SCIENTIFIC OPINION

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Safety and efficacy of a tincture derived from *Verbascum thapsus* L. 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 *Verbascum thapsus* L. (great mullein tincture) when used as a sensory feed additive for all animal species. The product is a water/ethanol ([redacted]) solution, with a dry matter content of approximately 2.8%. The product contains on average 0.216% polyphenols and 0.093% flavonoids. Since 82% of the dry matter fraction of the additive remains uncharacterised, the FEEDAP Panel cannot identify a safe level for the use of the additive for all animal species. Considering the uncertainty in the composition of the additive, and in the absence of information on the toxicological properties of the tincture, the FEEDAP Panel is unable to conclude on the safety for the consumers following the use of the tincture as flavouring in animal feed. 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. *V. thapsus* L. 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.

Keywords: sensory additives, *Verbascum thapsus* L., tincture, great mullein tincture, 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.

The European Commission received a request from Manghebati SAS[2] for authorisation of the product great mullein tincture (*Verbascum thapsus* 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 great mullein tincture, when used under the proposed conditions of use (see Section 3.2.3).

1.2. Additional information

The tincture from *V. thapsus* L. (great mullein tincture) is not currently authorised as a feed additive in the European Union.

The European Medicines Agency (EMA) issued an assessment report on *V. thapsus* L., *Verbascum densiflorum* Bertol. (*Verbascum thapsiforme* Schrad) and *Verbascum phlomoides* L. flos (EMA, 2018a). The traditional use of mullein flowers (from *V. thapsus* L.; *V. densiflorum* Bertol.; *V. phlomoides* L.) as herbal substance and herbal preparations (teas) in medicinal products is described in EMA's herbal monograph (EMA, 2018b).

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[3] in support of the authorisation request for the use of great mullein 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 total polyphenols, total phenolic acids in great mullein 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 429/2008[5] 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 identity, characterisation and conditions of

[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-2010-0350.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2010-0350_great_mullein_tincture.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.

use of feed additives (EFSA FEEDAP 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 studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

This opinion deals with the assessment of a tincture derived from *V. thapsus* L. (also known as great mullein or common mullein) when used as sensory additive (functional group: flavouring compounds) in feed animal species.

3.1. Origin and extraction

Verbascum is a genus of flowering plants in the family of Scrophulariaceae. The genus contains over one hundred accepted species, mostly native to Europe, particularly the Mediterranean region, and to parts of Asia. Several species are traditionally considered to have medicinal properties including *Verbascum thapsus* L.[6] often referred to by its trivial name of the common or great mullein (Turker and Gurel, 2005).

The tincture is produced from [redacted] *V. thapsus* by extended extraction with a water/ethanol mixture ([redacted]) for 1–6 weeks ([redacted]).[7] Solids are then removed by pressing and the resultant solution is clarified by filtration.

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 975 kg/m^3 (range: 972–977 kg/m^3) and a pH of 5.27 (range: 5.25–5.30).[8] It is a water/ethanol solution, which contains on average 0.216% polyphenols and 0.093% flavonoids.

The solvent represents about 97.2% of the additive leaving a dry matter content of about 2.8% (Table 1). The dry matter consists of ash and a plant-derived organic fraction, which contains polyphenols (on average 0.216%), and separately determined phenolic acids (on average 0.093%, expressed as chlorogenic acid equivalents).[8] In the phenolic acids fraction, five compounds were detected using a reagent specific for flavonoids and phenolic acids, but they were not individually identified. 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, 2.26% of the tincture, corresponding to the 82% of the dry matter (DM) fraction and to the 91% of the organic fraction remains uncharacterised.

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

Constituent	Method	Percentage of tincture	
		Mean	Range
Proximate analysis			
Dry matter	Gravimetry	2.76	2.55–3.00
Ash	Gravimetry	0.28	0.26–0.29
Organic fraction	By difference	2.48	2.29–2.71
Solvent	100%-dry matter	97.24	97.00–97.45

[6] Technical dossier/Supplementary information January 2019/Annex II_9.
[7] Technical dossier/Supplementary information January 2019/ Section_II_Identity [redacted].
[8] Technical dossier/Supplementary information January 2019/ Section_II_Identity and Annex II_4.

Constituent	Method	Percentage of tincture	
		Mean	Range
Characterisation of the organic fraction			
Total polyphenols	Folin–Ciocalteu	0.2162	0.1942–0.2341
Total flavonoids(a)	HPTLC	0.0934	0.0840–0.1075

HPTLC: high-performance thin-layer chromatography.
(a): At least five compounds detected.[9]

3.2.1.1. Impurities

No information on the concentrations of undesirable compounds in the tincture was submitted. The applicant controls contamination at the level of the raw material (dried plants). Specifications are set with suppliers covering heavy metals (cadmium < 1 mg/kg, mercury < 0.1 mg/kg and lead < 10 mg/kg) and arsenic (< 2 mg/kg), pesticides residues (identity unspecified) and microbial contamination (total plate count, unspecified, yeasts and moulds were < 10^5 colony forming unit (CFU)/g, *Salmonella* spp. and *Escherichia coli* absent). A single example of a 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 states that heavy metals, pesticides and microbial contamination have never been detected.

The applicant made a literature search on the composition of *V. thapsus* and its extracts.[11] Although the presence of iridoid glycosides was reported in water extract of the fresh whole plant (Warashina et al., 1991), no attempt was made to detect their presence in the additive. Iridoid glycosides are considered substances of concern, as the aglycone formed after hydrolysis is able to covalently bind to proteins, mainly to albumin (Kim et al., 2000).

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 data was provided to support this claim.

3.2.3. Conditions of use

The additive is intended for use in feed for all animal species. The applicant proposes a maximum concentration of 50 g tincture/kg complete feed. A minimum content is not proposed.

3.3. Safety

The safety assessment is based on the highest use level proposed by the applicant (50 g tincture/kg complete feed).

3.3.1. Safety for the target species

The applicant did not provide specific tolerance or toxicological studies in laboratory animals to support the safety of the additive for the target species. In the absence of tolerance studies and/or data from repeated dose toxicity 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 individual known components of the tincture (EFSA FEEDAP Panel, 2017b).

At the maximum proposed use level of 50 g tincture/kg in feed, the concentration of the total phenolic fraction (0.2162%, measured by the Folin–Ciocalteu method) would be 108 mg/kg feed. At least five phenolic acids could be separated and quantified (as chlorogenic acid equivalents, accounting for maximum 0.006% each, corresponding to 3 mg/kg). Although the individual compounds were not identified, phenolic acids are assigned to Cramer Class I. The available data indicate that individual phenolic compounds would exceed about 10-fold 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).

[9] Technical dossier/Supplementary information January 2019/Annex II_10.
[10] Technical dossier/Supplementary information January 2019/Annex II_2.
[11] Technical dossier/Supplementary information January 2019/Annex II_5.

At the maximum proposed use level (50 g/kg complete feed), the use of the additive is not considered safe for the target species.

The unidentified fraction of the tincture (2.26%) would represent 1,130 mg/kg feed at the highest supplementation level. Although it can be assumed that the uncharacterised fraction would contain carbohydrates and other plant polymer derived compounds,[12] this assumption is not supported by analytical data. There is also evidence in the literature of the presence of iridoid glycosides (aucubin) in the plant which are likely to be extracted into the tincture.

In the absence of data on the full characterisation of the additive and considering the uncertainty in the composition of 82% of the DM fraction of the additive, the FEEDAP Panel cannot identify a safe level for the use of the additive for all animal species. The Panel further notes that such a high inclusion level would have an impact on the nutritional value of the diet.

3.3.2. Safety for the consumer

The phenolic compounds present in the additive 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 82% of the dry matter fraction, including the potential presence of iridoid glycosides. In the absence of information on the toxicological properties of the tincture, the FEEDAP Panel is unable to conclude on the safety for the consumers following the use of the tincture derived from *V. thapsus* 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

V. thapsus 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 *V. thapsus* L. as a flavour in animal feed is not expected to pose a risk for the environment.

3.4. Efficacy

Mullein (flowers) and its extracts (infusion and decoction) are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2010).

Since *V. thapsus* 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

In the absence of data on the full characterisation of the additive, and considering that there is uncertainty in the composition of 82% of its DM fraction, the FEEDAP Panel cannot identify a safe level for the use of the additive for all animal species under the proposed conditions of use (maximum application rate of 50 g/kg complete feed for all animals).

Considering the uncertainty in the composition of the additive and in the absence of information on the toxicological properties of the tincture, the FEEDAP Panel is unable to conclude on the safety for the consumers following the use of the tincture derived from *V. thapsus* L. as flavouring in animal feed.

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 *V. thapsus* L. as a flavour in animal feed is not expected to pose a risk for the environment.

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

Since *V. thapsus* 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. Great mullein tincture for all animal species. Submitted by Manghebati S.A.S.
21/02/2011	Reception mandate from the European Commission
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
15/03/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for target species, safety for the consumer and safety for the user*
14/05/2018	Comments received from Member States
12/03/2019	Reception of supplementary information from the applicant - Scientific assessment remained suspended
13/08/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives - Scientific assessment re-started
12/11/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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EMA (European Medicines Agency), 2018a. Assessment report on *Verbascum thapsus* L., V. *densiflorum* Bertol. (V. *thapsiforme* Schrad) and V. *phlomoides* L., flos. EMA/HMPC/611531/2016. Committee on Herbal Medicinal Products (HMPC). 27 March 2018. Available online: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-verbascum-thapsus-l-v-densiflorum-bertol-v-thapsiforme-schrad-v-phlomoides-l_en.pdf

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Abbreviations

CFU	colony forming units
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 Methods of Analysis for Great Mullein tincture (*Verbascum thapsus* L.)

In the current application, authorisation is sought under Article 4(1) for the botanically defined *great mullein 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 and total phenolic acids as major constituents. The *feed additive* is intended to be incorporated directly into *feedingstuffs* alone or in combination with other flavouring substances (flavouring *premixtures*). The Applicant did not propose any minimum or maximum content of *great mullein tincture* in *feedingstuffs*.

The Applicant did not indicate any phytochemical marker and did not provide any corresponding method, but other methods aiming at the identification/characterisation of the *feed additive*.

The Applicant characterised the *feed additive* by determination of: loss on drying and ash content (gravimetry); total polyphenols (spectrophotometry); and total phenolic acids (high-performance thin-layer chromatography (HPTLC)).

Furthermore, the Applicant has provided as reference a typical HPTLC profile of *great mullein tincture*'s phenolic acids with the aim to provide a tool for the unambiguous identification of the *feed additive*. A detailed description of the characteristics of the profile has been included.

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 *great mullein 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.