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Safety and efficacy of a tincture derived from *Artemisia vulgaris* L. (Mugwort tincture) when used as a sensory additive in feed for all animal species

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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 a tincture from *Artemisia vulgaris* L. (Mugwort tincture) when used as a sensory feed additive for all animal species. The product is a water/ethanol solution, with a dry matter content of approximately 1.7%. The product is specified to contain a minimum of 0.01% hydroxycinnamic acid derivatives (expressed as chlorogenic acid). However, since the 74% of the dry matter fraction of the additive remains uncharacterised, the FEEDAP Panel cannot conclude on the safety of the additive at the proposed use levels of up to 400 mg/kg complete feed for all animal species or for the consumer. 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. *A. vulgaris* is native to Europe. Consequently, the use of a tincture derived from the plant 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 Manghebati SAS² for authorisation of the product Mugwort tincture (*Artemisia vulgaris* L.), when used as a feed additive for all animal species (category: sensory additives; functional group: flavouring compounds).

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 8 February 2018.

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 Mugwort tincture, when used under the proposed conditions of use (see Section 3.2.3).

1.2. Additional information

The tincture from *Artemisia vulgaris* L. (Mugwort tincture) is not currently authorised as a feed additive.

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 Mugwort tincture as a feed additive.

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.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phytochemical markers in Mugwort tincture in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁴

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the tincture is in line with the principles laid down in Regulation (EC) No 429/2008⁵ and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA, 2009), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b) and Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008).

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 – BP 42133 – 35221 Chateaubourg Cedex.

³ FEED dossier reference: FAD-2010-0401.

⁴ The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2010-0401-Mugworttincture.pdf>

⁵ 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.

3. Assessment

This application covers a tincture derived from *A. vulgaris* L. when used as sensory additive (functional group: flavouring compounds) in feed for animal species.

3.1. Origin and extraction

Artemisia is a genus of flowering plants in the family of Asteraceae (Compositae), which is native to temperate Europe, Asia and northern Africa. The genus contains many accepted species. The most commonly encountered species in Europe are *A. absinthium*, *A. dracunculus* and *A. vulgaris*. The trivial name Common Mugwort or just Mugwort is usually taken to apply to *A. vulgaris* but may be used to refer to other species.

The tincture is produced from the fragmented aerial parts of *A. vulgaris* by extended extraction with a water/ethanol mixture, [REDACTED] for [REDACTED].⁶ After this period, the tincture is [REDACTED]

3.2. Characterisation

3.2.1. Characterisation of the tincture

The tincture is a brown liquid, with a characteristic odour. It has an average density of 940 kg/m³ and a pH of 6.5. It is a water/ethanol [REDACTED] solution, which is specified to contain a minimum of 0.01% hydroxycinnamic derivatives (expressed as chlorogenic acid).⁷

The solvent represents about 98.3% of the additive leaving a dry matter content of about 1.7% (Table 1). The dry matter consists of ash and a plant-derived organic fraction, which contains polyphenols (0.104%), and separately determined phenolic acids (0.023%, expressed as chlorogenic acid equivalents).⁸ Table 1 summarises the results of the characterisation of the organic fraction in five batches of the additive. As a proximate analysis of the tincture was not provided, 1.24% of the tincture, corresponding to the 74% of the dry matter fraction and to the 92% of the organic fraction remains uncharacterised.

Table 1: Major constituents of a tincture derived from *Artemisia vulgaris* L. based on the analysis of five batches (mean and range)

Constituent	Method	Percentage of tincture	
		Mean (%)	Range (%)
Proximate analysis			
Dry matter	Gravimetry	1.66	1.51–1.87
Ash	Gravimetry	0.32	0.22–0.45
Organic fraction	By difference	1.34	1.13–1.65
Solvent	100%-dry matter	98.34	98.13–98.49
Characterisation of the organic fraction			
Total polyphenols	Folin–Ciocalteu	0.104	0.0730–0.1594
Total phenolic acids ^(a)	HPTLC	0.026	0.013–0.108
Chlorogenic acid	HPTLC	0.0056	0.0028–0.0136
α - and β -thujone	HPTLC	< 0.005	< 0.005
1,8-cineole	HPTLC	0.0011	0.0007–0.0013

HPTLC: high-performance thin-layer chromatography.

(a): At least seven compounds detected.⁹

3.2.1.1. 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 (dried plants). Specifications are set

⁶ Technical dossier/Supplementary information January 2019/Section II_Identity [REDACTED]

⁷ Technical dossier/section II/Annex II_9_ French Pharmacopoeia monograph.

⁸ Technical dossier/Supplementary information January 2019/Section II_Identity and Annex II_3.

⁹ Technical dossier/Supplementary information January 2019/Annex II_6.

with suppliers covering heavy metals (cadmium < 1 mg/kg, mercury < 0.1 mg/kg and lead < 5 mg/kg), benzo[a]pyrene (< 10 µg/kg) and polycyclic aromatic hydrocarbons (< 50 µg/kg), pesticides residues and microbial contamination. A single certificate of analysis of the raw material (aerial parts) showing compliance was provided.¹⁰ 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.

The applicant performed a literature search on the composition of *A. vulgaris* and its extracts.¹¹ Among the compounds identified, α -thujone, β -thujone, camphor and 1,8-cineole are reported in the EFSA Compendium of botanicals as substances of concern for the essential oil obtained from the aerial parts of *A. vulgaris* (EFSA, 2012). Thujones were detected at concentrations of 268.5 mg/kg, 1,8-cineole 75.4 mg/kg and camphor 5.9 mg/kg in the raw material prior to extraction (analysis of a single sample).¹⁰ Subsequent analysis by high-performance thin-layer chromatography (HPTLC) of the five batches of the tincture prepared from the raw material did not detect α -thujone or β -thujone (limit of detection (LOD) 0.005% of tincture), and 1,8-cineole was found only at a concentration of 0.001% (Table 1). No attempt was made to detect camphor in the tincture. The applicant argued that as camphor is less water soluble than 1,8-cineole, its concentration in the tincture would be lower than that of 1,8-cineole.

3.2.2. Shelf-life

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.

3.2.3. Conditions of use

The additive is intended for use in feed for all animal species. The applicant proposes a minimum concentration of 2 mg tincture/kg complete feed and maximum concentration of 400 mg tincture/kg complete feed.

3.3. Safety

The safety assessment is based on the highest proposed use level (400 mg tincture/kg complete feed).

3.3.1. 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 or its individual components, the threshold of toxicological concern (TTC) is applied to derive maximum safe feed concentrations for the known individual components of the tincture (EFSA FEEDAP Panel, 2017b).

At the maximum proposed use level of 400 mg tincture/kg in feed, the concentration of the total phenolic fraction (0.104%, measured by the Folin–Ciocalteu method) would be 0.42 mg/kg feed. At least seven phenolic acids could be separated and quantified (as chlorogenic acid equivalents, accounting for maximum 0.025% each, corresponding to 0.1 mg/kg). Since phenolic acids are assigned to Cramer Class I and the data indicate that none of the individual compounds would exceed the threshold value for Cramer Class I (ranging from 0.3 mg/kg feed for poultry to 1.5 mg/kg feed for salmonids and dogs) no concern for the target species arises from the phenolic fraction.

At the maximum proposed use level, the concentration of 1,8-cineole in feed would be 4.4 µg/kg feed, that of α - and β -thujone (belonging to Cramer Class II) would be below 20 µg/kg feed. Since none of these components would exceed the threshold value for Cramer Class II (ranging from 0.1 mg/kg feed for poultry to 0.5 mg/kg feed for salmonids and dogs), the presence of these impurities is not considered of concern for the target species.

The unidentified fraction of the tincture (1.21%) would result in 4.84 mg/kg feed. Although it can be assumed that the uncharacterised fraction would contain carbohydrates and other plant-derived polymer compounds,¹² this assumption is not supported by analytical data.

¹⁰ Technical dossier/Supplementary information January 2019/Annex II_10.

¹¹ Technical dossier/Supplementary information January 2018/Annex II_4.

¹² Technical dossier/Supplementary information_January 2019/Section II_Annex II_4_Bibliographic data concerning chemical composition of plant and plant extract.

In the absence of data on the full characterisation of the additive and considering that there is uncertainty in the composition of 74% of the dry matter fraction of the additive, the FEEDAP Panel cannot conclude on the safety of the additive for the target species.

3.3.1.1. Conclusions on safety for the target species

Since the 74% of the dry matter fraction of the additive remains uncharacterised, the FEEDAP Panel cannot conclude on the safety of the tincture derived from *A. vulgaris* L. under the proposed conditions of use.

3.3.2. Safety for the consumer

The phenolic compounds, present in the additive at concentrations below the thresholds for Cramer Class I compounds, will be readily metabolised and excreted and are not expected to accumulate in animal tissues and products. Consequently, no concern for the consumer is expected from the phenolic fraction.

However, uncertainty remains on the unknown composition of the 74% of the dry matter fraction. Therefore, the FEEDAP Panel is unable to conclude on the safety for the consumers following the use of the tincture derived from *A. vulgaris* L. as flavouring in animal feed.

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 or a skin sensitiser.

3.3.4. Safety for the environment

Artemisia vulgaris L. is native to Europe where it grows wild as well as being cultivated for commercial and decorative purposes. Use of the tincture derived from *A. vulgaris* L. as a flavour in animal feed is not expected to pose a risk for the environment.

3.4. Efficacy

Artemisia (mugwort) and its extracts are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2010), by the Flavour and Extract Manufacturers Association (FEMA) with the reference number 3115 and by the Council of Europe (CoE) with the reference number 72 (Mugwort tincture).

Since *A. vulgaris* L. 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.

4. Conclusions

Since the 74% of the dry matter fraction of the additive remains uncharacterised, the FEEDAP Panel cannot conclude on the safety of the tincture derived from *A. vulgaris* L. (Mugwort tincture) at the proposed use levels of up to 400 mg/kg complete feed for all animal species and for the consumer.

In the absence of data, no conclusions can be drawn on the potential of the tincture to be a dermal/eye irritant or a skin sensitiser.

Use of the tincture derived from *A. vulgaris* L. as a flavour in animal feed is not expected to pose a risk for the environment.

Since *A. vulgaris* L. and its 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 the tincture under application.

Documentation provided to EFSA/Chronology

Date	Event
01/12/2010	Dossier received by EFSA. Mugwort tincture for all animal species. December 2010. Submitted by Manghebat S.A.S.
23/02/2011	Reception mandate from the European Commission

Date	Event
02/03/2012	EFSA informed the applicant that, in agreement with the European Commission and 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
08/02/2018	Application validated by EFSA – Start of the scientific assessment
09/03/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation, safety for target species, safety for the consumer and safety for the user, analytical methods</i>
14/05/2018	Comments received from Member States
26/07/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
02/08/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
04/10/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
HACCP	hazard analysis and critical control points
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 Mugwort tincture

In the current application, authorisation is sought under Article 4(1) for the botanically defined *Mugwort 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, the *feed additive* is sought to be used for all animal species and categories.

The *feed additive* is a mixture of naturally occurring chemical components including total polyphenols, total phenolic acids, chlorogenic acid, alpha- and beta-thujones and eucalyptol as the major constituents, and it is intended to be incorporated directly into *feedingstuffs* or through flavouring premixtures without minimum or maximum limits.

The Applicant did not provide a method for the determination of the phytochemical marker, but submitted other methods aiming at the identification/characterisation of the *feed additive* (*Mugwort tincture*).

The Applicant proposed to characterise the *feed additive* (*Mugwort tincture*) by determination of loss on drying, ash content (measured by gravimetry), total polyphenols (measured by spectrophotometry), total phenolic acids, chlorogenic acid, alpha- and beta-thujones and eucalyptol (measured by high-performance thin-layer chromatography (HPTLC)). According to the Applicant, the use of the HPTLC profiles as a fingerprint of the *feed additive* is more reliable than the analysis of individual phytomarkers at an established range.

For the identification/characterisation of the *feed additive*, the EURL considers the methods based on gravimetry, spectrophotometry and HPTLC proposed by the Applicant as fit-for-purpose.

Furthermore, the Applicant did not provide experimental data or analytical method for the determination of *Mugwort tincture* in *premixtures* and *feedingstuffs* as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.