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Safety and efficacy of cumin tincture (*Cuminum cyminum* L.) when used as a sensory additive for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed
(EFSA FEEDAP Panel),

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of cumin tincture (*Cuminum cyminum* L.) when used as a sensory feed additive for all animal species. The product is a water ethanol solution, which contains 0.002–0.003% of 4-isopropylbenzaldehyde (cuminaldehyde). The FEEDAP Panel concludes that the additive cumin tincture is safe at the maximum proposed use level (30 µL/kg complete feed or water for drinking) for all animal species. No safety concern would arise for the consumer from the use of cumin tincture up to the highest safe level in feeds. No specific data were provided by the applicant regarding the safety of the additive for users. In the absence of data, no conclusions can be drawn on the additive's potential to be a dermal/eye irritant or a skin sensitiser. The use of cumin tincture at the maximum proposed dose is not considered to be a risk for the environment. Since the major components of the additive are recognised to provide flavour in food and its function in feed would be essentially the same, no demonstration of efficacy is considered necessary.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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.

The European Commission received a request from the company Manghebati SAS² for authorisation of the product cumin tincture (*Cuminum cyminum* L.), when used as a feed additive for all animal species (category: sensory additives; functional group: flavourings).

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). 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 13 March 2015.

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 cumin tincture (*C. cyminum* L.), when used under the proposed conditions of use (see Section 3.2.3).

1.2. Additional information

Cumin tincture from *C. cyminum* L. is not authorised as a feed additive. Cumin oil from *C. cyminum* L. 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).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier³ in support of the authorisation request for the use of cumin tincture (*C. cyminum* L.) as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008⁴ and the applicable EFSA guidance documents.

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) used the data provided by the applicant to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of cumin tincture in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁵

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of cumin tincture (*C. cyminum* L.) is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 2009), Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Technical Guidance for assessing the safety of feed additives for the environment

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 Manghebati SAS, Zone de la Basse Haye, 35220, Chateaubourg, France.

³ FEED dossier reference: FAD-2014-0024.

⁴ 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.

⁵ The full report is available on the EURL website <https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2014-0024.pdf> <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2014-0024?search&form-return>

(EFSA, 2008), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c).

3. Assessment

The additive under assessment is a water/ethanol [REDACTED] tincture of *C. cyminum* L. and is intended for use as a sensory additive (flavouring) in feed and in water for drinking.

3.1. Origin and extraction

Cuminum is a genus of flowering plants in the family of Apiaceae, native to regions ranging from the east Mediterranean to south Asia. The genus contains four accepted species. The most commonly encountered is *C. cyminum* L., which is the source for the cumin seeds used as spice and is also used as medicinal plant. The main producer and consumer of cumin is India. Other producers are Middle East and North African countries.

The tincture is produced from the crushed dried seeds by extraction with water/ethanol mixture, [REDACTED].

3.2. Characterisation

3.2.1. Characterisation of the tincture

The additive is a brown liquid, with a characteristic cumin odour. It has a density of 975 kg/m³. By specification, the product is a water ethanol [REDACTED] solution, which contains 0.002–0.003% of 4-isopropylbenzaldehyde (cuminaldehyde).

The solvent represents about 98% of the additive (loss on drying ranged between 97.9% and 98.1%). The remaining 2% are plant-derived compounds, among which are carbohydrates (< 0.2%), lipids (0.002%), polyphenols (0.044%), flavonoids (0.026%, expressed as chlorogenic acid equivalents), and volatile compounds (0.02%).⁶ Table 1 summarises the results of proximate analysis and of the characterisation of the organic fraction in five batches of the additive.

Table 1: Major constituents of cumin tincture (*Cuminum cyminum* L.) based on the analysis of five batches (mean and range)

	Method	Percentage of tincture	
		Mean (%)	Range (%)
<i>Proximate analysis</i>			
Loss on drying	Gravimetry	98.03	97.92–98.08
[REDACTED]	Gravimetry	[REDACTED]	[REDACTED]
Carbohydrate	HPAEC-PAD	< 0.2	< 0.2
Lipids		0.0021	0.0008–0.0025
Total		98.44	98.30–98.52
<i>Characterisation of the organic fraction</i>			
Polyphenolic content	Folin–Ciocalteu	0.044	0.036–0.056
Total flavonoids ^(a)	HPTLC	0.026	0.023–0.029
Chlorogenic acid	HPTLC	0.0013	0.0011–0.0014
Total volatile fraction	GC-FID	0.02	–

HPAEC-PAD: high-performance anion exchange chromatography with pulsed amperometric detection; HPTLC: high-performance thin layer chromatography; GC-FID: gas chromatography with flame ionisation detection.

(a): At least nine compounds detected and quantified accounting for maximum 0.006% each, expressed as chlorogenic acid equivalents.⁷

[REDACTED] The applicant provided the characterisation of one batch obtained by gas chromatography (GC) coupled with a flame ionisation detector (FID) and mass spectrometry (MS). Eighty-two peaks were detected,

⁶ Technical dossier/Supplementary information February 2018/Section_II_Identity and Annex II_2.

⁷ Technical dossier/Supplementary information February 2018/Annex II_9.

the majority of them accounts individually for less than 1% of the area under the curve. Besides cuminaldehyde (4-isopropylbenzaldehyde, 0.0024%), seven compounds (cuminalcohol 0.0024%, carvacrol 0.0002%, *p*-cymene (1-isopropyl-4-methyl benzene) 0.0001%, benzyl alcohol 0.0002%, linalool 0.0001%, *p*-menth-3-en-7-al 0.0007% and *p*-mentha-1,4-dien-7-ol (perillyl alcohol) 0.0005%) were identified by GC-MS in the volatile fraction and their content in the tincture was estimated by comparison of the areas of the GC-FID chromatogram.

3.2.2. Impurities

No information on the concentrations of undesirable compounds in the tincture is given. The applicant controls contamination at the level of the raw material (cumin seeds). Specifications are set with suppliers covering heavy metals, pesticides residues and microbial contamination. A single example of a certificate of analysis of cumin seeds showing compliance was provided.⁸ Analysis of impurities in the tincture apparently is made on irregular basis and does not form part of the Hazard Analysis and Critical Control Points (HACCP) plan.

3.2.3. Stability

The shelf life is declared to be at least 36 months, when stored in tightly closed containers under standard conditions. No separate or additional stability studies were performed.

3.2.4. Conditions of use

The additive cumin tincture is intended for use in feed and in water for drinking for all animal species. The applicant proposes a maximum concentration of 30 mL/tonne complete feed (or 30 μ L/kg feed, which corresponds to 29.25 mg cumin tincture/kg feed, considering the density of the additive of 975 kg/m³).⁹ Equivalent application rates to be used in water for drinking are not provided. No withdrawal period is foreseen.

3.3. Safety

The safety assessment is based on the highest use level proposed by the applicant (30 μ L/kg complete feed).

3.3.1. Safety for the target species

The tincture consists of 98% of a water/ethanol [REDACTED] mixture. The concentration of plant derived compounds is limited to 2% of the tincture, of which 0.4% was identified as ash. Among the identified organic compounds, 0.02% is volatile and 0.04% is phenolic in nature. The concentration of unidentified compounds in the tincture is about 1.5%.

With the exception of *p*-menth-3-en-7-al, all identified compounds in the volatile fraction are presently authorised as food flavourings without limitations¹⁰ and belong to Cramer Class I, as established by the EU Flavour Information System (FLAVIS). At the maximum proposed use level in feed of 30 μ L tincture/kg feed (about 30 mg/kg feed), the concentration of the identified volatile compounds would range between 0.03 and 0.72 μ g/kg feed, well below the threshold of toxicological concern (TTC) for Cramer Class I compounds, i.e. 1 mg/kg feed for poultry and pigs and 1.5 mg/kg feed for cattle, salmonids and non food-producing animals (EFSA FEEDAP Panel, 2012a). The concentrations of *p*-menth-3-en-7-al (0.21 μ g/kg feed) and the unidentified compounds in the volatile fraction (ranging from 0.0006 to 0.73 μ g/kg) are below TTC for Cramer Class III compounds, i.e. 0.05 mg/kg feed for poultry and pigs and 0.08 mg/kg feed for cattle, salmonids and non food-producing animals.

At the maximum proposed use level in feed of 30 μ L tincture/kg feed, the concentration of the total phenolic fraction (0.044%, measured by the Folin-Ciocalteu method) would be 13.2 μ g/kg feed. At least nine flavonoids could be separated and quantified (as chlorogenic acid equivalents, accounting

⁸ Technical dossier/Supplementary information February 2018/Annex II_3.

⁹ Technical dossier/Supplementary information February 2018/Section II_Identity.

¹⁰ 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.

for maximum 0.006% each). Since the flavonoid data indicate that no one individual compound would exceed the threshold value for Cramer Class III (0.05 mg/kg feed for poultry and pigs and 0.08 mg/kg feed for cattle, salmonids and non food-producing animals) no concern for the target species arises.

The unidentified fraction of the tincture (1.5%) would result in 0.45 mg/kg feed, which if present as a single compound, would be below the threshold for Cramer Class I but above the threshold values for Cramer Class II and III compounds. However, a literature survey did not identify compounds of concern (i.e. Cramer Class II or III) in extracts from cumin seeds. The water-soluble compounds are mainly glycosides of flavonoids, organic acids, vitamins, amino acids and protein.¹¹

3.3.1.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that cumin tincture (*C. cyminum* L.) is safe at the proposed maximum use level of 30 µL/kg feed or water for drinking for all animal species.

3.3.2. Safety for the consumer

All the identified compounds except *p*-menth-3-en-7-al are authorised as food flavourings without limitations.¹¹ The concentration of *p*-menth-3-en-7-al in feed is below the threshold value for Cramer Class III compounds and is considered safe. Owing to the metabolism and excretion in the target species, it is expected that the consumer exposure to *p*-menth-3-en-7-al from food of animal origin would be much lower than the levels given to the target species.

The active compounds present in the additive will be readily absorbed, metabolised and excreted. The individual identified compounds are not expected to accumulate in animal tissues and products. Considering the low concentration of the additive in feed or water for drinking, residues remaining in food products derived from animals fed with the additive are unlikely to be detectable. Consequently, no concern for the consumer is expected.

3.3.2.1. Conclusions on safety for the consumer

The use of the additive at the maximum proposed concentration of 30 µL/kg feed or water for drinking is safe for the consumer of products derived from animals fed with the additive.

3.3.3. Safety for the user

No specific data were provided by the applicant regarding the safety of the additive for users and, consequently, no conclusions can be drawn on the additive's potential to be a dermal/eye irritant.

The Panel notes that the additive contains a variety of compounds, known to cause allergic reactions in sensitive persons. Therefore, sensitisation may occur in users handling the additive, even in its diluted form.

3.3.4. Safety for the environment

Considering the composition of the additive and the maximum proposed use levels in feed (30 µL/kg) the maximum concentration of all plant derived material together (2%) in feed is 0.06 mg/kg. This amount is not likely to change the concentration of compounds from cumin in the environment. Use of the cumin tincture in animal production is not expected to pose a risk for the environment.

3.4. Efficacy

Under the terms Regulation (EC) No 1334/2008 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'. Consequently, there is no specific European Union authorisation for any *C. cyminum* L. extract when used to provide flavour in food. However, cumin and its extracts are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by the Flavour and Extract Manufacturers Association (FEMA) with the reference number 2340.

Since cumin seeds and its extracts are universally 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.

¹¹ Technical dossier/Section II/Annex_II_7 Bibliographic data concerning the chemical composition of cumin seeds and cumin seeds extracts.

4. Conclusions

Cumin tincture (*C. cyminum* L.) is safe at the proposed maximum use level of 30 µL/kg in feed or water for drinking for all animal species.

No concerns for consumer safety were identified following the application of cumin tincture (*C. cyminum* L.) at the maximum proposed use level in animal nutrition.

No specific data were provided by the applicant regarding the safety of the additive for users. In the absence of data no conclusions can be drawn on the additive's potential to be a dermal/eye irritant or a skin sensitiser.

Use in animal production of cumin tincture (*C. cyminum* L.) is not expected to pose a risk for the environment.

Since cumin seeds and their extracts 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 cumin tincture.

Documentation provided to EFSA

- 1) Cumin tincture (*Cuminum cyminum* L.) for all animal species and categories. December 2014. Submitted by Manghebati sas.
- 2) Cumin tincture (*Cuminum cyminum* L.) for all animal species and categories. Supplementary information. February 2018. Submitted by Manghebati sas.
- 3) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for cumin tincture.
- 4) Comments from Member States.

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Abbreviations

EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavour and Extract Manufacturers Association
FLAVIS	the EU Flavour Information System
GC-FID	gas chromatography coupled with flame ionisation detector
GC-MS	gas chromatography-mass spectrometry
HACCP	hazard analysis and critical control points
HPAEC-PAD	high-performance anion exchange chromatography with pulsed amperometric detection
HPTLC	high-performance thin layer chromatography
TTC	threshold of toxicological concern

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for cumin tincture

In the current application authorisation is sought under article 4(1) for the *Cumin Cyminum* L. (Cumine Tincture) under the category/functional group 2(b) 'Sensory additives'/flavouring compounds' according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the feed additive for all animal species and categories. According to the Applicant the product is a brown liquid tincture obtained from *Cumin Cyminum* L., containing 0.5 to 1.2% 4-iso-propylbenzaldehyde, later referred as the phytochemical marker. The feed additive is intended to be used directly into feedingstuffs or through premixtures and into water for drinking, with no proposed minimum or maximum concentration levels.

For the quantification of the phytochemical marker in the feed additive, the Applicant submitted a method based on gas chromatography coupled with flame ionisation detection (GC-FID). This method is derived from the ISO 9301 standard dedicated to the characterisation of oil from Cumin seed. The Applicant reported a relative standard deviation for repeatability (RSDr) of 3.2% when analysing preparations of Cumine Tincture containing 24.2 mg/kg 4-iso-propylbenzaldehyde. Based on the experimental evidence provided the EURL recommends the GC-FID method for official control to quantify the phytochemical marker (4-iso-propylbenzaldehyde) in the feed additive.

The Applicant did not provide any experimental method or data for the quantification of added *Cumin Cyminum* L. (Cumine Tincture) in premixtures, feedingstuffs and water. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Cumin Cyminum* L. (Cumine Tincture) in premixtures, feedingstuffs and water.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.