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Safety and efficacy of a feed additive consisting of a tincture derived from the fruit of *Petroselinum crispum* (Mill.) Fuss (parsley tincture) for use in all animal species (FEFANA asbl)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Andrew Chesson, Johannes Westendorf, Paola Manini, Fabiola Pizzo and Birgit Dusemund

Abstract

Following a request from the European Commission, 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 derived from the fruit of *Petroselinum crispum* (Mill.) Fuss (parsley tincture) when used as a sensory feed additive for all animal species. The product is a [redacted] solution, with a dry matter content of approximately 0.82%. The product contained 0.0198% polyphenols (of which 0.0085% were flavonoids), apiole (0.0083%), elemicin (0.0015%) and myristicin (0.0011%). The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the parsley tincture is safe at the maximum proposed use levels of 200 mg/kg complete feed for horses and 50 mg/kg complete feed for all other animal species. The FEEDAP Panel considered that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount which is considered safe when consumed via feed. No safety concern would arise for the consumer from the use of parsley tincture up to the maximum proposed use levels in feed. Parsley tincture should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser. When handling the additive, exposure of unprotected users to apiole, elemicin and myristicin cannot be excluded. Therefore, to reduce the risk, the exposure of the users should be minimised. The use of parsley tincture as a flavour in animal feed was not expected to pose a risk for the environment. Since the fruit of *P. crispum* and its preparations were recognised to provide flavour in food and their function in feed would be essentially the same, no demonstration of efficacy was considered necessary.

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Keywords: sensory additives, flavouring compounds, *Petroselinum crispum* (Mill.) Fuss, parley tincture, apiole, elemicin, myristicin

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Correspondence: feedap@efsa.europa.eu

Panel members: Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

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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. In addition, Article 10(2) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of seven years after the entry into force of this Regulation.

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2,3] for authorisation/re-evaluation of 29 preparations (namely dill herb oil, dill seed extract, dill tincture, dong quai tincture, celery seed oil, celery seed extract (oleoresin), celery tincture, hares ear tincture, caraway seed oil, caraway oleoresin/extract, coriander oil, cumin oil, taiga root extract (solvent-based, sb), taiga root tincture, fennel oil, fennel tincture, common ivy extract (sb), opoponax oil, ginseng tincture, parsley oil, parsley tincture, anise oil, anise tincture, ajowan oil, *Ferula assa-foetida* oil, anise star oil, anise star tincture, anise star terpenes and omicha tincture) belonging to botanically defined group (BDG) 02 – *Apiales/Austrobaileyales* when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). During the assessment, the applicant withdrew the application for nine preparations (namely dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract, opoponax oil,[4] parsley oil, hares ear tincture, taiga root extract (sb), ajowan oil[5] and celery tincture[6]). These preparations were deleted from the register of feed additives.[7] During the course of the assessment, this application was split and the present opinion covers only one out of the 20 remaining preparations under application: parsley tincture (*Petroselinum crispum* (Mill.) Fuss) for all animal species.

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) and under Article 10(2) (re-evaluation of an authorised 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 24 June 2019.

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 parsley tincture (*P. crispum*), when used under the proposed conditions of use (see Section 3.2.2).

The remaining 19 preparations belonging to botanically defined group (BDG) 02 – *Apiales/ Austrobaileyales* under application are assessed in separate opinions.

1.2. Additional information

The subject of the assessment is the feed additive consisting of parsley tincture, intended for use as a sensory additive (functional group: flavouring) for all animal species.

A tincture from *Petroselinum sativum* Hoffm. (or *P. crispum* Mill. or *P. hortense* L.) (parsley tincture) 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). *P. sativum* and *P. hortense* are no longer considered to have taxonomic standing and both are

[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] On 13/03/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1,050 Brussels, Belgium.
[3] On 27 February 2019, EFSA was informed by the applicant about the transfer of contact point for this application to Manghebati SAS, zone de la Basse Haye– BP 42133–35,221 Chateaubourg Cedex.
[4] On 27 February 2019, EFSA was informed by the applicant about the withdrawal of the applications on dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract, and opoponax oil.
[5] On 2 April 2020, EFSA was informed by the applicant about the withdrawal of the applications on parsley oil, hares ear tincture, taiga root extract (sb), ajowan oil.
[6] On 9 December 2020, the applicant informed EFSA about the withdrawal of the application on celery tincture.
[7] Register of feed additives, Annex II, withdrawn by OJ L162, 10.05.2021, p. 5.

considered synonyms for *Petroselinum crispum* (Mill.) Fuss. Tinctures derived from parsley have not been assessed as feed additives in the EU.

There is no specific EU authorisation for any *P. crispum* preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008[8] 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'.

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[9] in support of the authorisation request for the use of parsley tincture from *P. crispum* as a feed additive.

The FEEDAP Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 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.

Some of the components of the tincture under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings (CDGs). The applicant submitted a written agreement to reuse the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 02.[10]

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance/agent in animal feed. The evaluation report is related to the methods of analysis for each feed additive included the group BDG 02 (Apiales and Austrobaileyales). In particular, for the characterisation of parsley tincture the EURL recommended methods based on spectrophotometry (for the determination of total polyphenols in the feed additive) and high-performance thin-layer chromatography (HPTLC) (for the determination of the content of total flavonoids and of the phytochemical markers myristicin and apiole in the feed additive).[11]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of parsley tincture from *P. crispum* is in line with the principles laid down in Regulation (EC) No 429/2008[12] and the relevant guidance documents: Opinion of the Scientific Committee on harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic (EFSA, 2005), Statement on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed (EFSA SC, 2012), Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA SC, 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), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a), Statement on the

[8] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Regulation (EC) No 1601/91 of the Council, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34.

[9] FEED dossier reference: FAD-2010-0221.

[10] Technical dossier FAD-2010-0335/Supplementary information February 2018/2018-01-30_SInReply_cardamom.

[11] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0221_en

[12] 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.

genotoxicity assessment of chemical mixtures (EFSA SC, 2019b), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA SC, 2019c) and General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021).[13]

3. Assessment

The additive under assessment, parsley tincture, is derived from the fruit of *P. crispum* (Mill.) Fuss and is intended for use as a sensory additive (functional group: flavouring compounds) in feed and water for drinking for all animal species.

3.1. Origin and extraction

P. crispum is a biennal herb belonging to the Apiaceae family and is commonly referred as to parsley or garden parsley. It is native to central and eastern Mediterranean regions, but has become naturalised in many other parts of Europe and elsewhere. The apical parts of the plant are used as an herb in cooking and parsley roots are used as a vegetable.

The tincture is produced from the washed seeds of *P. crispum* by extended extraction for 3 weeks under ambient conditions with a [redacted]. After this period, the tincture is recovered by pressing to separate solid and liquid phases and the extracted solution is then clarified by filtration.

3.2. Characterisation

3.2.1. Characterisation of the tincture

The tincture is a brown liquid, with a characteristic slightly green odour. It has an average density of [redacted] and a pH of 6.11 (5.88–6.29).[14] It is soluble in water.

Table 1 summarises the results of proximate analysis of five batches of the additive.[15] The solvent represents about 99.2% of the additive leaving a dry matter (DM) content of about 0.82%. The dry matter consists of inorganic material measured as ash (23.5%) and a plant-derived organic fraction of 76.5%, which includes protein, lipids and 'carbohydrates.'

Table 1: Proximate analysis of a tincture derived from the fruit of *Petroselinum crispum* (Mill.) Fuss based on the analysis of five batches (mean and range in %, w/w)

Constituent	Mean	Range
	% (w/w)	% (w/w)
Dry matter	0.82	0.75–0.84
Ash	0.19	0.18–0.20
Organic fraction	0.62	0.56–0.73
Proteins	0. 11	0.09–0.13
Lipids	0.007	0.005–0.009
'Carbohydrates'(1)	0.51	0.47–0.60
Solvent	99.18	99.06–99.25

(1): 'carbohydrates' (by difference) include secondary plant metabolites, such as phenolic compounds.

The constituent defined as 'carbohydrates' in Table 1 describes 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 carbohydrate present.

The fraction of secondary metabolites was characterised in the same batches of the tincture and the results are summarised in Table 2. The tincture was shown to contain polyphenols (0.0198%) determined by spectrophotometry (at 760 nm) and expressed as gallic acid equivalents. At least nine

[13] https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf
[14] Technical dossier/Supplementary information October 2020/Annex_II_3_Results of analysis.
[15] Technical dossier/Supplementary information October 2020.

unidentified flavonoids (0.0085%) were separately determined by HPTLC expressed as chlorogenic acid equivalents.[16] The concentration of apiole (0.0021–0.0056%) and myristicin (0.0014%) was also determined by HPTLC in the same five batches of the tincture and expressed in myristicin equivalents.[17,18]

From published literature, it is known that, apart from the components specified in Table 2, phenolic acids, such as chlorogenic acid, caffeic acid and ferulic acid, have been identified in the fruit of *P. crispum* (Tadros et al., 2017). Moreover, according to Alol et al. (2012), in the flavonoid fraction of the fruit, apigenin is the major component.

The EFSA Compendium of botanicals reports the presence of elemicin, apiole and myristicin in fruit as substances of concern (EFSA, 2012).[19] The applicant performed a literature search to identify substances of concern in *P. crispum* and its botanical preparations, essential oils and aqueous and ethanol-water extracts.[20] Apiole (7.05–65.4%) and myristicin (7.6–44%) were identified as major components of the essential oils from the fruit. Elemicin was reported in samples of two essential oils (2.2–4.8%). No information on the occurrence of substances of concern in ethanol-water extracts was retrieved.

The applicant provided analytical data by gas chromatography–mass spectrometry (GC–MS) which showed that the content of elemicin in five batches was 13.65–15.69 mg/kg and that of apiole 76.74–91.61 mg/kg. In the same batches, the concentration of myristicin was between the limit of detection (LOD, 3.5 mg/kg) and the limit of quantification (LOQ, 10.5 mg/kg).[21] There is no specification defining limit values for undesirable compounds in the tincture.

The identified secondary metabolites account only on average for 3.9% of the dry matter content of the tincture (range: 3.43–4.18%).

Table 2: Characterisation of the fraction of secondary metabolites of a tincture derived from the fruit of *Petroselinum crispum* (Mill.) Fuss based on the analysis of five batches (mean and range, results expressed as % of the tincture, w/w)

Constituent	Method	Mean	Range
		% (w/w)	% (w/w)
Total polyphenols	Folin–Ciocalteu	0.0198	0.0184–0.0217
Flavonoids	HPTLC	0.0085	0.0038–0.0118
Apiole	GC–MS	0.0083	0.0077–0.0092
Elemicin	GC–MS	0.0015	0.0014–0.0016
Myristicin(1)	GC–MS	–	–

HPTLC: high-performance thin-layer chromatography; GC–MS: gas chromatography–mass spectrometry.
(1): Between the limit of detection (LOD, 3.5 mg/kg) and the limit of quantification (LOQ, 10.5 mg/kg).

The applicant controls contamination at the level of the raw material, including knowledge of the cultivation conditions and pesticides applied. Specifications are set with suppliers covering cadmium < 1 mg/kg, mercury < 0.1 mg/kg and lead < 5 mg/kg, pesticides, polycyclic aromatic hydrocarbons (< 10 μg/kg benzo(a)pyrene, < 50 μg/kg for the sum of benzo(a)pyrene, benzo(a)anthracene, benzo (b)fluorantene and chrysene) and microbial contamination.[22] One certificate of analysis of the raw material (parsley seeds) showing compliance with specifications was provided.[23] Analysis of impurities in the tincture is made on irregular basis and does not form part of the Hazard Analysis and Critical Control Points (HACCP) Plan.

3.2.2. Stability

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 to support this claim.

[16] Technical dossier/Supplementary information October 2020/ Section_II_Identity and Annex II_3.
[17] Technical dossier/Supplementary information October 2020/ Annex II_8_Detailed report of myristicin quantification.
[18] Technical dossier/Supplementary information October 2020/ Annex II_9_Detailed report of apiole quantification.
[19] Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals.
[20] Technical dossier/Supplementary information October 2020/Annex II_4_Bibliographic data concerning chemical composition of parsley and parsley extracts.
[21] Technical dossier/Supplementary information March 2021.
[22] Technical dossier/Supplementary information October 2020/Annex II_6_Parsley seed (raw material)_TDS.
[23] Technical dossier/Supplementary information October 2020/Annex_II_5_ Parsley seed (raw material)_COA.

3.2.3. Conditions of use

The additive is intended for use in feed and in water for drinking for all animal species. The applicant proposes a maximum concentration of 50 mg parsley tincture/kg complete feed for all animal species, except for horses, for which the proposed use is 200 mg/kg complete feed. No use level has been proposed by the applicant for the use in water for drinking.

3.3. Safety

The safety assessment is based on the highest proposed use levels in feed, which are 200 mg/kg complete feed for horses and 50 mg/kg complete feed for all other species.

No studies to support the safety for target animals, consumers or users were performed with the additive under assessment.

The additive under assessment, parsley tincture, is a mixture consisting of 99.2% (w/w) of a water/ethanol mixture. The concentration of plant-derived compounds is about 0.8% (w/w) of the tincture. The dry matter includes ash, protein, lipids and carbohydrates, which are not of concern, and are not further considered.

Among the secondary plant metabolites, total phenolic compounds including flavonoids were quantified but not identified. They will be assessed based on considerations at the level of the assessment group (see Section 3.3.3.1). These compounds are readily metabolised and excreted and are not expected to accumulate in animal tissues and products.

The additive contains trace concentrations of myristicin (3.5–10.5 mg/kg), apiole (76.74–91.61 mg/kg) and elemicin (13.65–15.69 mg/kg), which are genotoxic and probably carcinogenic. All these compounds are *p*-allylalkoxybenzenes, structurally related to safrole, estragole and methyleugenol. Information on the absorption, distribution, metabolism and excretion (ADME) and on the toxicology of *p*-allylalkoxybenzenes is summarised in the next sections.

3.3.1. Absorption, distribution, metabolism and excretion of *p*-allylalkoxybenzenes

The additive contains three alkoxy-substituted allylbenzenes, apiole, myristicin and elemicin, with apiole present at the highest concentration. Apiole and myristicin are methylenedioxy-substituted allylbenzenes, whereas elemicin is a methoxy-substituted allylbenzene.

In 2009, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the data from experimental and human studies of ADME of safrole, myristicin, estragole and methyleugenol and other alkoxy-substituted allylbenzenes and concluded that they are rapidly absorbed after consumption by the oral route. The distribution, evaluated in animals with labelled compounds, is also rapid. Excretion of low doses is almost complete within 24 h as CO_2 in exhaled air and in urine as hydroxylated metabolites and their conjugated derivatives. At higher doses, excretion in exhaled air decreases while the urinary fraction of metabolites increases.

A comprehensive review of metabolic studies performed in experimental animals was made by JECFA (WHO, 2009), which identified three main biotransformation pathways for the metabolism of alkoxy-substituted allylbenzenes (either methylenedioxy- or methoxy-substituted):

1) O-Demethylenation of safrole, myristicin and apiole and O-demethylation of the one or more of the methoxy substituents of estragole, methyleugenol or elemicin followed by excretion of the hydroxylated compounds in the conjugated forms. The O-dealkylation pathway is predominant at low doses in humans, mice and rats.
2) Epoxidation of the double bond in the allyl side-chain forming the 2′,3′-epoxide which is then hydrolysed by the epoxide hydrolase producing the diol or is conjugated with glutathione.
3) A bioactivation pathway of methylenedioxy- or methoxy-substituted allylbenzenes produced by the hydroxylation of the alkene side-chain forming the 1′-hydroxy metabolite which can be conjugated with either glucuronic acid or sulfate or can undergo isomerisation. The sulfate conjugate of the 1′-hydroxy metabolite is considered the ultimate metabolite which is the hepatotoxic and hepatocarcinogenic agent of some of these compounds in rodents. The sulfate conjugate is unstable, and hydrolysis generates a reactive electrophilic intermediate which binds to proteins and DNA. The formation of protein and DNA adducts in liver is dose dependent as demonstrated *in vivo*. At low doses the O-demethylenation of myristicin and safrole is by far the predominant pathway, giving rise to dihydroxyallylbenzene metabolites that are readily excreted either free or as sulfate or glucuronic acid conjugates. At high

doses in rodents, the O-demethylenation pathway becomes saturated, and 1′-hydroxylation and epoxidation of the allyl side-chain become more prevalent.

The application of physiologically based kinetic (PBK) models predicted that in rat liver the formation of the 1′-sulfoxy metabolite is about 3 times lower for apiole than for safrole (Alajlouni et al., 2016). Similarly, for elemicin the formation of the DNA reactive 1′-sulfoxymetabolite was predicted to be 11- and 2-fold lower as compared to the formation of the 1′-sulfoxymetabolites of estragole and methyleugenol, respectively (van den Berg et al., 2012). For myristicin, the PBK models for rat predict the formation of 1′-sulfoxymyristicin to be 1.5-fold higher for myristicin than for safrole at low dose of 0.05 mg/kg body weight (bw) and 2.2-fold higher for myristicin than for safrole at dose level of 100 mg/kg bw (Al-Malahmeh et al., 2017).

3.3.2. Toxicology of *p*-allylalkoxybenzenes

The tincture contains myristicin, apiole and elemicin, compounds which belong to the class of *p*-allylalkoxybenzene. They are structurally related to compounds with experimentally proven genotoxicity and carcinogenicity in rodents like safrole, estragole and methyleugenol (as reviewed in EC, 2002; EMA, 2021; IARC, 2018).

Although myristicin, parsley apiole and elemicin did not induce the formation of hepatic tumours in newborn male mice, after intraperitoneal (i.p.) injection, 1′-hydroxyelemicin had hepatocarcinogenic activity at high doses (Miller et al., 1983, as referenced in EMA, 2021). In addition, myristicin and elemicin were also shown to form DNA adducts, although the potency was lower than that of methyleugenol, estragole and safrole, whereas for parsley apiole DNA adducts were below the LOQ *of 1 pmol/mg DNA* (Phillips et al., 1984, Randerath et al., 1984; Zhou et al., 2007, as referenced in EMA, 2021). The two *in vivo* studies resulted in the same order of potency (i.e. methyleugenol > safrole > estragole > myristicin > elemicin > dillapiole).

Based on the above considerations on the relative potency of *p*-allylalkoxybenzenes and on the mode of action, the FEEDAP Panel selected the benchmark dose (BMD) lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) of 22.2 mg/kg bw per day derived from a carcinogenicity study in rats with methyleugenol (NTP, 2000) by applying model averaging (Suparmi et al., 2019), as the reference point for the assessment group of *p*-allylalkoxybenzenes (EFSA FEEDAP Panel, 2022).

3.3.3. 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, the approach to the safety assessment of the mixture is based on its individual components or groups of components. For the group assessment of phenolic compounds and flavonoids, in the absence of data, the threshold of toxicological concern (TTC) is applied to derive maximum safe feed concentrations for the whole groups in the tincture (EFSA FEEDAP Panel, 2017b).

3.3.3.1. Phenolic compounds including flavonoids

Among the secondary metabolites, 0.020% are polyphenols including 0.009% flavonoids.

At the maximum proposed use level of 50 mg parsley tincture/kg complete feed, the highest concentration of the fraction of polyphenols after subtraction of values for flavonoids (≤ 0.016% of the tincture, measured by the Folin–Ciocalteu method) would be 0.008 mg/kg feed. Although the individual compounds were not identified, the occurrence of phenolic acids, such as chlorogenic acid, caffeic acid and ferulic acid have been described in literature for fruit of *P. crispum* (see Section 3.2.1). These compounds are assigned to Cramer Class I and the available data indicate that their concentration would be two orders of magnitude 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 200 mg/kg complete feed, the highest concentration of polyphenols would be 0.031 mg/kg feed, which is well below the maximum acceptable concentration of 1.3 mg/kg feed for Cramer Class I compounds in feed for horses. Therefore, no concern for the target species arises from polyphenols other than flavonoids in parsley tincture.

At least nine unidentified flavonoids were detected and quantified (as chlorogenic acid equivalents) accounting together for ≤ 0.012% of the tincture. At the maximum proposed use level of 50 mg parsley tincture/kg complete feed, this would correspond to 0.006 mg/kg feed. Although the individual

compounds were not identified, flavonoids are assigned to Cramer Class III. The available data indicate that flavonoids would be at least 3-fold below the 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 200 mg/kg complete feed, the highest concentrations of flavonoids would be 0.024 mg/kg feed, which is below the maximum acceptable concentration of 0.07 mg/kg for Cramer Class III compounds in feed for horses. Therefore, the presence of flavonoids is not considered of concern for the target species.

Overall, no concern for the target species arises from the phenolic fraction and the presence of flavonoids.

3.3.3.2. Myristicin, apiole and elemicin

Low concentrations of apiole (76.74–91.61 mg/kg) and elemicin (13.65–15.69 mg/kg) were detected in all batches of the additive under assessment. In the same batches, the concentration of myristicin was between the LOD (3.5 mg/kg) and the LOQ (10.5 mg/kg).

At the maximum proposed use level of 50 mg parsley tincture/kg complete feed, the highest concentration of apiole, elemicin and myristicin would be up to 0.0046, 0.0008 and 0.0005, mg/kg complete feed, respectively.[24] The corresponding values for horses at the proposed use level of 200 mg/kg complete feed would be 0.018, 0.003 and 0.0021 mg/kg complete feed.

The individual intake for apiole, elemicin myristicin and their combined intake calculated for the target species at the maximum proposed use level in feed (50 mg parsley tincture/kg complete feed for all animal species, except horses, for which the proposed use is 200 mg/kg complete feed) are reported in Table 3.

Table 3: Individual intake of apiole, elemicin and myristicin, their combined intake calculated for the target animal categories at the maximum proposed use level of the additive in feed

Target species	Daily feed intake	Body weight	Use level	Apiole intake[(a)]	Elemecin intake[(a)]	Myristicin Intake[(a)]	Combined intake[(a)]
	kg DM/day	kg	mg/kg	µg/kg bw per day			
Chickens for fattening	0.158	2	50	0.413	0.072	0.047	0.532
Laying hens	0.106	2	50	0.277	0.048	0.032	0.357
Turkey for fattening	0.176	3	50	0.307	0.053	0.035	0.395
Piglet	0.88	20	50	0.230	0.040	0.026	0.296
Pig for fattening	2.2	60	50	0.192	0.033	0.022	0.247
Sow lactating	5.28	175	50	0.158	0.027	0.018	0.203
Veal calf (milk replacer)	1.89	100	50	0.092	0.017	0.011	0.120
Cattle for fattening	8	400	50	0.105	0.018	0.012	0.135
Dairy cows	20	650	50	0.161	0.028	0.018	0.207
Sheep/goat	1.2	60	50	0.105	0.018	0.012	0.135
Horse	8	400	200	0.418	0.073	0.048	0.539
Rabbit	0.1	2	50	0.261	0.045	0.030	0.337
Salmon	0.0021	0.12	50	0.091	0.016	0.010	0.118
Dog	0.25	15	50	0.087	0.015	0.010	0.112
Cat	0.06	3	50	0.105	0.018	0.012	0.135
Ornamental fish	0.00054	0.012	50	0.024	0.004	0.003	0.030

DM: dry matter; bw: body weight.

(a): The intake values of elemicin and apiole and dillapiole are calculated considering the highest analysed concentration in the tincture, the value for myristicin assuming it to be equivalent to the LOQ of 10.5 mg/kg.

[24] The concentration of myristicin, apiole and elemicin in feed were calculated considering the highest concentration measured in the tincture for apiole (0.0092%) and elemicin (0.0016%), whereas for myristicin its concentration in the tincture was assumed to be equivalent to the LOQ of 10.5 mg/kg of the GC–MS method (corresponding to 0.00105%).

Apiole, elemicin and myristicin belong to the same structural group (*p*-allylalkoxybenzenes) and share the same metabolic pathways, particularly the formation of the reactive 1′-sulfoxymetabolite (see Section 3.3.1) and the same mode of action. Although the available data do not allow to derive a relative potency factor, they indicate that these compounds are less potent than methyleugenol, estragole and safrole with respect to their genotoxicity/carcinogenicity effects (see Section 3.3.2). However, in the current assessment, myristicin, apiole and elemicin are grouped together in a worst-case scenario and considered equally potent to methyleugenol.

The FEEDAP Panel identified the $BMDL_{10}$ of 22.2 mg/kg bw per day derived from rodent carcinogenicity studies with methyleugenol (NTP, 2000; Suparmi et al., 2019), as the reference point for entire group of *p*-allylalkoxybenzenes (EFSA FEEDAP Panel, 2022). In the current assessment this reference point is also applied to myristicin, apiole and elemicin. When the estimated combined exposures to myristicin, apiole and elemicin for the different animal categories are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day, a combined margin of exposure (MOET) is calculated for the different target species.

The highest daily intake of myristicin, apiole and elemicin for the different target animal categories and the corresponding MOET are reported in Table 4.

Table 4: Compositional data, intake values (calculated for chickens for fattening at 50 mg/kg complete feed), reference points and margin of exposure (MOE) for myristicin, apiole and elemicin (if present in the additive at the corresponding limit of detection), and combined margin of exposure (MOET) for the assessment group *p*-allylalkoxybenzenes

Composition		**Exposure**		**Hazard characterisation**	**Risk characterisation**	
Assessment group	Highest conc. in the tincture	Highest feed conc.	Highest intake[(a)]	$BMDL_{10}$	MOE	MOET
Constituent	mg/kg	µg/kg	µg/kg bw per day	mg/kg bw per day	–	–
***p*-Allylalkoxybenzenes**						
Apiole	91.6	4.60	0.413	22.2	53,759	
Elemicin	15.7	0.80	0.072	22.2	309,114	
Myristicin	10.5	0.70	0.063	22.2	353,273	
MOET						41,737

(a): Intake calculations for the individual components are based on the use level of 50 mg/kg in feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point ($BMDL_{10}$) to the intake. The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

From the MOET for chickens for fattening, the MOET for *p*-allylalkoxybenzenes was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 5.

Table 5: Combined exposure and combined margin of exposure (MOET) for the assessment group *p*-allylalkoxybenzenes calculated at the maximum proposed use level of the additive in feed for target animal category

Animal category	Daily feed intake	Body weight	Use level	Combined Intake	MOET
	kg DM/day	kg	mg/kg	µg/kg bw per day	–
Chicken for fattening	0.158	2	50	0.532	41,737
Laying hen	0.106	2	50	0.357	62,212
Turkey for fattening	0.176	3	50	0.395	55,885
Piglet	0.88	20	50	0.296	74,937
Pig for fattening	2.2	60	50	0.247	89,114
Sow lactating	5.28	175	50	0.203	109,907
Veal calf (milk replacer)	1.89	100	50	0.120	173,538
Cattle for fattening	8	400	50	0.135	164,861
Dairy cow	20	650	50	0.207	106,362
Sheep/goat	1.2	60	50	0.135	164,861
Horse	8	400	200	0.539	41,215
Rabbit	0.1	2	50	0.337	65,944
Salmon	0.0021	0.12	50	0.118	183,179
Dog	0.25	15	50	0.112	193,954
Cat	0.06	3	50	0.135	164,861
Ornamental fish	0.00054	0.012	50	0.030	659,445

DM: dry matter; bw: body weight.

When the estimated exposures for the different animal categories are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day derived for methyleugenol by Suparmi et al. (2019) from a rodent carcinogenicity study (NTP, 2000, see Section 3.2.2), a MOET of at least 41,000 is calculated (see Table 5). The magnitude of this MOET is indicative of a low concern for the target species.

3.3.3.3. Conclusions on safety for the target species

The additive under assessment, parsley tincture, is safe up to maximum proposed use levels of 200 mg/kg complete feed for horses and 50 mg/kg complete feed for all other animal species.

The FEEDAP Panel considers that the use of the additive in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount which is considered safe when consumed via feed.

3.3.4. Safety for the consumer

Parsley, its fruit and their preparations are added to a wide range of food categories as spice or for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites values of 6.55 mg/kg bw per day for parsley, 0.0007 mg/kg bw per day for oil of parsley fruit and 0.002 mg/kg bw per day for parsley oleoresin (parsley leaf and parsley fruit oleoresin).

No data on residues in products of animal origin were made available for any of the constituents of the tincture. When considering the ADME of the individual components, the polyphenols, including flavonoids, present in the additive at concentrations below the thresholds for Cramer Class I compounds or Cramer Class III compounds, respectively, will be readily metabolised and excreted and are not expected to accumulate in animal tissues and products. For myristicin, apiole and elemicin, detected in low concentrations in the additive, the available data indicate that they are absorbed, metabolised and rapidly excreted and are not expected to accumulate in animal tissues and products (see Section 3.3.1).

Considering the above and the reported human exposure due to direct use of parsley, its fruit and their preparations in food (Burdock, 2009), it is unlikely that consumption of products from animals given parsley 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 parsley tincture up to the maximum proposed use levels in feed.

3.3.5. Safety for the user

No specific data were provided by the applicant regarding the safety of the additive for users.

The applicant provided information according to Classification, Labelling and Packaging (CLP) Regulation (EC) 1272/2008[25] concerning the presence of ethanol in the tincture.[26]

The additive under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser.

When handling the additive, exposure of unprotected users to apiole, elemicin and myristicin cannot be excluded. Therefore, to reduce the risk, the exposure of the users should be minimised.

3.3.6. Safety for the environment

P. crispum is a native species to Europe where it is widely grown both for commercial and decorative purposes. Therefore, the use of the tincture under the proposed conditions of use in animal feed is not expected to pose a risk for the environment.

3.4. Efficacy

Parsley (*P. crispum*), its fruit and its preparations are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009), by the Flavour and Extract Manufactures Association (FEMA) with the reference numbers 2,835 (parsley), 2,836 (parsley oil) and 2,837 (parsley oleoresin).

Since parsley fruit and its preparations 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.

4. Conclusions

Parsley tincture from the fruit of *P. crispum* (Mill.) Fuss may be produced from plants of different origins and by various processes resulting in preparations with different composition and toxicological profiles. Thus, the following conclusions apply only to parsley tincture which contains ≤ 92 mg/kg apiole, ≤ 16 mg/kg elemicin, ≤ 11 mg/kg myristicin and is produced from the seeds of *P. crispum* (Mill.).

The additive is safe at the maximum proposed use levels of 200 mg/kg complete feed for horses and 50 mg/kg complete feed for all other animal species. 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 which is considered safe when consumed via feed.

No safety concern would arise for the consumer from the use of parsley tincture up to the maximum proposed use levels in feed.

The additive under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser. When handling the additive, exposure of unprotected users to apiole, elemicin and myristicin cannot be excluded. Therefore, to reduce the risk, the exposure of the users should be minimised.

The use of parsley tincture as a flavour in animal feed is not expected to pose a risk for the environment.

Since the fruit of *P. crispum* and its preparations 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 assessment.

5. Recommendation

The specification should ensure that apiole, elemicin and myristicin concentrations should be as low as possible and should not exceed 92, 16 and 11 mg/kg parsley tincture, respectively.

[25] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. *OJ L 353, 31.12.2008, p. 1–1,355*.

[26] H319: causes serious eye irritation (relevant for dermal exposure).

6. Documentation provided to EFSA/Chronology

Date	Event
28/10/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 02 - Apiales and Austrobaileyales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)
09/11/2010	Reception mandate from the European Commission
26/02/2013	EFSA informed the applicant (EFSA ref. 7,150,727) that, 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, as agreed with the European Commission
24/06/2015	Technical hearing during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products": data requirement for the risk assessment of botanicals
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract, and opoponax oil
24/06/2019	Application validated by EFSA – Start of the scientific assessment
03/07/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization, safety for the target species, safety for the consumer, safety for the user, safety for the environment*
30/09/2019	Comments received from Member States
28/10/2020	Reception of supplementary information from the applicant (partial submission) - Scientific assessment remains suspended
22/06/2022	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization and safety for target species*
29/08/2022	Reception of supplementary information from the applicant (partial submission) - Scientific assessment remains suspended
16/09/2022	The application was split and a new EFSA-Q-2022-00570 was assigned to the preparation included in the present assessment
31/10/2022	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives - Scientific assessment re-started
22/11/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment for the preparation included in the present assessment. The assessment of other preparations is still ongoing

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Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
BDG	botanically defined group
BMD	benchmark dose
$BMDL_{10}$	benchmark dose (BMD) lower confidence limit for a benchmark response of 10%
bw	body weight
CAS	Chemical Abstracts Service
CDG	chemically defined group
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CLP	Classification, Labelling and Packaging
CYP450	cytochrome P450
DM	dry matter
EEIG	European economic interest grouping
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavour and Extract Manufactures Association
FFAC	Feed Flavourings authorisation Consortium of FEFANA (EU Association of Specialty Feed Ingredients and their Mixtures)
FLAVIS	The EU Flavour Information System
GC–MS	gas chromatography–mass spectrometry

HACCP	Hazard Analysis and Critical Control Points
HPTLC	high-performance thin-layer chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
MOE	margin of exposure
MOET	combined margin of exposure (total)
NOAEL	no observed adverse effect level
NTP	national toxicology program
PBK	physiologically based kinetic
sb	solvent-based
SC	EFSA Scientific Committee
TTC	threshold of toxicological concern
UF	uncertainty factor
WHO	World Health Organization