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Safety of a feed additive consisting of a tincture derived from *Verbascum thapsus* L. (great mullein tincture) for use in all animal species (MANGHEBATI SAS)

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Abstract

The tincture derived from *Verbascum thapsus* L. (great mullein tincture) is intended to be used as a sensory additive in feed for all animal species. The product is a water/ethanol solution, with a dry matter content of ~ 2.8% and contains on average 0.216% polyphenols including 0.093% flavonoids. According to a previous assessment, the additive was not characterised in full and about 82% of the dry matter fraction remained uncharacterised (representing 2.26% of the tincture). There was also uncertainty on the potential presence of iridoid glycosides in the tincture. Therefore, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) could not conclude on the safety of the additive at the proposed use levels of up to 50 g/kg complete feed for all animal species or for the consumer. The applicant has provided new data which show that the unidentified fraction consists of crude fibre, other carbohydrates, and protein. The tincture also contains aucubin (0.004%). Considering the genotoxic potential of aucubin and other related iridoids, no conclusions can be drawn for long-living animals (pets and other non-food producing animals, horses and animals for reproduction). For short-living animals (animals for fattening), the FEEDAP Panel concludes that the tincture is safe at the maximum proposed use level of 50 mg/kg complete feed and that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed. No safety concerns would arise for the consumer from the use of the tincture up to the highest safe level in animal nutrition. 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.

Keywords: sensory additives, flavouring compounds, *Verbascum thapsus* L., great mullein tincture, safety, aucubin

Requestor: European Commission

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 defined the term of the authorisation by the Commission.

The European Commission (EC) received a request from MANGHEBATI SAS[2] for the authorisation of the product *Verbascum thapsus* L. (great mullein tincture) when used as a feed additive for all animal species (category: Sensory additives; functional group: flavouring compounds; Table 1).

Table 1: Description of the substance

Category of additive	Sensory additives
Functional group of additive	Flavourings
Description	*Verbascum thapsus* L. (great mullein tincture)
Target animal category	All animal species
Applicant	MANGHEBATI SAS
Type of request	New opinion

On 12 November 2019, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product could not conclude on the safety of the product for the following reasons:

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 dry matter (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 consumer 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.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of Authority's opinion. The new data have been received on 18 May 2020.

In view of the above, the Commission asks the Authority to deliver a new opinion on the safety of *V. thapsus* (great mullein tincture) as a feed additive for all animal species based on the additional data submitted by the applicant.

1.2. Additional information

The subject of the assessment is a feed additive consisting of a tincture derived from *V. thapsus* L. (great mullein tincture) intended for use as a sensory additive (flavouring compound) for all animal species.

The FEEDAP Panel issued an opinion on the safety and efficacy of a tincture derived from *V. thapsus* L. when used as a sensory additive in feed for all animal species (EFSA FEEDAP Panel, 2019). Since the 82% of the dry matter fraction of the additive remained uncharacterised, the FEEDAP Panel could not conclude on the safety of the additive at the proposed use levels of up to 50 g/kg complete feed for all animal species or for the consumer.

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

[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.

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 to a previous application on the same product.[4]

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 phytochemical markers in great mullein tincture in animal feed are valid and applicable for the current application.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of great mullein tincture is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Guidance on the identity, characterisation and conditions of 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), Guidance on the assessment of the biological relevance of data in scientific assessments (EFSA Scientific Committee, 2017), 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 Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee, 2019c).

3. Assessment

The additive under assessment, great mullein tincture, is produced from [redacted] of *V. thapsus* L. by extended extraction with a water/ethanol mixture [redacted].

The additive is intended to be used as a sensory additive (functional group: flavouring compounds) in feed for all animal species.

In the previous opinion (EFSA FEEDAP Panel, 2019), the FEEDAP Panel concluded that at the maximum proposed use level (50 g/kg complete feed), the use of the additive was not considered safe for the target species. At the maximum proposed use level of 50 g tincture/kg feed, a safety concern arose from the phenolic fraction, which would exceed about 10-fold the maximum acceptable concentration in feed for Cramer class I compounds (ranging from 0.3 mg/kg feed for poultry to 1.5 mg/kg feed for dogs) and from the unidentified fraction of the tincture (2.26%) which would result in 1,130 mg/kg feed at the highest supplementation level. The FEEDAP Panel also noted that 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 addition, in the absence of data on the full characterisation of the additive and considering the uncertainty surrounding the composition of 82% of the dry matter fraction of the additive, the FEEDAP Panel could not conclude on the safety for the consumer. In the absence of specific data regarding the safety of the additive for users, no conclusions could be drawn on the potential of the tincture to be a dermal/eye irritant or a skin sensitiser (EFSA FEEDAP Panel, 2019).

The applicant has now provided new data to address the issues previously identified regarding the characterisation and the safety of the additive, including a clarification on the use level in feed and water for drinking.

[3] FEED dossier reference: FAD-2020-0044.
[4] FEED dossier reference: FAD-2010-0350.
[5] 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
[6] 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.
[redacted]

3.1. Characterisation of the tincture

3.1.1. Proximate analysis

In the initial application, the additive was shown to contain about 97.2% water/ethanol solvent and a dry matter content of 2.8%. The identified constituents of the dry matter fraction were ash (0.28% of the tincture) and polyphenols (0.216%) including 0.093% (flavonoids). Some 2.26% of the tincture, corresponding to the 82% of the dry matter fraction and to the 91% of the organic fraction remained uncharacterised (EFSA FEEDAP Panel, 2019).

The applicant has now submitted a proximate analysis of five batches of the additive[8] and the results are shown in Table 2. The analysis fully accounts for the dry matter content of the additive. The fraction of polyphenols accounts on average for 7.8% of the dry matter fraction of the tincture (range: 7.6–8.0%) and the other plant constituents for the remaining 92.2%.

Table 2: Proximate analysis of a tincture derived from *Verbascum thapsus* L. based on the analysis of five batches (mean and range). The results are expressed as % (w/w)

Constituent	Mean	Range
	% (w/w)	% (w/w)
Dry matter	2.76	2.55–3.00
Ash	0.28	0.26–0.29
Organic fraction	2.48	2.29–2.71
Proteins	0.29	0.28–0.30
Lipids	0.005	0.002–0.006
'Carbohydrates'[(a)]	2.19	1.99–2.42
Solvent	97.24	97.00–97.45

(a): The constituents described as 'carbohydrates' represent the fraction of organic matter remaining after subtraction of the values for protein and lipids. It contains a variety of plant-derived compounds including phenolic compounds, in addition to any carbohydrates present.

3.1.2. Substances of concern

To address the potential presence of substances of concern in the tincture, namely iridoid glycosides, the applicant performed a literature search on the chemical composition of *V. thapsus* and its extracts.[9]

Kahraman et al. (2010) analysed 65.8 g of a dried extract obtained from 586.2 g air dried powder flowers of *V. mucronatum* Lam. They determined a total of 301.4 mg identified iridoids representing 0.46% of the dried extract. Aucubin (103 mg) was the major of four iridoid glycosides followed by catalpol (62.1 mg), ajugol (48.6 mg) and lasianthoside I (6.7 mg). Furthermore, two saponins, llwensisaponin A (51.5 mg) and llwensisaponin C (14.7 mg), along with a phenylethanoid glycoside, verbascoside (14.8 mg), were isolated.

Alba et al. (2014) report from their analysis of leaves from *V. thapsus* that the percent of leaf dry weight composed of aucubin was consistently lower than the percent composed of catalpol.

Another publication reported the presence of ajugol, derivatives of ajugol and 6-*O*-(α-L-rhamnopyranosyl)-catalpol but not aucubin or its derivatives in a water extract of the whole fresh plants of *V. thapsus* originating from Japan (Warashina et al., 1991).

Verbascoside (0.016%), iridoid glycosides, e.g. harpagoside (0.014%) and ajugol (0.006%), and iridoids, e.g. genipin (0.016%), were also detected in a dried methanolic extract of *V. thapsus* (7 kg leading to 60.5 g crude extract), with total iridoids representing 0.07% of the dry extract (Hussain et al., 2009).

From this search, it appeared that a maximum of 0.6% iridoid glycosides had been reported to occur in the aerial parts or flowers of *V. thapsus* or related species. Other typical secondary metabolites of *V. thapsus* reported were phenylethanoid glycosides (e.g. verbascoside) and triterpenoid saponins (e.g. thapsuin A and hydroxythapsuin A) occurring in low levels (Alipieva et al., 2014).

[8] Technical dossier/Supplementary information April 2021.
[9] Technical dossier/May 2020/Annex_1_Great mullein tincture_Manghebati.

The additive under assessment was analysed for its content of aucubin as main representative of the iridoid glycosides. The content of aucubin, determined by high-performance liquid chromatography (HPLC) in five batches of the tincture, was on average 0.0040% (range 0.0031–0.0057%, w/w), corresponding to 0.15% (0.12–0.21%) of the dry matter fraction of the tincture.[8] The occurrence of other iridoid glycosides, such as catalpol, ajugol or harpagoside, iridoids such as genipin, triterpenoid saponins and phenylethanoid glycosides (e.g. verbascoside) was not investigated.

3.1.3. Conditions of use

The applicant has modified the conditions of use of the additive from a maximum of 50 g/kg complete feed to 50 mg tincture/kg complete feed or water for drinking for all animal species except horses, for which the maximum proposed use level is 600 mg/kg complete feed.

3.2. Safety

Among the secondary plant metabolites in the tincture, the analytical data provided confirm the presence of the iridoid glycoside aucubin. Although the occurrence of other iridoid glycosides, such as catalpol, ajugol or harpagoside was not investigated, they are likely to occur in the tincture at similar concentrations as aucubin.

Furthermore, phenolic compounds including flavonoids were quantified but not identified. They will be assessed based on considerations at the level of the assessment group considering the revised proposed use levels in feed (see Section 3.2.4.1). These compounds will be readily metabolised and excreted and are not expected to accumulate in animal tissues and products.

Other secondary metabolites, which have been reported to occur in low levels in *V. thapsus* and are likely to be present in trace amount in the tincture under assessment are phenylethanoid glycosides (e.g. verbascoside) and triterpenoid saponins. These compounds are not expected to be of concern when ingested at the concentrations that are likely to arise from the use of the tincture in feed (EFSA, 2009; EFSA FEEDAP Panel, 2019) and are not further considered.

The following sections focus on iridoid glycosides identified by the literature search performed by the applicant and particularly on aucubin, analytically determined in the tincture.

3.2.1. Absorption, distribution, metabolism and excretion

The applicant submitted a literature search on the absorption, distribution, metabolism and excretion (ADME) of iridoid glycosides.[10,11]

The applicant provided a summary of pharmacokinetic parameters of aucubin based on a review recently published (Zeng et al., 2020). From some *in vivo* studies therein described, aucubin administered by oral route showed to be rapidly absorbed, being the bioavailability of about 20%. The low oral bioavailability may be attributed to pH-instability in the gastric fluid, poor gastrointestinal (GI) absorption due to low lipophilicity, and the possible metabolism in the GI mucosa or in the liver (so called first-pass effect).

After oral administration, the half-life of aucubin ranged from about 2 h to 7 h, depending on whether administered as a pure compound or in preparations like tea or plant extracts. It is also referred that aucubin is widely distributed in the organism after intravenous administration, including kidney (with the highest concentration), liver, hearth, spleen, lung and brain, being rapidly excreted.

Kim et al. (2000) investigated the fate of ^{3}H-aucubin after i.v. and oral application to rats. After i.v. injection, the serum concentration showed a normal exponential decrease and the compound was excreted mostly unchanged. After oral application, the serum concentration showed a plateau which did not change over many hours. It was demonstrated that the radioactivity was covalently attached to serum albumin and other protein molecules.

The applicant also provided some additional data on related iridoid glycosides. The available data (Bai et al., 2019; Bhattamisra et al., 2020) indicate that catalpol administered by oral route to rats at 50 mg/kg body weight (bw) is rapidly absorbed (T_{max} at 1.3 h with a C_{max} of 23 ng/mL), has a good bioavailability and is fast eliminated (half-life of ~ 1.2 h) owing to its hydrophilic nature. In the review, it is also reported that the highest distribution of catalpol was detected in kidney, post dosing of 5 min, followed by other highly perfused tissue, such as liver, heart and lung.

[10] Technical dossier/May 2020/Annex_2_Greart mullein tincture_Manghebati.
[11] Technical dossier/Supplementary information April 2021/Complementary bibliographic search on the potential genotoxicity of aucubin.

3.2.2. Toxicology

The applicant did not provide toxicological studies to support the safety of the additive under assessment but provided a literature search on the toxicity of catalpol, aucubin and ajugol[12] and a complementary search on the genotoxicity of aucubin.[11] By the analysis of this search, the FEEDAP Panel identified some publications providing supportive information for a possible genotoxic mode of action of aucubin, while some publications investigating the potential beneficial effects of iridoid glycosides were considered not relevant for the current assessment. Evidence on potential genotoxic activity is reported below.

3.2.3. Genotoxicity

Aucubin is unstable at the acidic pH of the stomach and can be degraded by the glycosidases of the intestinal wall and liver, leading to the aglycone. The aglycone of aucubin is very reactive and the opening of the 6-membered ring can lead to a dialdehyde. This structure is able to form Schiff-Bases with amino groups of proteins (Kim et al., 2000) and possibly also with DNA-bases.

The literature search provided by the applicant showed that the aucubin aglycone can bind to DNA and aucubin can inhibit DNA polymerase and RNA polymerase (Lee et al., 2001) as well as topoisomerase I (Gálvez et al., 2005). These are key enzymes involved in replication, repair, transcription and topology of DNA. The Panel noted that increase of DNA damage resulting from the inhibition of these enzymes is extensively documented in the scientific literature.

Quantitative Structure–Activity Relationship (QSAR) analysis performed using the OECD toolbox[13] of the aglycones of aucubin, catalpol and ajugol did not identify structural alerts, except for the presence of an epoxide for catalpol. However, when considering the dialdehyde, generated by the opening of the 6-membered ring, alerts for DNA binding, *in vitro* and *in vivo* mutagenicity were detected for all the compounds.

In the absence of experimental data on the genotoxicity of aucubin, based on the available data on the reactivity of aucubin or its aglycone with proteins and DNA, the FEEDAP Panel considers it likely that aucubin could damage DNA, suggesting a potential for genotoxicity. The other iridoid glycosides likely to be present may have the same or similar properties.

3.2.4. Safety 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.

For the components, for which no concern for genotoxicity has been identified the TTC values of Cramer structural class I-III were assigned (EFSA FEEDAP Panel, 2017b).

For the components that have the potential to be genotoxic mutagens, i.e. aucubin and related iridoid glycosides, the TTC concept is applied in a specified way depending on the lifespan of the target species and the biological relevance of genotoxicity and carcinogenicity as endpoints (EFSA Scientific Committee, 2017):

- For long-living animals (pets and other non-food producing animals, horses and reproduction animals), considering their long lifespan and the likelihood to develop cancer, the threshold of the TTC of 0.0025 μg/kg bw per day is applied. This value has been established for potential DNA-reactive mutagens and/or carcinogens in human risk assessment (EFSA Scientific Committee, 2019c) and is considered applicable in this context.
- Due to their short lifespan cancer risk is not a relevant concern for short-living animals under farming conditions (animals for fattening). For those animals, the TTC for non-genotoxic substances is applied when comparing estimated exposures with the relevant thresholds established based on non-neoplastic endpoints.

3.2.4.1. Phenolic compounds including flavonoids

Among the identified secondary metabolites, 0.22% is phenolic in nature including 0.09% flavonoids (as chlorogenic acid equivalents).

[12] Technical dossier/May 2020/Annex_2_Great mullein tincture_Manghebati.
[13] http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm

At the maximum proposed use level of 50 mg tincture/kg complete feed for all species except horses, the concentration of the phenolic fraction after subtraction of values for flavonoids (on average 0.12% and up to 0.13%, measured by the Folin–Ciocalteu method) would be on average 0.06 mg/kg feed and at maximum 0.07 mg/kg feed. Although the individual compounds were not identified, phenolic acids are assigned to Cramer Class I. The available data indicate that phenolic compounds would be well below the maximum acceptable concentration in feed for Cramer Class I (ranging from 0.3 mg/kg feed for poultry to 1.5 mg/kg feed for salmonids and dogs). For horses, at the maximum proposed use level of 600 mg/kg feed the average concentration of polyphenols would be 0.74 mg/kg feed (maximum 0.80 mg/kg feed), which is below the maximum acceptable concentration of 1.3 mg/kg for Cramer Class I compounds in feed for horses.

At least five unidentified flavonoids were detected and quantified (as chlorogenic acid equivalents) accounting together for 0.09% on average (maximum 0.11%). At the proposed use level of 50 mg tincture/kg complete feed this would correspond to maximum 0.05 mg/kg feed. The available data indicate that flavonoids would be in the range of maximum acceptable concentrations in feed for Cramer Class III (ranging from 0.02 mg/kg feed for poultry to 0.08 mg/kg feed for salmonids and dogs). For horses, at the maximum proposed use level of 600 mg/kg feed the average concentrations of flavonoids would be 0.56 mg/kg feed (maximum 0.65 mg/kg), which is about eightfold higher than the maximum acceptable concentration of 0.07 mg/kg for Cramer Class III compounds in feed for horses.

When used in water for drinking, the intake of the additive via water would be 2–3 times higher than the intake via feed for poultry, pigs and rabbits (EFSA FEEDAP Panel, 2010). At the maximum proposed use level of 50 mg/kg for the use in water for drinking, the concentration of polyphenols including flavonoids would be above the maximum acceptable concentration for these species.

3.2.4.2. Aucubin and related iridoid glycosides

Low concentrations of aucubin (on average: 0.004%, maximum 0.006%) were detected in the additive under assessment. The use of the tincture at the proposed use levels of 50 mg/kg complete feed would result in an average concentration of 2 μg aucubin/kg feed (maximum 3 μg/kg feed). The corresponding value for horses, at proposed use level of 600 mg/kg complete feed is 24 μg aucubin/kg feed (maximum 34 μg/kg). The average and maximum intake of aucubin for the different animal categories is reported in Table 3.

Table 3: Target animal intake of aucubin (as μg/kg bw per day) at the maximum proposed use level of the additive in feed for each species. The values of aucubin in feed are calculated considering the average and the maximum analysed values in the additive

Animal category	Daily feed intake	Body weight	Use level	Aucubin	
				Average	Maximum
	kg DM/day	kg	mg/kg	μg/kg bw per day	
Chicken for fattening	0.158	2	50	0.180	0.256
Laying hen	0.106	2	50	0.120	0.172
Turkey for fattening	0.176	3	50	0.133	0.190
Piglet	0.88	20	50	0.100	0.143
Pig for fattening	2.2	60	50	0.083	0.119
Sow lactating	5.28	175	50	0.069	0.098
Veal calf (milk replacer)	1.89	100	50	0.043	0.061
Cattle for fattening	8	400	50	0.045	0.065
Dairy cow	20	650	50	0.070	0.100
Sheep/goat	1.2	60	50	0.045	0.065
Horse	8	400	600	0.545	0.777
Rabbit	0.1	2	50	0.114	0.162
Salmon	0.0021	0.12	50	0.040	0.057
Dog	0.25	15	50	0.038	0.054
Cat	0.06	3	50	0.045	0.065
Ornamental fish	0.00054	0.012	50	0.010	0.015

DM: dry matter; bw: body weight.

Long-living animals (pets and other non-food producing animals, horses and animals for reproduction)

For long-living animals, the intake of aucubin ranges from 0.010 μg/kg bw per day in ornamental fish to 0.545 μg/kg bw per day in horses (maximum intake in the range 0.015–0.777 μg aucubin/kg bw per day). These intake levels are on average 4 to 218-fold (6 to 311-fold when considering the maximum intake values) higher than the TTC value of 0.0025 μg/kg bw per day established for potential DNA reactive mutagens and/or carcinogens in human risk assessment (EFSA Scientific Committee, 2019c). Exposure to total iridoid glycosides and iridoids is expected to be higher.

For long-living animals, the TTC value is exceeded, and generation of further data on genotoxicity and/or carcinogenicity would be required to reach a conclusion on potential adverse health effects. In the absence of such studies with aucubin and related iridoid glycosides, no conclusion can be drawn on the safety of great mullein tincture for long-living animals.

Short-living animals (fattening animals)

For short-living animals, the TTC based on non-cancer endpoints has been applied. For these species, the average intake of aucubin ranges from 0.040 μg/kg bw per day in salmonids to 0.180 μg/kg bw per day in chickens for fattening (maximum intake in the range 0.057–0.256 μg aucubin/kg bw per day). These average intake levels are 8- to 38-fold (6- to 26-fold when considering the maximum intake values) lower than the TTC value for Cramer class III compounds (1.5 μg/kg bw per day), indicating that there is a low probability of adverse effects. Exposure to total iridoid glycosides and iridoids is expected to be higher, but still remaining below the TTC value for Cramer class III compounds.

When used in water for drinking at the maximum proposed use level of 50 mg/kg, the intake of aucubin and related iridoid glycosides of non-ruminants would be below the TTC value for Cramer class III compounds (EFSA FEEDAP Panel, 2010). For ruminants, when considering aucubin and related iridoid glycosides, the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

3.2.4.3. Conclusions on safety for the target species

Considering the possible genotoxic potential of aucubin, no conclusions can be drawn on the safety of great mullein tincture for long-living animals (pets and other non-food producing animals, horses and animals for reproduction).

For short-living animals (animals for fattening), the FEEDAP Panel concludes that the additive under assessment, great mullein tincture is safe at the maximum proposed use level of 50 mg/kg complete feed. The FEEDAP Panel considers that the use in water for drinking in short-living animals is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

3.2.5. Safety for the consumer

The flowers of *V. phlomoidea* L., *V. thapsiforme* Schrad and *V. thapsus* L. are used to prepare infusions and decoctions, including traditional medicinal herbal teas, and to flavour alcoholic beverages (Burdock, 2009; EMA 2018a,b). Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites values of < 1.00 mg/kg bw per day for the flowers.

For the safety for the consumer, the FEEDAP Panel concluded in 2019 that the phenolic compounds present in the additive would be readily metabolised and excreted and were not expected to accumulate in animal tissues and products. Consequently, no concern for the consumer was expected from the phenolic fraction.

For aucubin and the other related iridoid glycosides, the available data indicate that some of them are absorbed to a limited extent, metabolised and rapidly excreted and are not expected to accumulate in animal tissues and products (see Section 3.2.1).

Considering the reported human exposure due to direct use of mullein flowers (1 mg/kg bw per day) (Burdock, 2009) and traditional medicinal use (EMA 2018a,b) it is unlikely that consumption of products from animals given great mullein tincture at the proposed maximum use level would significantly increase human background exposure.

Consequently, no safety concern would be expected for the consumer from the use of the tincture up to the highest safe use level in animal nutrition.

3.2.6. Safety for user

The applicant did not provide additional data on the safety for the user. The FEEDAP Panel is unable to revise its previous conclusion that "no conclusions can be drawn on the additive's potential to be a dermal/eye irritant or a skin sensitiser."

4. Conclusions

The tincture derived from *V. thapsus* (great mullein tincture) is safe at the maximum proposed use level of 50 mg/kg complete feed for short-living animals (animals for fattening). The FEEDAP Panel considers that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed. Considering the genotoxic potential of aucubin and other related iridoid glycosides, no conclusions can be drawn for long-living animals (pets and other non-food producing animals, horses and animals for reproduction).

No safety concern would arise for the consumer from the use of the tincture up to the highest safe level in animal nutrition.

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.

5. Documentation as provided to EFSA/Chronology

Date	Event
18/05/2020	Dossier received by EFSA. *Verbascum thapsus* L. (great mullein tincture) for all animal species. Submitted by Manghebati S.A.S.
03/06/2020	Reception mandate from the European Commission
26/06/2020	Application validated by EFSA – Start of the scientific assessment
11/09/2020	Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. Issues: Characterisation of the additive, safety for target species and consumers
03/05/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
24/06/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
DM	dry matter
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GI	gastrointestinal
HPLC	high-performance liquid chromatography
OECD	Organisation for Economic Co-operation and Development
QSAR	Quantitative Structure-Activity Relationship
SC	EFSA Scientific Committee
TTC	threshold of toxicological concern