pharmacopoeia. Monograph 01/2008:1153. European Directorate for the Quality of Medicines and Health.

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

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

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

WHO (World Health Organization), 1999. WHO Food Additives Series: 42. Safety evaluation of certain food additives. Prepared by the fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva, 1998. Available online. https://apps.who.int/iris/handle/10665/42216

WHO (World Health Organization), 2000. Evaluation of certain food additives. Fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO technical report series, no. 891. Geneva, 9–18 June 1998. https://apps.who.int/iris/handle/10665/42245

WHO (World Health Organization), 2009. WHO food additives series 60: Safety evaluation of certain food additives. Prepared by the 69th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO, Geneva, pp. 351–460. Available online: https://inchem.org/documents/jecfa/jecmono/v60je01.pdf

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
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
JECFA	Joint FAO/WHO Expert Committee of Food Additives
GC–MS	gas chromatography–mass spectrometry
HACCP	Hazard Analysis and Critical Control Points
HPTLC	high performance thin layer chromatography
LOD	limit of detection
LOQ	limit of quantification
MOE	margin of exposure
MOET	combined margin of exposure (total)
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