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Safety and efficacy of feed additives consisting of an essential oil and tincture from the berries of *Juniperus communis* L. (juniper oil and juniper tincture) for use in all animal species (FEFANA asbl)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of juniper oil and juniper tincture obtained from the berries of *Juniperus communis* L., when used as sensory additives for all animal species. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that juniper oil is safe up to the maximum proposed use levels in complete feed of 5 mg/kg for laying hens, 15 mg/kg for veal calves (milk replacer) and cattle for fattening and 20 mg/kg for ornamental fish. For the other species, the calculated safe concentrations in complete feed were 4 mg/kg for chickens for fattening, 5 mg/kg for turkeys for fattening, 7 mg/kg for piglets, 8 mg/kg for pigs for fattening, 10 mg/kg for sows, dairy cows, 16 mg/kg for sheep, goats and horses, 6 mg/kg for rabbits, 17 mg/kg for salmonids, 18 mg/kg for dogs and 3 mg/kg for cats. These conclusions were extrapolated to other physiologically related species. For any other species, the additive was considered safe at 3 mg/kg complete feed. The FEEDAP Panel concluded that juniper tincture is safe up to the maximum proposed use level in feed of 45 mg/kg complete feed or water for drinking for all animal species. No concerns for consumers and the environment were identified following the use of the additives to the highest safe level in feed. The additives under assessment should be considered as irritants to skin and eyes, and as skin and respiratory sensitisers. Since the berries of *J. communis* and their preparations are recognised to flavour food and their function in feed would be the same as that in food, no further demonstration of efficacy was considered necessary.

Keywords: sensory additives, flavouring compounds, *Juniperus communis* L., juniper oil, juniper tincture, α-pinene, tannins

Requestor: European Commission

Question number: EFSA-Q-2010-01516 (new EFSA-Q-2023-00181)

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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 7 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] for authorisation/re-evaluation of 10 preparations (namely juniper oil, juniper berry extract (water-based, wb) and juniper tincture from *Juniper communis* L., cedarwood Texas oil from *Juniperus mexicana* Schiede, pine oil and pine tincture from *Pinus pinaster* Soland, pine oil white from *Pinus* spp., e.g. *P. sylvestris* L., pine needle oil from *Abies alba* Mill., *Abies sibirica* Ledeb., gingko extract (wb) and gingko tincture from *Gingko biloba* L.) belonging to botanically defined group (BDG) 18 – Gymnosperms (Coniferales, Ginkgoales) when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). During the assessment, the applicant withdrew the application for juniper berry extract (wb), pine oil[3] and pine needle oil.[4] These preparations were deleted from the register of feed additives.[5] During the course of the assessment, the application was split, and the present opinion covers only two out of the seven remaining preparations under application: juniper oil and juniper tincture (*Juniperus communis* L.) 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 11 February 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 products juniper oil and juniper tincture (*Juniperus communis* L.), when used under the proposed conditions of use (see Sections 3.2.1.3 and 3.3.1.3).

The remaining five preparations belonging to botanically defined group (BDG) 18 – Gymnosperms (Coniferales, Ginkgoales) under application are assessed in separate opinions.

1.2. Additional information

Juniper berry oil and juniper tincture from *Juniper communis* L. are 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). They have not been assessed as feed additives in the EU.

There is no specific EU authorisation for any *J. communis* L. preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008[6] flavouring preparations produced from food, may be used without an evaluation and approval as long as 'they do not, on the

[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 13/3/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1050 Brussels, Belgium.
[3] On 27 February 2019, EFSA was informed by the applicant about the withdrawal of the applications on juniper berry extract (wb) and pine oil.
[4] On 18 March 2021, EFSA was informed by the applicant about the withdrawal of the application on pine needle oil.
[5] Register of feed additives, Annex II, withdrawn by OJ L162, 10.5.2021, p. 5.
[6] 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.

basis of the scientific evidence available, pose a safety risk to the health of the consumer, and their use does not mislead the consumer.'

'Juniper (Juniperi pseudo-fructus)' is described in a monograph of the European Pharmacopoeia 10.5 (PhEur, 2021a) as the dried ripe cone berry of *Juniperus communis* L. with a minimum content of 10 mL/kg of essential oil (anhydrous drug).

'Juniper oil (Juniperi aetheroleum)' is described in a monograph of the European Pharmacopoeia 10.5 (PhEur, 2021b) as essential oil obtained by steam distillation from the ripe, non-fermented berry cones of *Juniperus communis* L.

For veterinary medicinal uses, the European Medicines Agency (EMA) published a summary report on Juniperi fructus (EMA, 1999). For human traditional medicinal uses, the EMA published an herbal monograph and an assessment report on *Juniperus communis* L., aetheroleum (EMA, 2010a,b) and an addendum to the assessment report in 2020 (EMA, 2020). The EMA also published an herbal monograph and an assessment report on *Juniperus communis* L., pseudo-fructus (EMA, 2009a,b), both revised in 2022 (EMA, 2022a,b).

Many of the individual components of the essential oil have been already assessed as chemically defined flavourings for use in feed and food by the FEEDAP Panel, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) and the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). The flavouring compounds currently authorised for feed[7] and/or food[8] use, together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000[9], and the corresponding EFSA opinion are listed in Table 1.

Table 1: Flavouring compounds already assessed by EFSA as chemically defined flavourings, grouped according to the chemical group (CG) as defined in Commission Regulation (EC) No 1565/2000, with indication of the EU Flavour Information System (FLAVIS) number and the corresponding EFSA opinion

CG	Chemical group	Product – EU register name (common name)	FLAVIS no	EFSA opinion,* Year
06	Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers	Linalool	02.013	2012a
		α-Terpineol	02.014	
		4-Terpineol	02.072	
		α-Terpinyl acetate	09.015	
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	*d,l*-Borneol	02.016	2016a
		d-Fenchone[(a)]	07.159	
		d,l-Bornyl acetate	09.017	
16	Aliphatic and alicyclic ethers	1,8-Cineole (eucalyptol)	03.001	2012a; 2021
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	Limonene[(b),(c)]	01.001	2008, EFSA (AFC)
		1-Isopropyl-4-methylbenzene (p-cymene)	01.002	2015
		Terpinolene	01.005	
		α-Phellandrene	01.006	
		1-Isopropenyl-4-methylbenzene	01.010	
		α-Terpinene	01.019	

[7] European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03.pdf

[8] Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1.

[9] Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8.

CG	Chemical group	Product – EU register name (common name)	FLAVIS no	EFSA opinion,* Year
		γ-Terpinene	01.020	
		d-Limonene	01.045	
		Pin-2(10)-ene (β-pinene)	01.003	2016b
		Pin-2(3)-ene (α-pinene)	01.004	
		β-Caryophyllene	01.007	
		Myrcene	01.008	
		Camphene	01.009	
		δ-3-Carene	01.029	
		δ-Cadinene[(b),(d)]	01.021	2011, CEF
		β-Cubebene[(b),(d)]	01.030	
		δ-Elemene[(b)]	01.039	
		Germacra-1(10),4(14),5-triene[(b),(d)]	01.042	
		3,7,10-Humulatriene[(b),(d)]	01.043	
		Longifolene[(b),(d)]	01.047	
		α-Muurolene[(b),(d)]	01.052	
		1,1,7-Trimethyl tricyclo [2.2.1.0.(2.6)]heptane (tricyclene)[(b),(d)]	01.060	
		4(10)-Thujene (sabinene)[(b)]	01.059	2015b, CEF
32	Epoxides	β-Caryophyllene epoxide	16.043	2014, CEF

(*): FEEDAP opinion unless otherwise indicated.
(a): Present in the additive as a mixture of enantiomers (d,l-fenchone or (±)-fenchone). JECFA and EFSA evaluated the enantiomer d-fenchone [07.159] for use in food and in feed (EFSA FEDAP Panel, 2016a).
(b): Evaluated for use in food. According to Regulation (EC) 1565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.
(c): JECFA and EFSA evaluated d-limonene [01.045] (EFSA, 2008). d-Limonene [01.045] and l-limonene [01.046] were also evaluated for use in feed (EFSA FEEDAP Panel, 2015).
(d): Evaluated applying the 'Procedure' described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). No longer authorised for use as flavours in food, as the additional toxicity data requested (EFSA CEF Panel, 2011) were not submitted and the CEF Panel was unable to complete its assessment (EFSA CEF Panel, 2015b).

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[10] in support of the authorisation request for the use of juniper oil and juniper tincture from *J. communis* L. as a feed additive. The dossier was received on 02 March 2023 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00181.[11]

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.

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings. The applicant submitted a written agreement to use the data submitted for the assessment of chemically defined flavourings (dossiers, publications and

[10] Dossier reference: FAD-2010-0320.
[11] The original application EFSA-Q-2010-01516 was split on 2/3/2023 and a new EFSA-Q-2023-00181 was generated.

unpublished reports) for the risk assessment of preparations belonging to BDG 18,[12] including the current one/s under assessment.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phytochemical marker in the additive. The evaluation report is related to the methods of analysis for each feed additive included in BDG 18 – Gymnosperms (Coniferales, Ginkgoales). In particular, for the characterisation of juniper oil, the EURL recommended a method based on gas chromatography with flame ionisation detection (GC-FID) for the quantification of the phytochemical marker pin-2(3)-ene (hereinafter referred as to α-pinene) in *juniper berry oil*. For the tincture, the EURL recommended a spectrophotometric method of the European Pharmacopeia (1/2008:20814) for the quantification of the phytochemical marker *tannins* (expressed as pyrogallol) in *juniper tincture*.[13]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of juniper oil and juniper tincture from *J. communis* L. is in line with the principles laid down in Regulation (EC) No 429/2008[14] and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 2009), Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012); Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012c); Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d); 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 Scientific Committee, 2019a); Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b); Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee, 2019c).

3. Assessment

The two additives under assessment are juniper oil and juniper tincture obtained from the berries of *Juniperus communis* L.. They are intended for use as sensory additives (functional group: flavouring compounds) for all animal species.

3.1. Origin and extraction

Juniperus communis L., or the common juniper, is a coniferous shrub or tree belonging to the Cupressaceae family. It is found throughout the Northern Hemisphere and is said to have the widest geographical distribution of any woody plant. Size is very variable from a small prostrate spreading shrub to a large tree. Juniper is dioecious and seeds are borne on the female plant in berry-like fleshy seed cones which turn purple-black when ripe. The ripe seed cone is edible and used as a flavouring in a variety of foods. It is an essential ingredient of gin and other liquors. It has a long history of use in many traditional systems of medicine to treat a range of disorders.

The essential oil is extracted from ripe fresh, dried or fermented berries (seed cones) by steam distillation. Released volatile compounds are collected and condensed and then separated from the aqueous fraction by decantation.

The tincture is obtained by maceration of whole dried cone berries in a water/ethanol mixture (35/65, v/v) for a period of 2 h at 35°C under stirring. The ratio of dry raw material to solvent is 1:10 (w/v). Following extraction, the tincture is obtained by pressing to remove solid material and clarified by filtration.

[12] Technical dossier/Supplementary information/Letter dated 31/1/2023.
[13] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0320_en
[14] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

3.2. Juniper oil

3.2.1. Characterisation of the juniper oil

Juniper oil is a light yellow clear mobile liquid, with a characteristic aroma. In five batches of the additive, the refractive index (20°C) ranged between 1.474 and 1.475 (average: 0.857), the density (20°C) between 855 and 863 kg/m^3 (average: 857 kg/m^3) and the optical rotation (20°C) between −14° and −11° (average: −12°).[15] Juniper oil is identified with the single Chemical Abstracts Service (CAS) number 8002-68-4, the European Inventory of Existing Commercial Chemical Substances (EINECS) number 283-68-3, the Flavor Extract Manufacturers Association (FEMA) number 2604 and Council of Europe (CoE) number 249.

For juniper oil, the product specifications used by the applicant are based on those developed by the International Organisation for Standardization (ISO) 8897:2010 for oil of juniper berry (*J. communis* L.),[16] adapted to reflect the concentrations of the main volatile components. Four components contribute to the specifications as shown in Table 2, with α-pinene selected as phytochemical marker. The analysis of five batches of the additive showed compliance with the specifications when analysed by GC-FID and expressed as percentage of gas chromatographic peak area (% GC area).[17] The applicant provided the full characterisation of the volatile constituents in five batches obtained by gas chromatography–mass spectrometry (GC–MS).[18] The four compounds accounted for about 64.8% on average (range 63.4–66.1%) of % GC area (Table 2).

Table 2: Main constituents of the essential oil from the berries of *Juniperus communis* L. as defined by specifications: batch to batch variation based on the analysis of five batches. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent	CAS No	FLAVIS No	% GC area		
EU register name			Specification	Mean	Range[(a)]
α-Pinene (pin-2(3)-ene)	80-56-8	01.004	25–45	41.0	39.6–44.2
Myrcene	125-35-3	01.008	3–22	10.6	4.9–12.9
Sabinene (4(10)-thujene)	3387-41-5	01.059	4–20	8.2	7.5–9.7
β-Pinene (pin-2(10)-ene)	127-91-3	01.003	1–12	5.0	4.3–7.3
Total				64.8	63.4–66.1

EU: European Union; CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.
(a): The values given for the Total are the lowest and the highest values of the sum of the components in the five batches analysed.

In total, up to 71 constituents were detected, 68 of which were identified and accounted on average for 99.8% (99.8–100%) of the % GC area. Besides the four compounds indicated in the product specifications, 13 other compounds were detected at individual levels >0.5% and are listed in Table 3. These 17 compounds >0.5% together accounted on average for 94.4% (93.4–97.5%) of the % GC area. The remaining 51 compounds (ranging between 0.001% and 0.5%) and accounting for 5.6% are listed in the footnote.[19] Based on the available data on the characterisation, juniper oil is considered a fully defined mixture (EFSA Scientific Committee, 2019a).

[15] Technical dossier/Supplementary information February 2021/Annex_II_juniper_berry_oil_CoA_chromatogram.
[16] Technical dossier/Supplementary information February 2021/Annex_III_SIn_reply_juniper_berry_oil_white_ISO.
[17] Technical dossier/Supplementary information February 2021/SIn_reply_juniper_berry oil/GC-FID analysis: α-pinene (41–42%), myrcene (8.1–15.1%), sabinene (7.24–8.64%), and β-pinene (4.83–9.42%).
[18] Technical dossier/Supplementary information February 2021/Annex_II_ juniper_berry_oil_CoA_chromatogram.
[19] Additional constituents: 19 components < 0.5% and > 0.1%: α-phellandrene, camphene, α-cubebene, γ-muurolene, longifolene, α-terpineol, α-copaene, β-cubebene, α-muurolene, γ-cadinene, α-selinene, bicyclogermacrene, γ-elemene, trans-β-farnesene, bicyclosesquiphellandrene, pseudolimonene, β-selinene, *d,l*-bornyl acetate and α-cadinene; 32 components < 0.1%: cubebol, trans-cadina-1(6),4-diene, cubenene, m-camphorene, δ-3-carene, linalool, alloaromadendrene, 1,1,7-trimethyltricyclo[2.2.1.0.(2.6)]heptane, selina-3,7(11)-diene, α-fenchene, 1-isopropenyl-4-methylbenzene, cubenol, α-terpinyl acetate, δ-elemene, α-cadinol, δ-amorphene, 1-epi-cubenol, α-longipinene, β-sesquiphellandrene, zonarene, β-caryophyllene epoxide, 1,8-cineole, T-muurolol, p-camphorene, α-amorphene, *d,l*-borneol, β-copaene, fenchone, aromadendrene, cadina-1 (6),4-diene (isomer), m-cymene and selina-3,6-diene.

Table 3: Other constituents of the essential oil from the berries of *Juniperus communis* L. accounting for >0.5% of the composition (based on the analysis of five batches) not included in the specifications. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent EU register name	CAS No	FLAVIS No	% GC area	
			Mean	Range[(a)]
d-Limonene	5989-27-5	01.045	6.43	4.39–7.05
β-Caryophyllene	87-44-5	01.007	5.42	4.23–6.02
Germacra-1(10),4(14),5-triene	23986-74-5	01.042	3.09	2.62–4.33
4-Terpinenol	562-74-3	02.072	2.91	2.59–3.60
1-Isopropyl-4-methylbenzene	99-87-6	01.002	2.66	2.20–4.22
γ-Terpinene	99-85-4	01.020	2.45	2.20–2.98
3,7,10-Humulatriene	6753-98-6	01.043	1.61	1.12–3.18
δ-Cadinene	41929-05-0	01.021	1.43	0.97–1.67
Terpinolene	586-62-9	01.005	0.96	0.63–1.10
α-Thujene	2867-05-2	–	0.89	0.71–1.07
α-Terpinene	99-86-5	01.019	0.61	0.49–0.78
Germacrene B	15423-57-1	–	0.61	0.26–0.96
β-Elemene	33880-83-0	01.045	0.55	0.38–0.70
Total			29.6	28.6–31.4

EU: European Union; CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.
(a): The values given for the Total are the lowest and the highest values of the sum of the components in the five batches analysed.

The applicant made a literature search for the chemical composition of *J. communis* and its preparations and the identity of any recognised substances of concern.[20] Two compounds (α- and β-thujone) reported to occur in essential oils prepared from needles of *J. communis* were not detected in the oil under assessment (limit of detection, 0.001%).

3.2.1.1. Impurities

The applicant referred to the 'periodic testing' of some representative flavouring premixtures for mercury, cadmium, lead, arsenic, fluoride, dioxins and polychlorinated biphenyls (PCBs), organo-chloride pesticides, organo-phosphorous pesticides, aflatoxins (B1, B2, G1, G2) and ochratoxin A. However, no data have been provided on the presence of these impurities. Since juniper oil is produced by steam distillation, the likelihood of any measurable carry-over of all the above-mentioned elements is considered low, except for mercury.

3.2.1.2. Shelf-life

The typical shelf-life of juniper oil is stated to be at least 12 months, when stored in tightly closed containers under standard conditions (in a cool, dry place protected from light).[21] However, no data supporting this statement were provided.

3.2.1.3. Conditions of use

Juniper oil is intended to be added to feed for all animal species without a withdrawal period. Maximum use levels in complete feed were proposed for the animal species and categories listed in Table 4. No use level has been proposed by the applicant for the other target species not listed in Table 4. The additive is not intended for use in water for drinking.

[20] Technical dossier/Supplementary information February 2021/Literature_search_Juniper_berry_oil.
[21] Technical dossier/Section II.

Table 4: Conditions of use for the essential oil from the berries of *Juniperus communis* L.: Maximum proposed use levels in complete feed for the certain animal categories

Animal category	Maximum use level (mg/kg complete feed)
Chicken for fattening	10
Laying hen	5
Turkey for fattening	10
Piglet	20
Pig for fattening	10
Sow lactating	20
Veal calf (milk replacer)	15
Cattle for fattening	15
Dairy cow	15
Sheep/goat	20
Horse	20
Rabbit	20
Salmon	20
Dog	20
Cat	20
Ornamental fish	20

3.2.2. Safety

The assessment of safety of juniper oil is based on the maximum use levels proposed by the applicant for the species listed above (see Table 4).

Many of the major volatile components of juniper oil, accounting for about 88% of the % GC area, have been previously assessed and considered safe for use as flavourings, and are currently authorised for food[8] and feed[7] uses at individual use levels higher than those resulting from the intended use of the essential oil in feed. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Seven compounds listed in Table 1, δ-cadinene [01.021], β-cubebene [01.030], germacra-1(10),4 (14),5-triene [01.042], 3,7,10-humulatriene [01.043], longifolene [01.047], α-muurolene [01.052] and tricyclene [01.060] were evaluated in FGE25.Rev2 (EFSA CEF Panel, 2011) by applying the procedure described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). For these compounds, for which there is no concern for genotoxicity, EFSA requested additional subchronic toxicity data (EFSA CEF Panel, 2011). In the absence of such toxicological data, the EFSA CEF Panel was unable to complete its assessment (EFSA CEF Panel, 2015b). As a result, these compounds are not authorised for use as flavours in food. For these compounds, in the absence of toxicity data, the FEEDAP Panel applies the threshold of toxicological concern (TTC) approach or read-across from structurally related substances, as recommended in the Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a).

β-Elemene (0.89%) and α-thujene (0.55%) and 36 additional components, each accounting for < 0.5% of the % GC area, have not been previously assessed for use as flavourings. The FEEDAP Panel notes that 31 of them[22] are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 31 and 8 and a similar metabolic and toxicological profile is expected. These lipophilic compounds are expected to be rapidly absorbed from the gastro-intestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2016a,b). The remaining five compounds (cubebol, 1-epi-cubenol, T-muurolol, α-cadinol and cubenol), were screened for their genotoxic potential with the Organization for Economic Co-operation and Development (OECD)

[22] Fenchone, trans-β-farnesene, β-sesquiphellandrene, m-cymene, α-fenchene, α-cubebene, α-longipinene, α-copaene, β-copaene, selina-3,6-diene, α-amorphene, β-selinene, α-selinene, δ-amorphene, γ-cadinene, α-cadinene, selina-3,7(11)-diene, pseudolimonene, γ-elemene, m-camphorene, p-camphorene, aromadendrene, alloroaromadendrene, cadina-1(6),4-diene, trans-cadina-1(6),4-diene, γ-muurulene, bicyclosesquiphellandrene, bicyclogermacrene, zonarene, cubebene and germacrene B.

Quantitative Structure–Activity Relationship (QSAR) Toolbox. No alerts were identified for *in vitro* mutagenicity, for genotoxic and non-genotoxic carcinogenicity and for other endpoints.[23]

3.2.2.1. Safety for the target species

Tolerance studies in the target species and/or toxicological studies in laboratory animals made with the essential oil under application were not submitted.

In the absence of these data, the approach to the safety assessment of a mixture whose individual components are known is based on the safety assessment of each individual component (component-based approach). This approach requires that the mixture is sufficiently characterised and that the individual components can be grouped into assessment groups, based on structural and metabolic similarity. The combined toxicity can be predicted using the dose addition assumption within an assessment group, taking into account the relative toxic potency of each component (EFSA Scientific Committee, 2019a).

As the additive under assessment is a fully defined mixture (the identified components represent > 99.8% of the % GC area, see Section 3.2.1), the FEEDAP Panel applied a component-based approach to assess the safety for target species of the essential oil.

Based on considerations related to structural and metabolic similarities, the components were allocated to nine assessment groups, corresponding to the chemical groups (CGs) 6, 8, 16, 32 and 31, as defined in Annex I of Regulation (EC) No 1565/2000. For CG 31 ('aliphatic and aromatic hydrocarbons'), subassessment groups as defined in Flavouring Group Evaluation 25 (FGE.25) and FGE.78 were established (EFSA CEF Panel, 2015a,b). The allocation of the components to the (sub-) assessment groups is shown in Table 5 and in the corresponding footnote.

For each component in the assessment group, exposure of target animals was estimated considering the use levels in feed, the percentage of the component in the oil and the default values for feed intake according to the guidance on the safety of feed additives for target species (EFSA FEEDAP Panel, 2017b). Default values on body weight are used to express exposure in terms of mg/kg bw per day. The intake levels of the individual components calculated for chickens for fattening, the species with the highest ratio of feed intake/body weight per day, are shown in Table 5.

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification (Cramer et al., 1978). For some components in the assessment group, toxicological data were available to derive no observed adverse effect level (NOAEL) values. Structural and metabolic similarity among the components in the assessment groups were assessed to explore the application of read-across allowing extrapolation from a known NOAEL of a component of an assessment group to the other components of the group with no available NOAEL or, if sufficient evidence were available for members of a (sub-)assessment group, to derive a (sub-) assessment group NOAEL.

Toxicological data of subchronic studies, from which NOAEL values could be derived, were available for linalool [02.013] and terpineol[24] [02.230] in CG 6 (EFSA FEEDAP Panel, 2012c), 1,8-cineole in CG 16 (EFSA FEEDAP Panel, 2012b, 2021), myrcene [01.008], d-limonene [01.045], p-cymene [01.002] and β-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016a), and β-caryophyllene epoxide in CG 32 (EFSA CEF Panel, 2014).

Considering the structural and metabolic similarities, for the subgroup of terpinyl derivatives in CG 6, i.e. α-terpineol [02.014] and 4-terpinenol [02.072], the reference point was selected based on the NOAEL of 250 mg/kg bw per day available for terpineol [02.230] and d-limonene [01.045].

Similarly, the NOAELs of 44, 250, 154 and 222 mg/kg bw per day for the representative compounds of CG 31, myrcene [01.008], d-limonene [01.045], 1-isopropyl-4-benzene [01.002] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within subassessment group II (trans-β-farnesene), group III (γ-terpinene, terpinolene, α-terpinene, β-elemene, α-phellandrene and β-sesquiphellandrene), group IVe (m-cymene) and group V (α-pinene, sabinene, β-pinene, δ-cadinene, α-thujene, camphene, α-cubebene, longifolene, α-copaene, β-cubebene, γ-cadinene, α-selinene, α-cadinene, β-selinene, δ-3-carene, cubebene, tricyclene, selina-3,7 (11)-diene, α-fenchene, δ-amorphene, α-longipinene, zonarene, β-copaene and selina-3,6-diene)[25] (EFSA CEF Panel, 2015a,b).

[23] Technical dossier/Supplementary information February 2021/Annex_VI_Sin_reply Juniper_berry_oil_QSAR.

[24] Terpineol is a mixture of four isomers: α-terpineol [02.014], a mixture of *(R)*-(+)-α-terpineol and *(S)*-(−)-α-terpineol, β-terpineol, γ-terpineol and 4-terpinenol [02.072].

[25] Some of these compounds are not listed in Table 5 because their individual margin of exposure (MOE) was > 50,000.

For the remaining compounds,[26] NOAEL values were not available and read-across was not possible. Therefore, the TTC approach was applied (EFSA FEEDAP Panel, 2017b).

As the result of the hazard characterisation, a reference point was identified for each component in the assessment group based on the toxicity data available (NOAEL from *in vivo* toxicity study or read across) or from the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class (i.e. 3, 0.91 and 0.15 mg/kg bw per day, respectively, for Cramer Class I, II and III compounds, Munro et al., 1996). Reference points selected for each compound are shown in Table 5.

For risk characterisation, the margin of exposure (MOE) was calculated for each component as the ratio between the reference point and the exposure. For each assessment group, the combined (total) margin of exposure (MOET) was calculated as the reciprocal of the sum of the reciprocals of the MOE of the individual substances (EFSA Scientific Committee, 2019a,b). A MOET > 100 allowed for interspecies differences and intraspecies variability (as in the default 10 × 10 uncertainty factor). The compounds resulting individually in an MOE > 50,000 were not further considered in the assessment group as their contribution to the MOE(T) is negligible. They are listed in the footnote.[27]

The approach to the safety assessment of juniper oil for the target species is summarised in Table 5. The calculations were done for chickens for fattening, the species with the highest ratio of feed intake/body weight and represent the worst-case scenario at the use level of 10 mg/kg in feed.

Table 5: Compositional data, intake values, reference points and margin of exposure (MOE) for the individual components of juniper oil classified according to assessment groups

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS-no**	**Highest conc. in the oil**	**Highest feed conc.**	**Daily intake[(a)]**	**Cramer class[(b)]**	**NOAEL[(c)]**	**MOE**	**MOET**
Constituent	–	%	mg/kg	mg/kg bw/day	–	mg/kg bw/day	–	–
CG 6								
4-Terpinenol	2.072	3.6	0.360	0.0323	(I)	**250**	7,736	
Cubebol	–	0.2	0.020	0.0018	I	*3*	1,671	
T-Muurolol	–	0.04	0.004	0.0004	III	*0.15*	418	
α-Cadinol	–	0.04	0.004	0.0004	III	*0.15*	418	
1-epi-Cubenol	–	0.03	0.003	0.0003	I	*3*	11,139	
MOET CG 6								178
CG 8								
d,l-Bornyl acetate	09.017	0.12	0.012	0.0011	I	*3*	2,785	
Cubenol	–	0.06	0.006	0.0005	III	*0.15*	278	
d,l-Borneol	07.196	0.02	0.002	0.0002	I	*3*	16,709	
Fenchone	–	0.01	0.001	0.0001	II	*0.91*	10,137	
MOET CG 8								243
CG 31, II (Acyclic alkanes)								
Myrcene	01.008	12.9	1.290	0.1158	(I)	**44**	380	
trans-β-Franesene	–	0.21	0.021	0.0019	(I)	44	23,339	
MOET CG 31, II								374

[26] Cubebol, 1-epi-cubenol, T-muurolol and α-cadinol (CG 6); *d,l*-bornyl acetate, cubenol, *d,l*-borneol and fenchone (CG 8); γ-elemene, pseudolimonene, m-camphorene, δ-elemene and p-camphorene (CG 31, III); 1-isopropenyl-4-methylbenzene (CG 31, IVe); γ-muurolene, α-muurolene, bicyclogermacrene, bicyclosesquiphellandrene, trans-cadina-1(6),4-diene, alloroaromadendrene, α-amorphene, cadina-1(6),4diene (isomer) (CG 31, V); germacra-1(10),4(14),5-triene, 3,7,10-humulatriene amd germacrene B (CG 31, VI).

[27] Compounds included in the assessment groups but not reported in the table: linalool, α-terpineol and α-terpinyl acetate (CG 6); 1,8-cineole (CG 16); trans-β-farnesene (CG 31, II); β-sesquiphellandrene (CG 31, III); m-cymene (CG 31,IVe); longifolene, α-copaene, β-cubebene, γ-cadinene, α-selinene, α-cadinene, β-selinene, δ-3-carene, cubenene, tricyclene, selina-3,7(11)-diene, α-fenchene, δ-amorphene, α-longipinene, zonarene, β-copaene and selina-3,6-diene (CG 31, V); β-caryophyllene oxide (CG 32).

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS-no**	**Highest conc. in the oil**	**Highest feed conc.**	**Daily intake[a]**	**Cramer class[b]**	**NOAEL[c]**	**MOE**	**MOET**
CG 31, III (Cyclohexene hydrocarbons)								
d-Limonene	01.001	7.05	0.705	0.0633	(I)	**250**	3,950	
γ-Terpinene	01.020	2.98	0.298	0.0268	(I)	250	9,345	
Terpinolene	01.005	1.10	0.110	0.0099	(I)	250	25,316	
α-Terpinene	01.019	0.78	0.078	0.0070	(I)	250	35,703	
β-Elemene	–	0.70	0.070	0.0063	(I)	250	39,783	
α-Phellandrene	01.006	0.58	0.058	0.0052	(I)	250	48,014	
γ-Elemene	–	0.25	0.025	0.0022	I	*3*	1,337	
Pseudolimonene	–	0.18	0.018	0.0016	I	*3*	1,857	
m-Camphorene	–	0.14	0.014	0.0013	I	*3*	2,387	
δ-Elemene	–	0.04	0.004	0.0004	I	*3*	8,354	
p-Camphorene	–	0.03	0.003	0.0003	I	*3*	11,139	
MOET CG 31, III								419
CG 31, IVe (Benzene hydrocarbons, alkyl)								
p-Cymene	01.002	4.22	0.422	0.0379	(I)	**154**	4,065	
4-Isopropenyl-4-methylbenzene	01.010	0.007	0.007	0.0006	I	*3*	4,774	
MOET CG 31, IVe								2,193
CG 31, V (Bi-, tricyclic, non-aromatic hydrocarbons)								
α-Pinene	01.004	44.2	4.420	0.3968	(I)	222	559	
Sabinene	01.059	9.71	0.971	0.0872	(I)	222	2547	
β-Pinene	01.003	6.02	0.602	0.0540	(I)	222	4108	
β-Caryophyllene	01.007	7.25	0.725	0.0651	(I)	**222**	3411	
δ-Cadinene	01.021	1.67	0.167	0.0150	(I)	222	14808	
α-Thujene	–	1.07	0.107	0.0096	(I)	222	23,111	
Camphene	01.009	0.61	0.061	0.0055	(I)	222	40,540	
α-Cubebene	–	0.51	0.051	0.0046	(I)	222	48,488	
γ-Muurolene	–	0.39	0.039	0.0035	I	*3*	857	
α-Muurulene	01.052	0.31	0.031	0.0028	I	*3*	1,078	
Bicyclogermacrene	–	0.33	0.033	0.0030	I	*3*	1,031	
Bicyclosesquiphellandrene	–	0.21	0.021	0.0019	I	*3*	1,591	
trans-Cadina-1(6),4-diene	–	0.13	0.013	0.0012	I	*3*	2,571	
Alloroaromadendrene	–	0.08	0.008	0.0007	I	*3*	4,177	
α-Amorphene	–	0.04	0.004	0.0004	I	*3*	8,354	
Aromadendrene	–	0.02	0.002	0.0002	I	*3*	16,709	
Cadina-1(6),4-diene (isomer)	–	0.02	0.002	0.0002	I	*3*	16,709	
MOET CG 31, V								134
CG 31, VI (macrocyclic non aromatic hydrocarbons)								
Germacra-1(10),4(14),5-triene	01.042	4.33	0.433	0.0389	I	*3*	77	
3,7,10-Humulatriene	01.043	3.18	0.318	0.0285	I	*3*	105	
Germacrene B	–	0.96	0.096	0.0086	I	*3*	348	
MOET CG 31, VI								39

(a): Intake calculations for the individual components are based on the use level of 10 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 (no observed adverse effect level, NOAEL) 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.
(b): When a NOAEL value is available or read-across is applied, the allocation to the Cramer class is put into parentheses.
(c): Values **in bold** refer to those components for which the NOAEL value was available, values *in italics* are the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class, other values (plain text) are NOAELs extrapolated by using read-across.

As shown in Table 5, for all the assessment groups, the MOET was higher than 100, except for one group (CG 31, VI). From the lowest MOET of 39 for chickens for fattening, the MOET for the assessment group 'macrocyclic non aromatic hydrocarbons' (CG 31, VI) was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 6.

Table 6: Combined margin of exposure (MOET) for the assessment group 'macrocyclic non-aromatic hydrocarbons' (CG 31, VI) calculated for the different target animal categories at the proposed use level and maximum safe use levels in feed calculated to ensure a MOET ≥100 (500 for cats)

Animal category	Body weight (kg)	Feed intake (g DM/day)	Proposed use level (mg/kg feed)	Lowest MOET	Maximum safe use level (mg/kg feed)(1)
Chicken for fattening	2	158	10	39	4
Laying hen	2	106	5	116	5
Turkey for fattening	3	176	10	52	5
Piglet	20	880	20	35	7
Pig for fattening	60	2,200	10	83	8
Sow lactating	175	5,280	20	51	10
Veal calf (milk replacer)	100	1,890	15	108	15
Cattle for fattening	400	8,000	15	103	15
Dairy cow	650	20,000	15	66	10
Sheep/goat	60	1,200	20	77	15
Horse	400	8,000	20	78	15
Rabbit	2	100	20	31	6
Salmon	0.12	2.1	20	86	17
Dog	15	250	20	91	18
Cat	3	60	20	77	3(2)
Ornamental fish	0.012	0.054	20	308	20

(1): Complete feed containing 88% dry matter (DM), milk replacer 94.5% DM.
(2): The MOET for cats is increased to 500 because of the reduced capacity of glucuronidation.

At the proposed use levels in complete feed, the MOET exceeds the value of 100 for laying hens, veal calves, cattle for fattening and ornamental fish, but is below the value of 100 for the other species. For the other species, the maximum safe use levels in feed were calculated in order to ensure a MOET ≥ 100. Because glucuronidation is an important metabolic reaction to facilitate the excretion of the components of the essential oil, the use of juniper oil as additive in cat feed needs a wider margin of exposure. Considering that cats have a low capacity for glucuronidation (Court and Greenblatt, 1997; Lautz et al., 2021), a MOET of 500 is considered adequate. The maximum safe levels in feed are shown in Table 6.

3.2.2.2. Conclusions on safety for the target species

The FEEDAP Panel concludes that the use of juniper oil is safe up to the maximum proposed use levels in complete feed of 5 mg/kg for laying hens and other laying/breeding birds reared for egg production/reproduction, 15 mg/kg for veal calves (milk replacer), cattle for fattening and other ruminants for fattening and ruminants reared for milk production/reproduction (including pseudo-ruminants), and 20 mg/kg for ornamental fish. For the other species, the calculated safe concentrations in complete feed are 5 mg/kg for turkeys for fattening or reared for breeding, 4 mg/kg for chickens for fattening and other poultry for fattening or reared for laying/breeding, 7 mg/kg for

piglets and all pigs (Suidae) from sucking to fattening, 8 mg/kg for pigs for fattening, 10 mg/kg for sows and all Suidae for reproduction, dairy cows and other dairy ruminants and ruminants for reproduction (including pseudo-ruminants), 15 mg/kg for sheep, goats and horses, 6 mg/kg for rabbits, 17 mg/kg for salmonids and other minor fin fish, 18 mg/kg for dogs and 3 mg/kg for cats. For any other species, the additive is considered safe at 3 mg/kg complete feed.

3.2.2.3. Safety for the consumer

Juniper oil obtained by steam distillation of the berries of *J. communis* is added to a wide range of food for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavor ingredients (Burdock, 2009) cites values of 0.0006 mg/kg bw per day for juniper oil (FEMA 2604) and of 0.005 mg/kg bw per day for juniper berries (FEMA 2602).

The majority of the individual constituents of the essential oil under assessment are currently authorised as food flavourings without limitations and have been already assessed for consumer safety when used as feed additives in animal production (see Table 1).

No data on residues in products of animal origin were made available for any of the constituents of the essential oil. However, the Panel recognises that the constituents of juniper oil are expected to be extensively metabolised and excreted by the target species (see Section 3.2.2).

Considering the above and the reported human exposure due to the direct use of juniper berries and its preparations in food (Burdock, 2009), it is unlikely that consumption of products from animals given juniper oil at the proposed maximum use level would significantly increase human background exposure.

No safety concern would be expected for the consumer from the use of juniper oil up to the highest safe use level in feed.

3.2.2.4. Safety for the user

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

The applicant produced a safety data sheet[28] for juniper oil where hazards for users have been identified. There is limited evidence of mild skin irritation in humans and rabbits from the literature (Cosmetic Ingredient Review Expert Panel, 2001).

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

3.2.2.5. Safety for the environment

J. communis is a species native to Europe, where it is widely grown. The use of juniper oil in animal feed under the proposed conditions is not expected to pose a risk to the environment.

3.3. Juniper tincture

3.3.1. Characterisation of juniper tincture

The tincture under assessment has a density of 895–902 kg/m^3 (898 kg/m^3 on average, five batches). It is a water/ethanol (35/65, v/v) solution, specified to contain 20–200 mg/kg tannins (corresponding to 0.002–0.02%, w/w), which are selected as the phytochemical marker.[29]

Table 7 summarises the results of the proximate analysis of five batches of the additive expressed as % (w/w).[30] The solvent represents about 99% of the additive, and the dry matter (DM) content ranged between 0.03 and 1.1% (w/w).

[28] Technical dossier/ Supplementary Information February 2021/Annex_VIII_SIn reply_juniper_berry_oil_MSDS. Aspiration hazard (H304), hazards for skin corrosion/irritation (H315, category skin irritation 2), serious eye damage/eye irritation (H319, category eye irritation 2), skin sensitisation (H317, category 1).
[29] Technical dossier/Supplementary information February 2023/ SIn reply_juniper_tincture.
[30] Technical dossier/Supplementary information February 2023/Annex_I_Characterisation.

Table 7: Proximate analysis of juniper tincture derived from the dried ripe cone berries of *Juniperus communis* L. based on the analysis of five batches. The results are expressed as % of the tincture (w/w)

Constituent	Range
	% (w/w)
Dry matter	0.03–1.1
Lipids	< 0.5
Protein	< 0.1
Sugars (as sucrose)	< 0.5–1.1
Ash	< 0.1
Solvent (water/ethanol, 35/65, v/v)	98.90–99.97
Ethanol	70.5–72.1
Water	27.0–28.2

The fraction of secondary metabolites was characterised in the same batches of the additive. Polyphenols (expressed as gallic acid equivalents) were determined by spectrophotometry (at 760 nm), tannins (expressed as pyrogallol) were determined as described in the general method of the European Pharmacopoeia (PhEur, 2022).[30] The FEEDAP Panel notes that the concentration of tannins expressed as pyrogallol equivalents does not reflect the true concentration of tannins of different type. According to the PhEur Commentary (2022), this may be about four times higher depending on the type of tannins present in the specific botanical preparation. An analysis of flavonoids by high-performance liquid chromatography electrospray mass spectrometry (HPLC-ESI-MS)[31] investigated 19 flavonoids. All compounds were below the limit of quantification (LOQ). Hesperidin (< 0.002%), kaempferol (< 0.0009%), rutin (< 0.0003%) and tangeretin (< 0.0004–0.0008%) were detected in some of the batches. Unidentified flavonoids were detected in all the analysed batches and accounted for 0.0002% and 0.0016% (w/w) of the tincture. The essential oil present in the tincture was extracted with hexane and analysed by GC-FID. The chromatogram was similar to that of the essential oil, with α-pinene as the major compound.[32] The content of α-pinene (range: 0.7–57.7 mg/L) and the content of terpenes other than α-pinene (range: 0.7–29.7 mg/L, expressed as α-pinene) were determined by GC-FID analysis.[33] An analytical investigation of diterpene resin acids (labdane type, typically occurring in conifers) by GC–MS did not detect isocupressic acid or communic acid.[34] Analytical results are presented in Table 8 and are expressed as % (w/w).

Table 8: Characterisation of the fraction of secondary metabolites of juniper tincture derived from the dried ripe cone berries of *Juniperus communis* L. based on the analysis of five batches. The results are expressed as % (w/w) of juniper tincture

Constituent	Method	Mean	Range
		% (w/w)	% (w/w)
Polyphenols (as gallic acid equivalent)	UV (760 nm)	0.0187	0.0097–0.0327
Tannins (as pyrogallol)	UV, PhEur 2.8.14	0.0078	0.006–0.014
Flavonoids (unidentified)	HPLC-MS	0.0007	0.0002–0.0016
Volatiles	GC-FID	0.006	0.002–0.009
α-Pinene	GC-FID	0.0036	0.0001–0.0064
Terpenes (as α-pinene)	GC-FID	0.0016	0.0001–0.0033

The sum of identified secondary components accounted on average for 12.2% (range: 5.3–31%) of the dry matter fraction of the tincture and the other plant constituents (sugars, lipid, proteins) for about 70%.

[31] Technical dossier/Supplementary information February 2023/Annex_I_Characterisation. Flavonoids included in the analyses: avicularin, eriocitrine, hesperidine, hesperitin, hyperoside, isorhamnetin, kaempferol, luteolin, myricetin, naringenin, naringin, narirutin, neohesperidine, nobiletine, quercetin, quercitrin, rutin and tangeretin.
[32] Technical dossier/Supplementary information February 2023/Annex_VII_Essential_oil.
[33] Technical dossier/Supplementary information February 2023/Annex_V_Terpenes.
[34] Technical dossier/Supplementary information February 2023/Annex VI_Diterpenes.

According to existing monographs (PhEur Commentary, 2019; EMA, 2009a,b) provided by the applicant, the dried berries from *J. communis* L. contain a fraction of phenolic compounds including condensed tannins (3–5%), hydrolysable tannins, flavonoids and diterpenic resin acids of the labdane type.

3.3.1.1. Impurities

Data on impurities were provided for five batches of juniper tincture. Mercury, arsenic and cadmium were below the corresponding LOQ. The concentrations of lead were in the range 0.5–1.0 mg/kg. Pesticides were not detected in a multiresidue analysis.[35] Polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) were below the corresponding LOQ. The calculated upper bound for the sum of dioxins was < 0.03 ng WHO PCDD/F-TEQ (World Health Organisation polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) toxic equivalents (TEQ))/kg, the sum of dioxin and dioxin-like polychlorinated biphenyls (PCBs) was < 0.05 ng WHO PCCD/F + PCB TEQ/kg.[36] In three batches, mycotoxins were below the corresponding LOQ.[37]

Residual solvents potentially present in ethanol as impurities, i.e. methanol (84–127 mg/kg) and 2-propanol (< 500 mg/kg) were analysed in three batches[38] and were below the corresponding thresholds proposed by International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (EMA, 2010c).

Analysis of microbial contamination of three batches of juniper tincture indicated that *Salmonella* spp. was not detected in 25 g, *E. coli* and *Enterobacteriaceae* were < 1 and < 10 colony-forming unit (CFU)/g, respectively, and yeast and moulds < 10 CFU/g.[39]

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

3.3.1.2. Shelf-life

The applicant states that the typical shelf-life of flavourings is at least 12 months, when stored in tightly closed containers under standard conditions. However, no data supporting this statement were provided.

3.3.1.3. Conditions of use

Juniper tincture is intended for use in feed for all animal species, at a maximum use level of 50 μL/kg complete feed or water for drinking corresponding to 45 mg/kg complete feed or water for drinking.

3.3.2. Safety

The safety assessment of the additive is based on the highest proposed use level in feed (see Section 3.3.1.3).

No studies to support the safety for target animals, consumers and users were performed with the additive under assessment. The applicant provided a literature search on the toxicology and the pharmacological effects of preparations obtained from *J. communis*.[40] These pharmacological effects were not considered relevant for the safety assessment of juniper tincture as a flavouring additive.

The additive under assessment, juniper tincture, on average consists of about 99% (w/w) water/ethanol mixture. The concentration of plant-derived compounds is about 1% (w/w) of the tincture. The dry matter included minerals (expressed as ash), proteins, lipids and carbohydrates, which are of no safety concern and are not further considered.

Among the identified secondary plant metabolites (see Table 8), up to 0.033% (w/w) of the tincture is constituted by polyphenols expressed as gallic acid equivalents (of which up to 0.014% (w/w) are condensed and hydrolysable tannins expressed as pyrogallol and up to 0.0016% (w/w) are

[35] Technical dossier/Supplementary information February 2023/Annex_I_Characterisation. LOQ for heavy metals and arsenic: < 0.5 mg/kg for arsenic and cadmium, < 0.1 mg/kg for mercury; LOQ for individual pesticides: 0.001–0.005 mg/L.
[36] Technical dossier/Supplementary information February 2023/Annex_II_Dioxins and dioxin-like PCBs.
[37] Technical dossier/Supplementary information February 2023/Annex_VIII_Mycotoxins. LOQ for mycotoxins: < 0.1 μg/kg for aflatoxins B1, B2, G1 and G2, < 1 μg/kg for ochratoxin A, < 2 μg/kg for zearalenone, α- and β-zearalenone, HT2-toxin, T2-toxin cytrochalasin E and sterigmatocystin, < 5 μg/kg for nivalenol, fusarenon X and diacetoxyscirpenol, and < 10 for deoxynivalenol, deoxynivalenol-3-glycoside, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, citrinin, patulin and fumonisins B1, B2 and B3.
[38] Technical dossier/Supplementary information February 2023/Annex_IV_Solvents.
[39] Technical dossier/Supplementary information February 2023/Annex_III_Microbial contamination.
[40] Technical dossier/Supplementary information February 2022/References.

unidentified flavonoids), and up to 0.009% (w/w) by volatile terpenes, mainly α-pinene (up to 0.006%).

Condensed tannins will be poorly absorbed and excreted via the faeces. Polyphenols including hydrolysable tannins will be readily metabolised and excreted and are not expected to accumulate in animal tissues and products. Tannins do not raise concern for genotoxicity.

3.3.2.1. Safety for the target species

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

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 α-pinene, subchronic studies are available, from which a NOAEL can be derived. For the group assessment polyphenols (including the subgroups of tannins and flavonoids), in the absence of data, the TTC approach is applied to derive maximum safe feed concentrations for the whole group in the tincture (EFSA FEEDAP Panel, 2017b).

Polyphenol compounds including tannins and flavonoids

Among the secondary metabolites, 0.0327% are polyphenols including 0.014% tannins expressed as pyrogallol and 0.0016% flavonoids.

At the maximum proposed use level of 45 mg juniper tincture/kg complete feed, the highest concentration of the fraction of tannins (≤ 0.014% of the tincture, expressed as pyrogallol) would be 0.006 mg/kg feed. Although the individual compounds were not identified, tannins are assigned to Cramer Class III. The available data indicate that tannins would be 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). Therefore, the presence of tannins is not considered of concern for the target species also when considering that the true concentration of tannins may be about four times higher than the given pyrogallol value.

Several unidentified flavonoids were detected and quantified accounting together for ≤ 0.0016% of the tincture. At the maximum proposed use level of 45 mg juniper tincture/kg complete feed, this would correspond to 0.0007 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 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). Therefore, the presence of flavonoids is not considered of concern for the target species.

At the maximum proposed use level of 45 mg juniper tincture/kg complete feed, the highest concentration of the fraction of polyphenols after subtraction of values for tannins and flavonoids (≤ 0.017% of the tincture) would be 0.008 mg/kg feed. Polyphenols are assigned to Cramer Class I and the available data indicate that their concentration 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). Therefore, no concern for the target species arises from polyphenols other than tannins and flavonoids in juniper tincture.

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

Volatiles

At the maximum proposed use level of 45 mg juniper tincture/kg complete feed, the highest concentration of α-pinene (≤ 0.0064% of the tincture) would be 0.0029 mg/kg feed. These concentrations are several orders of magnitude below the concentrations in feed which were considered safe by the FEEDAP Panel in its opinion on chemical group 31, i.e., 5 mg/kg complete feed for all animal species (EFSA FEEDAP Panel, 2016a,b) based on a NOAEL of 222 mg/kg bw per day identified from a 90-day study in rat with β-caryophyllene. Therefore, no concern for the target species is expected.

For the other terpenes (≤ 0.0033% of the tincture), at the maximum proposed use level of 45 mg juniper tincture/kg complete feed, the highest concentration would be 0.0015 mg/kg feed. Although the individual compounds were not identified, terpenes are assigned to Cramer Class I. The available data indicate that terpenes would be below the maximum acceptable concentrations 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). Therefore, the presence of terpenes is not considered of concern for the target species.

The FEEDAP Panel concludes that the use of juniper tincture at the maximum proposed use level of 45 mg/kg in complete feed is safe for all animal species.

When used in water for drinking, the intake of the additive via water would be two to three times higher than the intake via feed for poultry, pigs and rabbits (EFSA FEEDAP Panel, 2010). The applicant proposed a maximum use level of 45 mg/kg for the use in water for drinking. Considering the very low levels of polyphenols (including flavonoids and tannins) and volatiles in the tincture, the FEEDAP Panel concludes that the use of juniper tincture at the maximum proposed use levels of 45 mg/kg in water for drinking is safe for all animal species.

Conclusions on the safety for the target species

Juniper tincture is safe for the target species up to the maximum proposed use level of 45 mg/kg in complete feed or water for drinking.

3.3.2.2. Safety for the consumer

Juniper berries from *J. communis* and their oil are added to a wide range of food for flavouring purposes (see Section 3.2.2.3).

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 and tannins and tannins, present in the additive at concentrations below the respective thresholds for Cramer Class I or Cramer Class III compounds, will be readily metabolised and/or excreted and are not expected to accumulate in animal tissues and products. Similarly, for α-pinene and other terpenes, the available data indicate that they are absorbed, metabolised and rapidly excreted and are not expected to accumulate in animal tissues and products.

Considering the above and the reported human exposure due to direct use of juniper berries and its preparations in food (Burdock, 2009), it is unlikely that consumption of products from animals given juniper tincture at the proposed maximum use level would significantly increase human background exposure.

No safety concern would be expected for the consumer from the use of juniper tincture up to the maximum proposed use level in feed.

3.3.2.3. 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[41] concerning the presence of ethanol in the tincture.[42]

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

3.3.2.4. Safety for the environment

J. communis is a species native to Europe, where it is widely grown. The use of juniper tincture in animal feed under the proposed conditions is not expected to pose a risk to the environment.

3.4. Efficacy

Juniper berries and juniper berry oil (*J. communis* L.) are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference numbers 2602 and 2604, respectively.

Since the berries of *J. communis* and their preparations were recognised to flavour food and their function in feed would be the same as that in food, no further demonstration of efficacy is considered necessary.

4. Conclusions

Juniper oil is safe up to the maximum proposed use levels in complete feed of 5 mg/kg for laying hens and other laying/breeding birds reared for egg production/reproduction, 15 mg/kg for veal calves

[41] 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. 1–1355.

[42] Technical dossier/Supplementary information February 2022/Annex_X_MSDS_juniper_tincture_MSDS. H319: moderate eye irritation.

(milk replacer), cattle for fattening and other ruminants for fattening and ruminants reared for milk production/reproduction (including pseudo-ruminants), and 20 mg/kg for ornamental fish. For the other species, the calculated safe concentrations in complete feed are 5 mg/kg for turkeys for fattening or reared for breeding, 4 mg/kg for chickens for fattening and other poultry for fattening or reared for laying/breeding, 7 mg/kg for piglets and all pigs (Suidae) from sucking to fattening, 8 mg/kg for pigs for fattening, 10 mg/kg for sows and all Suidae for reproduction, dairy cows and other dairy ruminants and ruminants for reproduction (including pseudo-ruminants), 15 mg/kg for sheep, goats and horses, 6 mg/kg for rabbits, 17 mg/kg for salmonids and other minor fin fish, 18 mg/kg for dogs and 3 mg/kg for cats. For any other species, the additive is considered safe at 3 mg/kg complete feed.

Juniper tincture is safe up to the maximum proposed use level of 45 mg/kg in complete feed or water for drinking for all animal species.

No concerns for consumers were identified following the use of juniper oil and juniper tincture up to the highest safe levels in feed for the target animals.

The additives under assessment should be considered as irritants to skin and eyes, and as skin and respiratory sensitisers.

The use of juniper oil and juniper tincture in animal feed under the proposed conditions is not expected to pose a risk to the environment.

Since the berries of *J. communis* and their 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.

5. Documentation provided to EFSA/Chronology

Date	Event
05/11/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 18 - Gymnosperms (Coniferales, Ginkgoales) for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)
14/12/2010	Reception mandate from the European Commission
26/02/2013	EFSA informed the applicant (EFSA ref. 7150727) 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
11/02/2019	Application validated by EFSA – Start of the scientific assessment
20/02/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for target species, safety for the consumer, safety for the user and environment*
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: Juniper berry extract (wb), Pine oil.
13/05/2019	Comments received from Member States
10/02/2021	Reception of supplementary information from the applicant - (partial dataset: juniper oil) - Scientific assessment remains suspended
01/02/2023	Reception of supplementary information from the applicant - (partial dataset: juniper tincture) - Scientific assessment remains suspended
02/03/2023	The application was split and a new EFSA-Q-2023-00181 was assigned to the preparation included in the present assessment
09/03/2023	Scientific assessment re-started for the preparations included in the present assessment
14/03/2023	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
22/03/2023	Opinion adopted by the FEEDAP Panel on juniper oil and juniper tincture (EFSA-Q-2023-00181). End of the Scientific assessment for the preparations included in the present assessment. The assessment of other preparations in BDG 18 is still ongoing

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Abbreviations

AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials
BDG	botanically defined group
bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony forming unit
CG	chemical group
CLP	Classification, Labelling and Packaging
CoE	Council of Europe
DM	dry matter
EEIG	European economic interest grouping
EINECS	European Inventory of Existing Chemical Substances
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 Extract Manufacturers Association
FFAC	Feed Flavourings authorisation Consortium of (FEFANA) the EU Association of Specialty Feed Ingredients and their Mixtures
FGE	Flavouring Group Evaluation
FLAVIS	the EU Flavour Information System
GC	gas chromatography

GC-FID	gas chromatography with flame ionisation detector
GC–MS	gas chromatography–mass spectrometry
HPLC-ESI-MS	high-performance liquid chromatography electrospray mass spectrometry
ISO	International standard organisation
LOQ	limit of quantification
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MOE	margin of exposure
MOET	combined margin of exposure (total)
NOAEL	no observed adverse effect level
OECD	Organization for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
PhEur	European Pharmacopoeia
QSAR	Quantitative Structure–Activity Relationship
SC	EFSA Scientific Committee
TEQ	toxic equivalent
TTC	threshold of toxicological concern
WHO	World Health Organization