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Safety and efficacy of a feed additive consisting of a preparation of essential oils of thyme and star anise, and quillaja bark powder (BIOSTRONG® 510 all natural) for all poultry species (Delacon Biotechnik GmbH)

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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 preparation consisting of essential oils of thyme and star anise, and quillaja bark powder (BIOSTRONG® 510 all natural) as a zootechnical feed additive (functional groups: digestibility enhancer; other zootechnical additives) for all poultry species. BIOSTRONG® 510 all natural is a preparation of partially microencapsulated essential oils, quillaja bark powder, dried herbs and dried spices. The additive contains estragole (up to [redacted]. For short-living animals, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) had no safety concerns when the additive is used at the recommended level of 150 mg/kg complete feed for chickens for fattening and other poultry species for fattening. For long-living animals, the use of the additive was considered of concern owing to the presence of estragole. No safety concern would be expected for the consumer and the environment from the use of the additive at the recommended use level in feed. The Panel concluded that the additive is corrosive to the eyes but not irritant to skin. It may be a respiratory irritant or dermal or respiratory sensitiser. When handling the additive, exposure of unprotected users to estragole [redacted] may occur. Therefore, to reduce the risk, the exposure of the users should be minimised. The additive BIOSTRONG® 510 all natural was considered efficacious for chickens for fattening at the use level 150 mg/kg complete feed. This conclusion was extrapolated to all poultry species for fattening or reared for laying/breeding. [redacted]

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Keywords: zootechnical additives, digestibility enhancers, BIOSTRONG® 510 all natural, thyme oil, star anise oil, estragole, poultry

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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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Delacon Biotechnik GmbH[2] for the authorisation of the additive consisting of a preparation of essential oils of thyme and star anise, and quillaja bark powder (BIOSTRONG® 510), when used as a feed additive for all avian species (category: zootechnical additives; functional groups: digestibility enhancer, other zootechnical additives).

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). The particulars and documents in support of the application were considered valid by EFSA as of 9 February 2022.

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 feed additive consisting of a preparation of essential oils of thyme and star anise, and quillaja bark powder (BIOSTRONG® 510), when used under the proposed conditions of use (see **Section 3.1.6**).

1.2. Additional information

In 2016, EFSA issued an opinion on the safety and efficacy of BIOSTRONG® 510, a feed additive containing thyme essential oil, quillaja bark powder, dried spices and herbs and a synthetic mixture of compounds mimicking star anise oil. The additive was intended for use in feed for chickens and minor avian species for fattening and rearing to the point of lay (EFSA FEEDAP Panel, 2016a). This additive is currently authorised for use in feed for chickens for fattening, chickens reared for laying and minor avian species for fattening and reared for laying (EC 4d15).

The present application is for an additive 'BIOSTRONG® 510 all natural' with essentially the same qualitative and quantitative composition, except that the synthetic mixture of compounds mimicking the star anise oil is replaced with the natural product, star anise oil [redacted] from the fruit of *Illicium verum* L. The current application is intended for all poultry species.

In 2019, the EFSA Panel on Food Additives and Flavourings (FAF) issued an opinion on the re-evaluation of Quillaia extract (E 999) as a food additive and safety of the proposed extension of use (EFSA FAF Panel, 2019).

Many of the individual volatile components of the additive under assessment have been already assessed as chemically defined flavourings for use in feed and food by the FEEDAP Panel, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) and the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). The list of flavouring compounds currently authorised for food[3] and feed[4] uses together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000[5] and the corresponding EFSA opinion is given in Table 1.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Delacon Biotechnik GmbH, Langwiesen 24, 4209, Engerwitzdorf, Austria.
[3] 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.
[4] 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
[5] Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8.

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

CG	Chemical group	Product (EU register name)	FLAVIS no	EFSA opinion,* year
06	Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters, with esters containing tertiary alcohols ethers	Linalool	02.013	2012a
		α-Terpineol	02.014	
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	d-Camphor[(a)]	07.215	2016b
16	Aliphatic and alicyclic ethers	1,8-Cineole	03.001	2012b, 2021a
18	Allylhydroxybenzenes	1-Methoxy-4-(prop-1(trans)-enyl)benzene (*trans*-anethole)	04.010	2011
		1-Methoxy-4-(1-propenyl) benzene (anethole)[(b)]	04.088	
21	Aromatic ketones, secondary	4-Methoxyphenylacetone[(b)]	07.087	EFSA (2008a) (AFC)
23	Benzyl alcohols/aldehydes/acids/esters/ acetals	4-Methoxybenzaldehyde (anisaldehyde)	05.015	2012c
25	Phenol derivatives containing ring-alkyl, ring-alkoxy and side chains with an oxygenated functional group	Thymol	04.006	2012d
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	Limonene[(b),(c)]	01.001	EFSA (2008b) (AFC)
		1-Isopropyl-4-methylbenzene (p-cymene)	01.002	2015
		Terpinolene	01.005	
		γ-Terpinene	01.020	
		Pin-2(10)-ene (β-pinene)	01.003	2016c
		Pin-2(3)-ene (α-pinene)	01.004	
		Myrcene	01.008	
		Camphene	01.009	

(*): FEEDAP opinion unless otherwise indicated.
(a): JECFA and EFSA evaluated the enantiomer d-camphor [07.159] (name in the register (1R,4R)-1,7,7-Trimethylbicyclo[2.2.1] heptan-2-one) for use in food (EFSA, 2008a,b) and in feed (EFSA FEEDAP Panel, 2016a).
(b): Evaluated for use in food only. According to Regulation (EC) 1565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.
(c): JECFA and EFSA evaluated d-limonene [01.045] (EFSA, 2008a,b). d-Limonene [01.045] and l-limonene [01.046] were also evaluated for use in feed (EFSA FEEDAP Panel, 2015).

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[6] in support of the authorisation request for the use of a preparation consisting of essential oils of thyme and star anise, quillaja bark powder and a mixture of herbs (BIOSTRONG® 510, all natural), as a feed additive. The dossier was received on 26 April 2021 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00344.

[6] FEED dossier reference: FAD-2021-0062.

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

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active substance thymol in animal feed are valid and applicable for the current application.[7]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of a preparation consisting of essential oils of thyme and star anise, quillaja bark powder and a mixture of herbs (BIOSTRONG® 510, all natural) is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012e), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b) and general approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021b).[9]

3. Assessment

The additive under assessment is a preparation consisting of essential oils of thyme and star anise, quillaja bark powder and a mixture of herbs (BIOSTRONG® 510 all natural). It is intended for use as zootechnical additive (functional group: digestibility enhancer, other zootechnical additives) in feed for all poultry species.

3.1. Characterisation

3.1.1. Characterisation of the additive

The additive BIOSTRONG® 510 all natural is a beige-brownish fine powder with an aromatic herbal flavour. It consists of [redacted] oils of thyme and star anise, quillaja bark powder, crushed dried herbs and spices and some excipients (Table 2). The applicant proposed a specification for the total essential oil content [redacted], for thymol [redacted], *trans*-anethole ([redacted]), [redacted], quillaja bark powder [redacted] and crushed dried herbs and spices [redacted].[10] Analysis of five batches of the additive showed compliance with these specifications (see Table 2). Quillaja bark (*Quillaja saponaria*) contains [redacted] saponins, which consist primarily of glycosides of quillaic acid.

The loss on drying is [redacted].[11]

[7] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2011-0036_en

[8] 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.

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

[10] Technical dossier/Section II/Table II.2.

[11] Technical dossier/Section II/Annex_II.1.3.1_Conf.

[12] Technical dossier/Section II/Annex_II.1.3.2_Conf.

Table 2: Composition of BIOSTRONG® 510 all natural, as specified and batch to batch variation based on the analysis of five batches

Ingredients	Content (mg/g)	Analysed (mg/g)
Essential oils	[redacted]	[redacted]
Thyme oil	[redacted] (thymol 2–4)	[redacted]
Star anise oil	[redacted] (*trans*-anethole 40–50)	[redacted]
Quillaja bark powder	[redacted]	[redacted]
Crushed dried herbs and spices	[redacted]	
[redacted]	[redacted]	
[redacted]	[redacted]	
[redacted]	[redacted]	
[redacted]	[redacted]	
Excipients	To 100%	
[redacted]	[redacted]	
[redacted]	[redacted]	
[redacted]	[redacted]	
[redacted]	[redacted]	

[redacted]

The applicant provided a characterisation of the volatile components of BIOSTRONG® 510 all natural, by analysis of five batches of the additive by gas chromatography–mass spectrometry (GC–MS).[12] [redacted].

[redacted]

[redacted]	[redacted]	[redacted]	[redacted]	
			[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

The fraction of essential oils contains up to estragole, . The presence of estragole (up to 5%) in the essential oil from the fruit of *I. verum* has been reported in the EFSA Compendium of botanicals.[13] Tisserand and Young (2014) also reported the occurrence of estragole (0.3–6.6%) in star anise oil. Methyleugenol was not detected in the additive (limit of detection, LOD 0.03 μg/g).

Three batches of the additive were analysed for impurities. [14] [15] [16] [17] [18] [19]

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

[13] Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals.
[14] Technical dossier/Section II/Annexes_II.1.4.1.3_Conf.
[15] Technical dossier/Section II/Annexes_II.1.4.1.4_Conf.
[16] Technical dossier/Section II/Annexes_II.1.4.1.2_Conf.
[17] Technical dossier/Section II/Annexes_II.1.4.1.1_Conf.
[18] Technical dossier/Section II/Annexes_II.1.4.1.5_Conf.
[19] Technical dossier/Section II/Table II.10 and Table II.11.

3.1.2. Characterisation of the active substances

The active substances in BIOSTRONG® 510 all natural predominantly derive from the thyme oil, star anise oil and quillaja bark. The crushed herbs and spices are also expected to contribute to the activity of the additive but to a lesser extent. Thymol is selected as the phytochemical marker.

Essential oil of thyme is obtained [redacted] The major component is thymol [redacted] with carvacrol [redacted].

Star anise oil from *Illicium verum* L. is obtained [redacted].[20] The major component is *trans*-anethole [redacted], with *cis*-anethole representing [redacted].

Quillaja bark powder contains [redacted] saponins, which consist primarily of glycosides of quillaic acid. A content of tannins to a maximum of 8% dry matter has been reported for quillaja bark extracts (WHO, 2005). Quillaja extract (E 999) is approved as food additive under Regulation (EU) 1129/2011.[21]

3.1.3. Physical properties of the additive

The additive BIOSTRONG® 510 all natural has a density of 1,000 kg/m^3 and a bulk density of 520 kg/m^3 [redacted], and a foam index of [redacted].

Stauber–Heubach data of three batches indicate a dusting potential ranging between 1.72 and 2.22 g/m^3. The particle size of the dust was measured by laser diffraction in the same batches. The fractions of particles < 1, 10 and 50 μm were 4.35%, 54.6% and 99.9% (v/v), respectively. No particles < 0.1 μm were detected.[22]

3.1.4. Manufacturing process

BIOSTRONG® 510 all natural is manufactured by [redacted][23]

3.1.5. Stability and homogeneity

The stability of the additive and feed containing the additive was assessed by monitoring the content of thymol.

3.1.5.1. Shelf-life

[redacted].[24]

3.1.5.2. Stability

The stability of BIOSTRONG® 510 all natural was tested in three batches of a vitamin/mineral premixture containing choline chloride. [redacted].[23]

The stability of BIOSTRONG® 510 all natural in mash feed was tested in three batches. [redacted].[23]

[20] Technical dossier/Section II/Annex_II.2.2.1.
[21] Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L 295, 12.11.2011, p. 1–177.
[22] Technical dossier/Supplementary information September 2022.
[23] Technical dossier/Section II/ Annex_II.3.1.1_Conf.
[24] Technical dossier/Section II/Annex_II.4.1.2.

3.1.5.3. Homogeneity

The capacity for homogeneous distribution of the additive in a premixture and in a complete feedingstuff before and after pelleting was measured in 10 subsamples of each taken at random. [redacted].[25]

3.1.6. Conditions of use

BIOSTRONG® 510 all natural is intended for use in feed for all poultry species at a recommended use level of 150 mg/kg complete feed.

3.2. Safety

To support the safety of the additive, the applicant carried out three structured database searches[26] using three single databases including PubMed, CAB Abstract and Veterinary Science Database. The three literature searches on star anise, thyme and Quillaja covered the period from 1999 until October 2019. Specific subject areas were added in order to restrict the search (such as safety for the different target animals, safety for user/workers, safety for consumers and safety for the environment). A detailed description of the iterations used, and the inclusion/exclusion criteria applied for the selection were provided.

The literature searches also addressed the individual components of the additive already evaluated in the previous assessment (α-pinene, anethole, 1,8-cineole and thymol). The literature searches did not identify any concern for the target species, the consumer and the environment.

The literature search addressed also estragole, one of the components of natural star anise oil, and methyleugenol despite it was not detected in the additive. [redacted] The information on the absorption, distribution, metabolism and excretion (ADME) and on the toxicology of estragole and methyleugenol is summarised in the next sections.

3.2.1. Absorption, distribution, metabolism, excretion and residues

Terpenoids

Most of the volatile components of BIOSTRONG® 510 all natural are terpenoids which are expected to be extensively metabolised. Mono and sesquiterpenes are lipophilic compounds, which are expected to be rapidly absorbed from the gastro-intestinal tract and oxidised to polar oxygenated metabolites (by the cytochrome P450 enzymes, alcohol dehydrogenases and aldehyde dehydrogenases). The resulting hydroxylated metabolites may be excreted as glucuronide- and sulfate-conjugates or undergo further oxidation, yielding more polar metabolites that are also excreted in conjugated form in the urine and bile. Oxidation of the double bonds leads to epoxide intermediates which are rapidly detoxified either by hydrolysis to yield diols or by conjugation with glutathione. The enzymes involved in the biotransformation pathways of these compounds are present in all the target species, including poultry species (reviewed in EFSA FEEDAP Panel, 2016c).

Estragole and other p-allylkoxybenzenes

Estragole is a lipophilic compound and, as such, readily and completely absorbed from the gastrointestinal tract in laboratory animals. Phase I metabolism is catalysed by 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 EC, 2001a; 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

[25] Technical dossier/Section II/Annex_II.4.2.
[26] Technical dossier/Section III/Annex_III_1.

on the quantity excreted in urine (Zangouras et al., 1981, Anthony et al., 1987 as referenced in EC, 2001a). 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 EC, 2001a). 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 EC, 2001a), it is consistently shown that as doses increase the urinary levels of 1′-hydroxyestragole as glucuronide significantly increases.

Similar metabolic pathways as for estragole have been described for the other *p*-allylalkoxybenzenes [redacted] and methyleugenol (EC, 2001b, as reviewed in WHO, 2009).

[redacted]

Residue study in laying hens

[redacted][27]

3.2.2. Toxicology

Star anise oil contains estragole ([redacted]) a compound with experimentally proven genotoxicity and carcinogenicity in rodents (as reviewed in EC, 2001; EMA, 2019). [redacted] EFSA compendium of botanicals[13]).

Estragole was included in the diet of female CD-1 mice at 0, 2.3 or 4.6 g/kg diet for 12 months. At least 50% of the animals in the exposed groups developed hepatic tumours by 18 months,[28] which were diagnosed as hepatoma 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, as referenced in EC, 2001a). The FEEDAP Panel notes that there is high uncertainty in deriving 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.[29]

Since estragole [redacted] share the same mode of action as methyleugenol, all belonging to the group of *p*-allylalkoxybenzenes, the FEEDAP Panel applies to estragole a $BMDL_{10}$ of 22.2 mg/kg body weight (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, 2022a). The FEEDAP Panel identified a no observed adverse effect level (NOAEL) of 10 mg/kg bw per day for non-neoplastic lesions (effect on liver and the glandular stomach) from a 90-day study in mice with

[27] Technical dossier/Section III/Annex_III_2_1_Conf.
[28] Incidence of hepatomas in female mice (0/50, 25/50, 35/50).
[29] 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.

methyleugenol (NTP, 2000), which is also applied to estragole and other p-allylalkoxybenzenes (EFSA FEEDAP Panel, 2022b).

3.2.3. Safety for the target species

[redacted]

3.2.3.1. Safety for chickens for fattening

In the previous opinion, the FEEDAP Panel evaluated two tolerance trials in chickens for fattening, both performed using a formulation of the additive containing natural star anise oil, which matches the additive under assessment (EFSA FEEDAP Panel, 2016a).

The FEEDAP Panel concluded that the supplementation of BIOSTRONG® 510 (an additive formulated with a synthetic mixture of compounds mimicking star anise oil, which did not contain estragole) was safe for chickens for fattening at the recommended use level of 150 mg/kg with a margin of safety of 10. The Panel also considered that this conclusion could be extended to chickens reared for laying and extrapolated to all minor poultry species for fattening or reared to point of lay at the same dose (EFSA FEEDAP Panel, 2016a).

3.2.3.2. Safety for laying hens

[redacted][30]

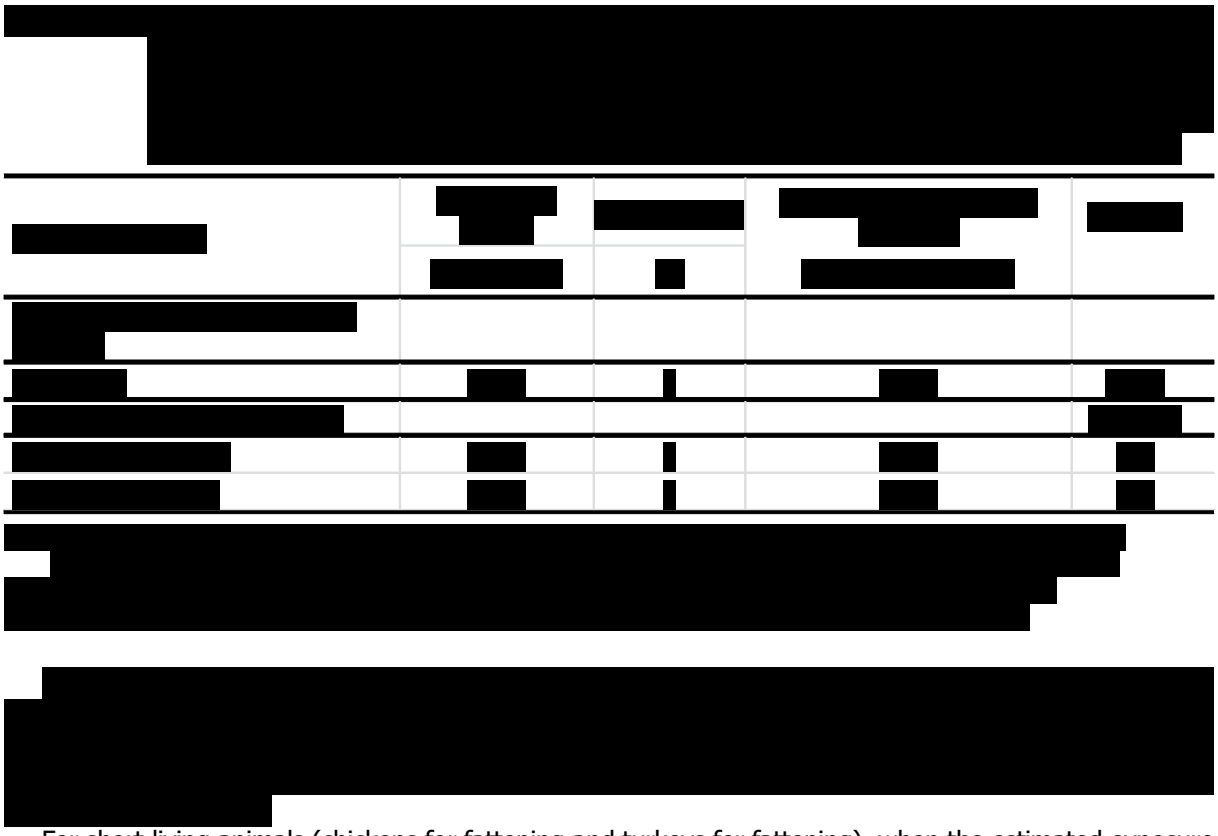
3.2.3.3. Estragole

[redacted]

For short-living animals, genotoxicity and carcinogenicity endpoints are not considered relevant; [redacted]

The highest daily intake of estragole [redacted] at the recommended use level of 150 mg/kg complete feed for the different target animal categories [redacted] are reported in Table 4.

[30] Technical dossier/Section III/Annex_III_1_1.



For short-living animals (chickens for fattening and turkeys for fattening), when the estimated exposure is compared to the NOAEL of 10 mg/kg bw per day for non-neoplastic endpoints identified from a 90-day study in mice with methyleugenol (NTP, 2000, see Section 3.2.2), [redacted] and is of no safety concern. This conclusion is extrapolated to other poultry species for fattening.

3.2.3.4. Conclusions on safety for the target species

Based on the outcome of the tolerance trials in chickens for fattening, the Panel concluded that the additive is tolerated in chickens for fattening or reared for laying/breeding and minor poultry species at the recommended conditions of use. No conclusion could be reached regarding the tolerance for laying hens or breeding hens.

Based on the MOET calculated considering the presence of estragole and [redacted] the FEEDAP Panel concludes that:

For laying hens, the MOET of [redacted] calculated at the recommended use level of 150 mg/kg complete feed is considered of concern (MOET < 10,000). This conclusion applies to breeding birds and birds reared for laying/breeding/reproduction.

For short-living animals, the Panel has no safety concern when the additive BIOSTRONG® 510 all natural is used at the recommended use level of 150 mg/kg complete feed. Taking also into account the results from the tolerance trials, the FEEDAP Panel concludes that the use of the additive at the recommended level is of no safety concern for chickens for fattening and other poultry species for fattening.

3.2.4. Safety for the consumer

Constituents of the additive other than estragole

In the previous opinion (EFSA FEEDAP Panel, 2015), the assessment of consumer safety was based on a formulation of the additive in which star anise oil was replaced by a mixture of pure compounds mimicking the oil, without estragole. In 2015, the FEEDAP Panel concluded that 'Considering the composition of BIOSTRONG® 510, the consumer exposure to any possible residues of the components of essential oils, quillaja bark, and the herbs and spices would be within the range of exposures considered safe for food use' and (...) 'that the use of BIOSTRONG® 510 as an additive in the feed for target animals would not measurably increase the exposure of consumers to any component of the additive and, therefore, would not present a risk for the consumer.'

The same conclusions apply to the individual volatile components present in BIOSTRONG® 510 all natural. [redacted].[31] [redacted] These results are consistent with those described in the former opinion for the residue studies in chickens for fattening, which showed that thymol, *trans*-anethole and α-pinene were not detected in meat and liver samples (EFSA FEEDAP Panel, 2016a).

Estragole

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

Similar to thymol, *trans*-anethole and α-pinene, the ADME data available for estragole 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). [redacted] suggesting that there is no carry over to tissues and products.

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 consumption of products from animals given the additive at the proposed maximum use level would cause a meaningful increase of human background exposure.

Consequently, no safety concern would be expected for the consumer from the use of BIOSTRONG® 510 all natural at the recommended use level in feed for all poultry species.

3.2.5. Safety for the user

3.2.5.1. Effect on respiratory system

Exposure of the respiratory system of users handling BIOSTRONG® 510 all natural can occur by inhalation of volatile components as well as dust of the additive. The dusting potential of the additive according to Stauber–Heubach is up to 2.2 g/m^3, and the particle size of the dust indicate that ~ 50% of the particles have a diameter below 10 μm and about 5% below 1 μm, which suggest a high possibility of exposure during handling. Thus, it is likely that the respiratory tract of users is exposed to the dust.

The additive contains components with a potential to irritate mucous membranes of the respiratory tract and to cause inflammation of lung tissue (saponins, [redacted]).

BIOSTRONG® 510 all natural also contains a variety of components with the potential to induce allergic reactions (dried crushed herbs, essential oils). This assumption is confirmed by experience of the applicant, who classifies the additive as a respiratory sensitiser on the basis of manufacturing experience.

3.2.5.2. Effect on eyes and skin

A Bovine Corneal Opacity and Permeability Test was conducted with BIOSTRONG® 510 all natural according to Organisation for Economic Co-operation and Development (OECD) Technical guidance (TG) 437 (2020).[32] The results show that the additive induced serious eye damage on the cornea and therefore should be classified as UN GHS Category I (Corrosive).

BIOSTRONG® 510 all natural was tested for skin irritation potential in an *in vitro* test (Episkin) conducted according to OECD TG 439.[33] The study results indicate that BIOSTRONG® 510 all natural should be classified as non-irritant (UN GHS No Category).

No data were provided on the sensitisation potential of BIOSTRONG® 510 all natural.[34]

[31] [redacted]

[32] Technical dossier/Supplementary information September 2022/Reply_EFSASIn_14SEP22_signed, pp. 66.

[33] Technical dossier/Supplementary information September 2022/Reply_EFSASIn_14SEP22_signed, pp. 92.

[34] A Skin Sensitisation - Dendritic Cell Line Activation study (OECD 442E) could not be performed since the additive is not soluble in any of the solvents suitable for the test at the concentration required.

When handling the additive, exposure of unprotected users to estragole [redacted] may occur. Therefore, to reduce the risk, the exposure of the users should be minimised.

3.2.5.3. Conclusions on safety for the user

On the basis of the studies submitted, the additive was shown to be corrosive to the eyes but not irritant to skin. The additive may be a respiratory irritant or dermal or respiratory sensitiser.

When handling the additive, exposure of unprotected users to estragole [redacted] may occur. Therefore, to reduce the risk, the exposure of the users should be minimised.

3.2.6. Safety for the environment

In the previous opinion, the FEEDAP Panel concluded that 'The additive contains compounds naturally present in the environment that will not result in a substantial increase in their concentration in the environment at the application rate of 150 mg BIOSTRONG® 510/kg feed for chickens for fattening'... and that 'the use of BIOSTRONG® 510 at the recommended use level is not considered to be a risk for the environment' (EFSA FEEDAP Panel, 2016a).

The same conclusions apply to the current assessment.

3.3. Efficacy

In the previous opinion, the FEEDAP Panel evaluated four long-term feeding studies and six short-term digestibility studies in chickens for fattening, both made with a formulation of the additive containing natural star anise oil, which matches the additive under assessment (EFSA FEEDAP Panel, 2016a). The studies showed that BIOSTRONG® 510 has the potential to improve the performance of chickens for fattening by means of better feed to gain ratio and/or higher weight gain at a dose of 150 mg/kg complete feed. The results of the digestibility studies, which indicated improved ileal digestibility, were consistent with the effects seen in the long-term efficacy studies. The conclusions were extended to chickens reared for laying and extrapolated to all minor poultry species for fattening or reared to point of laying at the same dose.

3.3.1. Efficacy in laying hens

The applicant provided two short-term and one long-term efficacy trials in laying hens to support the safety for the target animals. BIOSTRONG 510® all natural is intended to be used as a zootechnical additive, under the functional groups of digestibility enhancers and other zootechnical additives (improvement of performance parameters). The efficacy of zootechnical additives intending to improve the productive performance of the animals needs to be demonstrated by means of long-term efficacy trials, in agreement with the conditions established in the Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018). [redacted]

In the long-term trial, 150 25-week-old laying hens (Lohmann Brown) were distributed in 30 pens of 5 hens each, and randomly allocated to three dietary treatments (10 replicates per treatment).[35] The basal diet based on maize, wheat and soybean meal was either not supplemented (control) or supplemented with BIOSTRONG® 510 all natural to provide 150 or 1,500 mg/kg feed. The confirmation of the level of additive in feed was done by means of the analysis of thymol, considered as the reference marker.[36] The experimental diets were offered ad libitum in mash form for 168 days, and included an external marker for the digestibility study.

Mortality and health status were checked daily, and the most likely cause of death or reason for culling was recorded. The laying hens were weighed at the start of the trial (day 1). Thereafter, the body weight and feed intake were weekly monitored. The number of eggs, unsaleable eggs (dirty, cracked, shell less eggs, roughed or soft shelled) and the egg weight were daily recorded. The laying rate, egg mass per hen and the feed to egg mass ratio were calculated per week and for the overall period. The eggshell strength and egg yolk colour were measured every week, while other egg quality parameters (Haugh unit and egg yolk index) on a monthly basis. At the end of the trial (day 168), the hen with the bodyweight closest to the average of the pen was selected (10 hens per treatment), killed and the ileal content extracted. The feed and ileal samples were analysed for the content of dry

[35] Technical dossier/Section IV/Annex_IV_3_1.

[36] Analytical concentration of thymol in feeds: < LOD (0.03 mg/kg), 0.40 and 3.06 mg/kg feed for the control, 150 and 1,500 mg/kg diets, respectively. The expected level of thymol in feed were 0.3 (150) and 3.0 (1,500) mg/kg.

matter, crude protein, amino acids (lysine and methionine), crude fat, crude ash, calcium and phosphorus and the apparent ileal digestibility (AID) calculated.

The experimental data were analysed with an analysis of variance (ANOVA) with the treatment as fixed effect. A nonparametric Kruskal–Wallis test was applied when the normality of the data could not be assumed. Group means were compared with Tukey's test. Significance level was set at 0.05.

Mortality was 2%, 4% and 2% for the control, 150 and 1,500 mg/kg groups, respectively, and no differences were found between treatments. The supplementation of BIOSTRONG® 510 all natural at 150 and 1,500 mg/kg feed showed higher laying rate (Control = 90.97; 150 mg/kg = 92.03; 1,500 mg/kg = 92.05%) and better feed to egg mass ratio (2.20; 2.15; 2.12) compared to control. The group supplemented with 1,500 mg/kg also showed lower average daily feed intake (125.8; 125.0; 124.0 g) and higher egg weight (62.8; 63.3; 63.5 g) in comparison with the control. No detrimental effect of the additive was observed in any of the egg quality parameters measured or on the number of unsaleable eggs. The improvement of the laying performance observed was consistent with the higher AID of total amino acids, crude protein, crude ash, phosphorus and calcium observed in both supplemented groups in comparison with the control.

3.3.2. Conclusion on efficacy

`The FEEDAP Panel concludes that the additive BIOSTRONG 510® all natural shows potential to improve the zootechnical performance of chickens for fattening at the recommended level of 150 mg/kg complete feed. This conclusion can be extended to chickens reared for laying/breeding and extrapolated to all poultry species for fattening or reared for laying/breeding.

[redacted]

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[37] and good manufacturing practice.

4. Conclusions

The following conclusions apply only to BIOSTRONG 510® all natural, which contains [redacted] estragole [redacted]

Based on the outcome of the tolerance trials and the MOET calculated considering the presence of estragole [redacted] in the additive BIOSTRONG 510® all natural, the FEEDAP Panel concludes that:

For laying hens, the MOET of [redacted] calculated at the recommended level of 150 mg/kg complete feed is considered of concern (MOET < 10,000). This conclusion applies to breeding birds and birds reared for laying/breeding/reproduction.

For short-living animals, the Panel has no safety concern when the additive BIOSTRONG® 510 all natural is used at the recommended level of 150 mg/kg complete feed. Taking also into account the results from the tolerance trials, the FEEDAP Panel concludes that the use of the additive at the recommended level is of no safety concern for chickens for fattening and other poultry species for fattening.

No safety concern would be expected for the consumer from the use of BIOSTRONG® 510 all natural at the recommended use level in feed.

The additive is corrosive to the eyes but not irritant to skin. It may be a respiratory irritant or dermal or respiratory sensitiser. When handling the additive, exposure of unprotected users to estragole [redacted] may occur. Therefore, to reduce the risk, the exposure of the users should be minimised.

The use of the additive in poultry species is considered safe for the environment.

The additive BIOSTRONG® 510 all natural is considered to be efficacious at the recommended level of 150 mg/kg complete feed in feed for all poultry species for fattening or reared for laying/breeding.

[37] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

[redacted]

5. Recommendations

The specifications for the additive should ensure that the maximum concentrations for estragole [redacted] analysed/estimated for the additive under assessment are not exceeded.

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Abbreviations

AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
AID	apparent ileal digestibility
ANOVA	Analysis of Variance
BMD	benchmark dose
$BMDL_{10}$	benchmark dose (BMD) lower confidence limit for a benchmark response of 10%
BW	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony forming unit
CG	chemical group
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FAF	EFSA Panel on Food Additives and Flavourings

FAO	Food Agricultural Organization
FBO	food business operator
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FGE	food group evaluation
FLAVIS	The EU Flavour Information System
FLAVIS-no	FLAVIS number
GC	gas chromatography
GC–MS	gas chromatography–mass spectrometry
HACCP	hazard analysis and critical control points
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
MOE	Margin of exposure
MOET	combined margin of exposure
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxin
RH	relative humidity
SC	EFSA Scientific Committee
TEQ	toxic equivalent
TG	technical guidance
UN GHS	United Nations' Globally Harmonized System (of Classification and Labelling of Chemicals)
WHO	World Health Organization