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Safety and efficacy of a feed additive consisting of a tincture derived from the buds of *Pinus sylvestris* L. (pine tincture) for use in all animal species (FEFANA abl)

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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 a tincture from the buds of *Pinus sylvestris* L. (pine tincture) when used as a sensory additive in feed and water for drinking for all animal species. The product under assessment is a [redacted] solution, with a dry matter content of ~ 2.2%. The product contains on average 0.0882% polyphenols, of which 0.0222% are phenolic acids. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that pine tincture is safe at the maximum proposed use level of 50 mg/kg complete feed for all animal species. The FEEDAP Panel considers that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered safe when consumed via feed alone. No safety concern would arise for the consumer from the use of pine tincture up to the maximum proposed use level in feed. Pine tincture should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser. The use of pine tincture in animal feed was not expected to pose a risk for the environment. Since twigs of *P. sylvestris*, which are considered similar in composition to the source material for the production of pine tincture, are described to flavour food, no further demonstration of efficacy is deemed necessary.

Keywords: sensory additives, flavouring compounds, *Pinus sylvestris* L., pine tincture, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2010-01516 (new EFSA-Q-2023-00400)

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Table of contents

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)[2,3] 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) and pine oil.[4] These preparations were deleted from the register of feed additives.[5] During the course of the assessment, this application was split and the present opinion covers only one out of the eight remaining preparations under application: pine tincture from the buds of *Pinus sylvestris* L. for all animal species.

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

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 product pine tincture (*P. sylvestris* L.), when used under the proposed conditions of use (see **Section 3.2.3**).

1.2. Additional information

Pine tincture from *Pinus sylvestris* L. 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) and foreseen for re-evaluation. It has not been assessed as a feed additive in the EU.

[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 transfer of contact point for this application to Manghebati SAS, zone de la Basse Haye – BP 42133–35221 Chateaubourg Cedex.
[4] On 27 February 2019, EFSA was informed by the applicant about the withdrawal of the applications on juniper berry extract (wb) and pine oil.
[5] Register of feed additives, Annex II, withdrawn by OJ L162, 10.05.2021, p. 5.

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[6] in support of the authorisation request for the use of pine tincture from *P. sylvestris* as a feed additive. The dossier was received on 8 June 2023 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFPOanleSA-Q-2023-00400.[7]

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phytochemical marker in the additive. The evaluation report is related to the methods of analysis for each feed additive included the group BDG 18 (Ginkgoales). In particular, for the characterisation of pine tincture, the EURL recommended a method based on spectrophotometry and high-performance thin-layer chromatography (HPTLC) for the quantification of the phytochemical markers *total polyphenols* (expressed as gallic acid) and *total phenolic acids* (expressed as chlorogenic acid) in *pine tincture*.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of pine tincture from *P. sylvestris* is in line with the principles laid down in Regulation (EC) No 429/2008[9] 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, 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 efficacy of feed additives (EFSA FEEDAP Panel, 2018), 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 additive under assessment, pine tincture, is derived primarily from the buds of *Pinus sylvestris* L. and is intended for use as sensory additive (functional group: flavouring compounds) in feed and water for drinking for animal species.

3.1. Origin and extraction

Pinus sylvestris L. is an evergreen coniferous tree belonging to the *Pinaceae* family, commonly referred to as the Scots pine or Baltic pine. It is native to the temperate regions of Eurasia and is the only pine species native to the northern parts of Europe. Several varieties are recognised and have a standing in taxonomy. It is an important species in forestry, used for the production of sawn timber and pulp for the paper industry. Extracts of the buds (young cones) are found in some cosmetic and household products.

[6] FEED dossier reference: FAD-2010-0320.
[7] The original application EFSA-Q-2010-01516 was split on 8 June 2023 and a new EFSA-Q-2023-00400 was generated.
[8] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0320_en
[9] 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.

The tincture is primarily produced from the buds of *P. sylvestris* L. Although a small amount of other pine-derived material may be present, this does not include the pine needles.

Buds are extracted for 3 weeks under ambient conditions with a [redacted] and a [redacted]. The tincture is then 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

Pine tincture is a brown liquid, with a characteristic sweet pine odour. It has an average density of 970 kg/m^3 (range 969–971 kg/m^3) and a pH of 4.37 (range 4.19–4.62).[10]

Table 1 summarises the results of the proximate analysis of five batches of the additive.[11] The solvent represents on average 97.78% of the additive leaving a dry matter (DM) content of about 2.22%. The dry matter consists of inorganic material measured as ash (11.3% on average) and a plant-derived organic fraction (88.7%), which includes protein, lipids and 'carbohydrates+fibre'.

Table 1: Proximate analysis of a tincture derived from the aerial parts (buds) of *Pinus sylvestris* L. based on the analysis of five batches. The results are expressed as % of the tincture (w/w)

Constituent	Method	Mean	Range
		% (w/w)	% (w/w)
Dry matter	Gravimetry	2.22	1.93–2.46
Ash	Gravimetry	0.25	0.21–0.30
Organic fraction		1.97	1.69–2.23
Lipids[(a)]	LLE and gravimetry	0.004	0.003–0.005
Proteins	Dumas	–	< 0.08
'Carbohydrates+fibre'	By difference	1.89	1.60–2.15
Solvent	100%-DM	97.78	97.54–98.08

(a): Fraction extracted by liquid–liquid extraction and quantified by gravimetry.

The constituent defined as 'carbohydrates+fibre' 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 five batches of the tincture and the results are summarised in Table 2. The tincture was shown to contain on average 0.088% polyphenols (range: 0.070–0.102%) determined by spectrophotometry (at 760 nm) and expressed as gallic acid equivalents. Five unidentified phenolic acids were separately determined and semi-quantified (average: 0.022%, range: 0–0.038%) by high-performance thin layer chromatography (HPTLC) and expressed as chlorogenic acid equivalents.[12] The concentrations of rutin (9.83 mg/L, range: < LOD–10.13 mg/L) and ferulic acid (5 mg/L, range: 2.98–6.43 mg/L) were determined by high-performance liquid chromatography with diode array detector (HPLC-DAD) and that of α-terpineol (1.94 mg/L; range: 1.27–2.49 mg/L) by gas chromatography with flame ionisation detector (GC-FID).[11]

[10] Technical dossier/Supplementary information February 2020/Annex II_4_Results of analysis.
[11] Technical dossier/Supplementary information May 2023.
[12] Technical dossier/Supplementary information February 2020/ Section_II_Identity and Annex II_4.

Table 2: Characterisation of the fraction of secondary metabolites of a tincture derived from the aerial parts (buds) of *Pinus sylvestris* L. based on the analysis of five batches. The results are expressed as % (w/w) of pine tincture

Constituent	Method	Mean	Range
		% (w/w)	% (w/w)
Total polyphenols	Folin–Ciocalteu[(a)]	0.088	0.070–0.102
Total phenolic acids	HPTLC	0.037	n.d.–0.038
Rutin	HPLC-DAD	0.001	n.d.-0.001
Ferulic acid	HPLC-DAD	0.0005	0.0003–0.0006
α-Terpineol	GC-FID	0.00019	0.00013–0.00025

(a): Internal method based on European Pharmacopoeia (PhEur, 2022): chapter 2.8.14, Determination of tannins in herbal drugs.

The applicant also provided an analysis by gas chromatography with flame ionisation detector (GC-FID) which showed that the main terpenes present in essential oils derived from pine needles (α-pinene, β-pinene, limonene, β-caryophyllene and 1,8-cineole) are not present in the pine tincture under assessment.[13] This analysis confirmed that needles were not used to produce the tincture.

3.2.1.1. Substances of concern

The applicant performed a literature search to identify substances of concern in *P. sylvestris* and its aqueous and aqueous ethanol extracts.[14] The literature search did not identify any publication specifically related to preparations obtained from buds. Most of the information retrieved was related to composition of essential oils from pine needles and is considered of limited relevance. Several publications reported the presence of resin acids (abietic acid, neoabietic acid, dehydroabietic acid, levopimaric acid, palustric acid, pimaric acid, isopimaric acid, sandaracopimaric acid) in *P. sylvestris* wood (15–25 mg/g) and needles (about 5 mg/g) (Manninen et al., 2002; Ekeberg et al., 2006; Hovelstad et al., 2006). The relative pattern of resin acids in different parts (stem wood, stem wood thinning, sawdust, branch biomass and bark) of *P. sylvestris* from different climatic regions in Finland has been described by Verkasalo et al. (2022). Overall, the most abundant compounds were abietic acid (0.404–3.994 mg/g dw) and dehydroabietic acid (0.707–3.290 mg/g dw), followed by neoabietic acid (0–2.868 mg/g dw), levopimaric acid (0.012–2.796 mg/g dw), palustric acid (0–2.707 mg/g dw), pimaric acid (0.373–1.731 mg/g dw), isopimaric acid (0.270–1.135 mg/g dw), sandaracopimaric (0.091–0.307 mg/g dw). In branch biomasses, the concentrations of palustric acid (0–0.059 mg/g dw) and neoabietic acid (0–0.239 mg/g dw) were much lower compared to stem wood and other parts of the plant.

Analytical data on the content of resin acids in the tincture were not provided. In a worst-case scenario, an average concentration of 1.88% (range: 1.67–2.12%) is estimated for resin acids, which corresponds to the DM content of the tincture subtracted by the identified components (i.e. phenolic acids, ash and lipids).

3.2.1.2. Impurities

The applicant controls contamination at the level of the raw material. Specifications are set with suppliers covering cadmium < 1 mg/kg, mercury < 0.1 mg/kg, lead < 10 mg/kg and arsenic < 2 mg/kg, and the degree of microbial contamination.[15] Three certificates of analysis of the raw material (pine) showing compliance were provided (one for chemical impurities and two for microbial contamination).[16] Analysis of impurities in the tincture apparently is made on irregular basis and does not form part of the Hazard Analysis and Critical Control Points plan.

[13] Technical dossier/Supplementary information May 2023. Limit of detection (LOD): α-pinene (0.05 mg/L), β-pinene (0.074 mg/L), limonene (0.19 mg/L), β-caryophyllene (0.034 mg/L) and 1,8-cineole (0.035 mg/L).
[14] Technical dossier/Supplementary information February 2020/Annex II_6_Bibliographic data.
[15] Technical dossier/Supplementary information February 2020/Annex II_5_Technical data sheet_pine (raw material).
[16] Technical dossier/Supplementary information February 2020/Annex_II_6_COA_pine (raw material).

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. However, no data supporting this statement were provided.

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 pine tincture/kg complete feed or 50 mg/kg water for drinking for all animal species.

3.3. Safety

The safety assessment is based on the highest proposed use level in feed, which is 50 mg tincture/ kg complete feed.

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

The additive under assessment, pine tincture, on average consists of 97.78% (w/w) water/ethanol mixture. The concentration of plant-derived compounds is about 2.22% (w/w) of the tincture. The dry matter includes ash, lipids and carbohydrates, which are of no safety concern, and are not further considered.

Among the secondary plant metabolites, total polyphenols including flavonoids were quantified as a whole, but not identified. They will be assessed based on considerations at the level of the assessment group (see Section 3.3.4.1). These compounds will be readily metabolised and excreted and are not expected to accumulate in animal tissues and products.

Resin acids are potentially present in the tincture (see Section 3.2.1.1). Information on the absorption, distribution, metabolism and excretion (ADME) and on the toxicology of resin acids is summarised in the next sections, where reference is made to the Maximale Arbeitsplatz-Konzentration (MAK, maximum workplace concentration) Value Documentation for abietic acid in 'The MAK-Collection for Occupational Health and Safety Documentation and methods' (2013) and to the EFSA opinion on the re-evaluation of glycerol esters of wood rosin (E 445) as a food additive (EFSA ANS Panel, 2018).

3.3.1. Absorption, distribution, metabolism and excretion of resin acids

The ADME data for free resin acids (dehydroabietic acid, tetrahydroabietic acid and isopimaric acid) have been summarised in the Opinion of the ANS Panel on the re-evaluation of glycerol esters of wood rosin (E 445) as a food additive as follows: 'Experiments in rats orally administered with radiolabelled dehydroabietic acid revealed absorption of approximately 40% of the dose. Most of the radioactivity was excreted in bile and faeces, minor amounts were found in urine and only traces were exhaled. Tetrahydroabietic acid and isopimaric acid exhibited an excretion pattern similar to dehydroabietic acid' (EFSA ANS Panel, 2018).

As reported in the MAK Value Documentation (2013), when administered to male rabbits via gavage (about 666 mg abietic acid sodium salt/kg body weight at 3 kg body weight), 'abietic acid was oxidised on the isopropyl group at C-17, probably via a primary alcohol as intermediate, to form an acid (27% of the acid ether extract of the urine). The isopropyl group of dehydroabietic acid was oxidised to the primary or to the tertiary alcohol (15% or 58% of the acid ether extract) as well as to the allylic double bond (14% of the acid ether extract). 7-Oxodehydroabietic acid was demonstrable as a trace (only urine metabolites were investigated, no quantitative details on excretion)'.

3.3.2. Genotoxicity

The available genotoxicity studies have been reviewed in the MAK Value Documentation for abietic acid (2013) reporting negative results for the induction of gene mutations in bacteria (strains TA98, TA100, TA1535 and TA1548) by abietic acid (purity not specified), dehydroabietic acid (95%), levopimaric acid (98%), 7-oxodehydroabietic acid (95%), pimaric acid (85%), isopimaric acid (98%) and sandaracopimaric acid (90%), while neoabietic acid (purity 95%) showed a mutagenic activity in the absence and presence of metabolic activation in the strains TA98, TA100, TA1535 and TA1548, but not TA1537 (Douglas et al., 1980; Nestmann et al., 1979, as referenced in MAK Value Documentation, 2013). Resin acids, including neoabietic acid, induced mitotic gene conversion in

Saccharomyces cerevisiae D7. In addition, neoabietic acid and 7-oxodehydroabietic acid increased the frequency of tryptophane reversion, but not of histidine or homoserine reversions, in *Saccharomyces cerevisiae* XV185-14C (Nestmann and Lee, 1981, as referenced in MAK Value Documentation, 2013). Negative results with abietic acid were obtained in a test for differential killing in a DNA-polymerase-deficient *Escherichia coli* strain (no other details; Domanski, 1989). Pine resin ('retsina'), containing abietic acid as main component, did not induce sister chromatid exchanges and chromosomal aberrations in human lymphocyte cultures (Athanasiou and Bartsocas, 1980, as referenced in MAK Value Documentation, 2013).

The ANS Panel in 2018 assessed these studies and concluded that the positive results observed with neoabietic acid were of questionable relevance for the assessment of genotoxicity of glycerol esters of wood rosin (EFSA ANS Panel, 2018). Based on the negative results obtained with structurally related compounds and the lack of structural alerts for genotoxicity in neoabietic acid, the ANS Panel considered that the mutagenic response of neoabietic acid could be attributed to the presence of impurities in the lot tested as well as to cytotoxic effects leading to the formation of microcolonies of auxotrophic bacteria (i.e. not true histidine revertants), since no data on the clearance of the background lawn were reported.

Taking into consideration the available data and the limitations of the studies, the FEEDAP Panel agrees with the conclusions of the ANS Panel.

3.3.3. Toxicological studies

The toxicological data available for abietic acid and dehydroabietic acid have been reviewed in the MAK Value Documentation (2013).

The available data indicate that the acute oral toxicity of abietic acid and dehydroabietic acid is low. No adverse effects were observed in mice receiving oral doses of 250 mg abietic acid/kg bw per day for 28 days or in rats receiving 2,000 mg abietic acid/kg diet per day (corresponding to about 100 mg/kg bw per day) for 15 weeks (Domanski, 1989, as referenced in MAK Value Documentation, 2013).

No effects on body weight gain, food and water consumption were observed after feeding dehydroabietic acid at concentrations of 50, 500 or 5,000 mg/kg diet (corresponding to about 2.5, 25 or 250 mg/kg bw per day) to rats for 14 or 28 days. Gross pathology and microscopic examination of the organs (larynx, thyroid, heart, lungs, liver, spleen, kidneys and brain) did not yield unusual findings. No effects,[17] other than an increase in liver aniline hydroxylase activity and serum alkaline phosphatase activity in the high-dose group, which was only observed at 28 days, were recorded. Based on these effects, a NOAEL of 25 mg/kg bw per day was derived from this study in rats (Villeneuve et al., 1977, as referenced in MAK Value Documentation, 2013).

In contrast to abietic acid and the other resin acids described in Section 3.2.1.1, dehydroabietic acid has an aromatic ring. Although a NOAEL for abietic acid could not be derived from the available data, the experimental evidence indicated that this compound is less toxic than dehydroabietic acid. The FEEDAP Panel considered it very unlikely that toxicity studies with abietic acid would lead to a lower NOAEL compared to the NOAEL of 25 mg/kg bw per day for dehydroabietic acid. Therefore, the FEEDAP Panel selected this NOAEL of 25 mg/kg bw per day as the reference point for the group of resin acids.

3.3.4. Safety for the target species

In the absence of tolerance studies and/or data from repeated dose toxicity studies in laboratory animals performed with the additive under assessment, 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 including 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). For dehydroabietic acid, subchronic studies are available, from which a NOAEL of 25 mg/kg bw per day can be derived. This NOAEL is selected as the reference point for the assessment group resin acids.

[17] Haematological (haemoglobin, haematocrit, erythrocyte and leucocyte counts, differential blood count, mean erythrocyte volume and saturation index), biochemical parameters (serum cholesterol, bilirubin, blood urea nitrogen, sodium, potassium and alkaline phosphatase), urea and urine parameters (protein, glucose, blood, pH).

3.3.4.1. Phenolic compounds including flavonoids

Among the secondary metabolites, 0.088% on average are polyphenols including 0.022% phenolic acids and 0.001% rutin.

At the maximum proposed use level of 50 mg pine tincture/kg in feed, the highest concentration of the fraction of polyphenols (≤ 0.102% of the tincture) would be up to 0.051 mg/kg feed.

At least five phenolic acids were detected and quantified (as chlorogenic acid equivalents) accounting together for maximum 0.038%. At the proposed use level, this would correspond to 0.019 mg/kg. Although the individual compounds were not identified, phenolic acids are assigned to Cramer Class I. The available data indicate that phenolic acids 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, at the maximum proposed use level (50 mg/kg complete feed), no concern for the target species arises from phenolic acids.

At the maximum proposed use level of 50 mg pine tincture/kg complete feed, the highest concentration of the total polyphenolic fraction after subtraction of values for phenolic acids (≤ 0.065% of the tincture) would be up to 0.033 mg/kg feed. Although the individual compounds were not identified, flavonoids are assigned to Cramer Class III. The available data indicate that polyphenols other than phenolic acids would be in the same range of 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 polyphenols other than phenolic acids is not considered of concern for the target species.

Overall, no concern for the target species arises from polyphenols including the phenolic acid fraction.

3.3.4.2. Resin acids

Analytical data on the occurrence of resin acids in the additive were not provided. Therefore, the present assessment is based on the estimated highest value of 2.12% (w/w) (see Section 3.2.1.1).

At the maximum proposed use level of 50 mg tincture/kg complete feed, the highest concentration of resin acids in the additive (corresponding to the estimated value of 2.12%) would be 0.94 mg/kg complete feed, resulting in an intake of the target species ranging from 5.4 μg/kg bw per day in ornamental fish to 95 μg/kg bw per day in chickens for fattening. When the intake values of the target species are compared to the NOAEL of 25 mg/kg bw per day derived for dehydroabietic acid from a 28-day study in rat, a margin of exposure of at least 262 is calculated (see Table 3). Therefore, the presence of resin acids is not considered of concern for the target species.

Table 3: Target animal intake of resin acids (as μg/kg bw per day) and margin of exposure (MOE) calculated at the maximum proposed use level of the additive in feed (50 mg/kg complete feed)

	Body weight (kg)	Feed intake (g DM/day)	Resin acids intake[(a)] (μg/kg bw per day)	MOE[(b)]
Chicken for fattening	2	158	95	262
Laying hen	2	106	64	391
Turkey for fattening	3	176	71	353
Piglet	20	880	53	471
Pig for fattening	60	2,200	44	565
Sow lactating	175	5,280	36	687
Veal calf (milk replacer)	100	1,890	23	1,096
Cattle for fattening	400	8,000	24	1,036
Dairy cow	650	20,000	37	673
Sheep/goat	60	1,200	24	1,036
Horse	400	8,000	24	1,036
Rabbit	2	100	60	414
Salmon	0.12	2.1	21	1,184
Dog	15	250	20	1,243
Cat	3	60	24	1,036
Ornamental fish	0.012	0.054	5.4	4,603

bw: body weight; DM: dry matter.
(a): The intake values of resin acids are calculated based on the estimated concentration of 2.12% for resin acids.
(b): The MOE for resin acids is calculated as the ratio of the reference point to the intake: the NOAEL of 25 mg/kg bw per day derived from a 28-day study in rat with dehydroabietic acid was selected as the reference point for resin acids (Villeneuve et al., 1977, as referenced in MAK Value Documentation, 2013).

The FEEDAP Panel considers that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered safe when consumed via feed alone.

3.3.4.3. Conclusions on safety for the target species

The FEEDAP Panel concludes that pine tincture is safe at the maximum proposed use level of 50 mg/kg complete feed.

The FEEDAP Panel considers that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered safe when consumed via feed alone.

3.3.5. Safety for the consumer

Phenolic compounds, present in the additive at concentrations below the thresholds for Cramer Class I and III compounds, will either not be absorbed (condensed tannins with a high degree of polymerisation) or be readily metabolised and excreted and are not expected to accumulate in animal tissues and products (phenolic acids). The absorbed resin acids, present in the additive at residual levels, are expected to be metabolised and excreted and no appreciable residues will be present in food products. Consequently, no concern for the consumer is expected from the phenolic fraction.

3.3.6. 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[18] concerning the presence of ethanol in the tincture.[19] Since pine tincture is likely to contain resin acids, the additive may cause sensitisation reactions after skin contact. As reported in MAK (2013), 'Numerous studies exist on contact sensitisation and case reports in persons occupationally exposed to abietic acid in rosin (colophony)' and 'Restrictions in lung function and obstructive respiratory diseases have been described especially after longer term exposure to abietic acid or its oxidation and decomposition products in vapours of soldering fluxes containing rosin' (MAK, 2013).

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

3.3.7. Safety for the environment

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

3.4. Efficacy

According to Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009), needles and twigs of *P. sylvestris* are used to flavour food.

Since twigs are a material considered similar in composition to the source material for the production of pine tincture, no further demonstration of efficacy is deemed necessary.

4. Conclusions

Pine tincture from *Pinus sylvestris* L. 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 pine tincture which is produced from the aerial parts (buds) of *P. sylvestris* L.

[18] 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.
[19] H319: causes serious eye irritation (relevant for dermal exposure).

The FEEDAP Panel concludes that pine tincture is safe at the maximum proposed use level of 50 mg/kg complete feed for all animal species. The FEEDAP Panel considers that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered safe when consumed via feed alone.

No concerns for consumers were identified following the use of the additive at the use level considered safe in feed for the target animals.

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

The use of the additive under the proposed conditions of use is not expected to pose a risk to the environment.

Since twigs of *P. sylvestris*, which are considered similar in composition to the source material for the production of pine tincture, are described to flavour food, no further demonstration of efficacy is deemed 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*
13/05/2019	Comments received from Member States
20/02/2020	Reception of supplementary information from the applicant (partial dataset: pine tincture) - Scientific assessment remains suspended
09/02/2023	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*
14/03/2023	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
25/05/2023	Reception of supplementary information from the applicant (partial dataset: pine tincture) - Scientific assessment restarted
07/06/2023	The application was split and a new EFSA-Q-2023-00400 was assigned to the preparation included in the present assessment
04/07/2023	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

DM	dry matter
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
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
FL-no	FLAVIS number
HACCP	hazard analysis and critical control points
HPTLC	high performance thin layer chromatography
LOD	limit of detection
LOQ	limit of quantification
TTC	threshold of toxicological concern