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Safety and efficacy of a feed additive consisting of an extract of condensed tannins from *Schinopsis balansae* Engl. and *Schinopsis lorentzii* (Griseb.) Engl. (red quebracho extract) for use in all animal species (FEFANA asbl)

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

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of an extract of condensed tannins from *Schinopsis balansae* Engl. and *Schinopsis lorentzii* (Griseb.) Engl. (red quebracho extract) when used as a sensory additive in feed and water for drinking for all animal species. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the additive under assessment is safe up to the maximum proposed use levels of 400 mg/kg for chickens for fattening and other growing poultry, 600 mg/kg for laying hens and other laying/breeding birds kept for egg production/reproduction, 540 mg/kg for turkeys for fattening, 720 mg/kg for piglets, 860 mg/kg for pigs for fattening and other growing Suidae, 1,050 mg/kg for sows, 1,680 mg/kg for veal calves (milk replacer), 1,580 mg/kg for cattle for fattening and other growing ruminants, 1,030 mg/kg for dairy cows and other dairy ruminants, 1,580 mg/kg for sheep, goats, horses, 630 mg/kg for rabbits, 1,810 mg/kg for salmonids and other fin fish, 1,900 mg/kg for dogs and 3,000 mg/kg for ornamental fish. For cats, the calculated safe concentration in feed is 317 mg/kg complete feed. For all the other minor species, the additive is considered safe at 317 mg/kg complete feed. The FEEDAP Panel considered the use in water for drinking as safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed. No concerns for consumers were identified following the use of the additive at the maximum proposed use level in feed. The extract under assessment is not an eye irritant but in the absence of data, no conclusion can be drawn on its potential to be a skin irritant and a dermal and respiratory sensitiser. The use of the extract under the proposed conditions of use in feed was not expected to pose a risk for the environment. Since quebracho and its preparations were recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy was considered necessary.

Keywords: sensory additives, flavouring compounds, *Schinopsis balansae* Engl. and *Schinopsis lorentzii* (Griseb.) Engl., red quebracho extract, safety

Requestor: European Commission

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Correspondence: feedap@efsa.europa.eu

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

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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 seven years after the entry into force of this Regulation.

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation/re-evaluation of 20 preparations (namely buchu leaves oil, amyris oil, olibanum extract (water based, wb), olibanum tincture, lime oil, neroli bigarade oil, petigrain bigarade oil, petitgrain bigarade absolute, bitter orange extract of the whole fruit, lemon oil expressed, lemon oil distilled, orange oil, orange terpenes, mandarin oil, mandarin terpenes, grapefruit oil expressed, grapefruit extract (solvent-based, sb), grapefruit extract, quebracho extract (wb), cashew oil), belonging to botanically defined group (BDG) 8 - *Sapindales*, when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). During the assessment, the applicant withdrew the application for nine preparations.[3] During the course of the assessment, this application was split and the present opinion covers only one out of the 11 remaining preparations under application: red quebracho extract from *Schinopsis balansae* Engl. and *Schinopsis lorentzii* (Griseb.) Engl. 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 19 March 2018.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of red quebracho extract, when used under the proposed conditions of use (see Section 3.2.4).

The remaining 10 preparations belonging to botanically defined group (BDG) 8 - *Sapindales* under application have been assessed in separate opinions.

1.2. Additional information

A 'quebracho colorado condensed tannins extract' from *S. balansae* Engl. 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). It has not been assessed as a feed additive in the EU.

There is no specific EU authorisation for any *S. balansae* or *S. lorentzii* preparation when used to provide flavour in food.

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[4] in support of the authorisation request for the use of red quebracho extract from *S. balansae* or *S. lorentzii* as a feed additive.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] On 13/03/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1,050 Brussels, Belgium.
[3] On 27 February 2019, EFSA was informed about the withdrawal of the application on amyris oil, olibanum tincture, cashew oil, neroli bigarade oil, petitgrain bigarade absolute, mandarin terpenes, grapefruit oil expressed, grapefruit extract (sb), grapefruit extract.
[4] FEED dossier reference: FAD-2010-0322.

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.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phytochemical markers in the additives. The Executive Summary of the EURL report can be found in Annex A.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of red quebracho extract from *S. balansae* or *S. lorentzii* is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA SC, 2009), Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA SC, 2019b).

3. Assessment

The additive under assessment, 'quebracho colorado condensed tannins extract' hereinafter referred to as 'red quebracho extract', is obtained from the wood of *S. balansae* Engl. and *S. lorentzii* (Griseb.) Engl. It is intended for use as a sensory additive (functional group: flavouring compounds) in feed for all animal species.

3.1. Origin and extraction

Quebracho is a trivial name given primarily in Latin America to a number of tree species renowned for producing a dense hard wood. It is a name most usually associated with trees of the unrelated genera *Schinopsis* (a member of the Anacardiaceae) or Aspidosperma (a member of the Apocynaceae). Because of the colour of the wood, the epithet 'red quebracho' or 'colorado' is often applied to species of *Schinopsis*, in particular to *S. balansae* Engl. and *S. lorentzii* (Griseb.) Engl. In contrast, species of Aspidosperma are commonly referred to as 'white quebracho (quebracho-blanco)'. The term 'quebracho' may also be applied to herbal products derived from Aspidosperma spp. while the term 'quebracho colorado', as used in this application, is usually reserved for products derived from *Schinopsis* spp.

The additive under application is produced from the wood of either *S. balansae* or *S. lorentzii* sourced in South America. [redacted] to obtain a powder, which is then granulated.[7]

[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2010-0322-bdg08.pdf

[6] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[7] Technical dossier/Supplementary information February 2019/Annex_VI_Quebracho_Extract_flowchart_CONFIDENTIAL.

3.2. Characterisation

3.2.1. Characterisation of red quebracho extract

The additive consists of reddish-brown granules with a relative density of 500–600 kg/m^3 and a pH of 4.8 (at 10% solution). It is soluble in water but not in oil. The Chemical Abstracts Service (CAS) number 90106-04-0 is associated with *S. lorentzii*, ext.[8] Red quebracho extract contains by specification ≥ 70% (w:w) of condensed tannins (oligomeric/polymeric flavan-3-ols, also referred to as proanthocyanidins) and 5–12% water.

The applicant provided a measure of the content of the condensed tannins in five batches of the additive using an industry standard measure (ISO 14088) based on their tanning properties. The affinity of the condensed tannins in aqueous solution to the protein in hide powder is measured, with the percentage of dry matter irreversibly bound to the hide defined as tannin. The analysis of the five batches showed compliance with the proposed specification with the condensed tannins representing approximately 79% of the additive (Table 1).[9]

The same batches of the additive were also assayed for protein, lipid and ash content (Table 1).[10] Using the values for tannin and moisture provided by the ISO method and assuming that these additional analytes were found only in the non-tannin and insoluble fraction, summation accounts for 91% of the additive, with 9% remaining uncharacterised. Given the source of the additive, it is to be expected that this uncharacterised fraction would consist primarily of low molecular weight phenolic compounds and carbohydrates.

The gravimetric determination of ash was supported by a more extensive determination of organic and inorganic anions by ion chromatography, and cations by inductively coupled plasma/mass spectrometry. When summed these gave a mean value of 3.6% of the extract.[10]

Table 1: Analysis of red quebracho extract (*Schinopsis balansae* Engl. and *Schinopsis lorentzii* (Griseb.) Engl.) based on the analysis of five batches (mean and range). The results are expressed as % (w/w)

Constituent	Mean	Range
Analysis according to ISO 14088	% (w/w)	% (w/w)
Soluble fraction		
Condensed tannins	78.8	75.9–81.4
Non-tannins(1)	10.3	9.9–10.7
Insoluble fraction	1.78	0.8–3.1
Loss on drying	9.16	7.3–10.3
Total	100	
Gross analysis of the additive		
Protein	0.42	0.4–0.5
Fat (Lipids)	0.12	0.1–0.2
Ash	2.38	2.2–2.8

(1): Non-tannins: indirect gravimetry analysis after adsorption of tannins to hide powder.

One batch of the additive was further analysed by matrix-assisted laser desorption ionisation time of flight (MALDI-TOF)[11] to investigate the mass distribution of the flavanol oligomers contributing to the extract. Peaks interpretation indicated that the quebracho extract is composed of dimers (10.7%), trimers (38.2%), tetramers (25.3%), pentamers (11.3%), hexamers (8.3%) and heptamers (1.2%).[12] The MALDI-TOF analysis indicated that tannin oligomers are constituted predominantly by fisetinidol units and to a lesser extent by robinetinidol units (see Figure 1). The analysis also confirmed the presence of carbohydrate in the extract (5%).

[8] According to ECHA: Extractives and their physically modified derivatives such as tinctures, concretes, absolutes, essential oils, oleoresins, terpenes, terpene-free fractions, distillates, residues, etc., obtained from *Schinopsis lorentzii*, Anacardiaceae. https://echa.europa.eu/it/substance-information/-/substanceinfo/100.081.991.
[9] Technical dossier/Supplementary information February 2019/Annex_II.
[10] Technical dossier/Supplementary information February 2019/Annex_III.
[11] Technical dossier/Supplementary information February 2019/Annex_IV and Annex_V.
[12] Technical dossier/Supplementary information February 2019/Annex_IV.

The results of this analysis are consistent with the results published in the literature on the characterisations of quebracho extracts (*S. lorentzii* and *S. balansae*). Quebracho extracts were found to contain predominantly fisetinidol units (Venter et al., 2012; Crestini et al., 2016), with robinetinidol units also present (Vivas et al., 2004; Rabede et al., 2013).

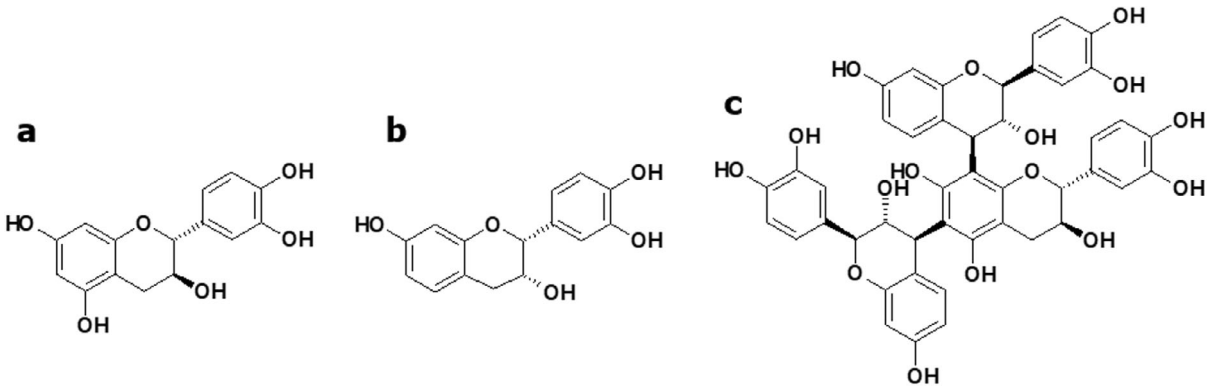

Figure 1: Monomeric units of the tannin fraction of red quebracho extract: catechin (a) and fisetinidol (b). Catechin is the starter unit, which is angularly bond to fisetinidol extender units, to form dimers, trimers or tetramers (c)

The applicant performed a literature search for substances of concern and for description of the chemical composition of *S. balansae* and *S. lorentzii* and their preparations.[13] No substances of concern were identified for the wood of *S. balansae* and *S. lorentzii*.

3.2.2. Impurities

Data on chemical and microbiological impurities were provided for five batches of red quebracho extract.[14] The concentration of mercury, cadmium, lead and arsenic was below the corresponding limit of detection (LOD) in all batches. In the same batches, pesticide residues were not detected in a multiresidue analysis. Individual polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) were not detectable. The sum of dioxin-like polychlorinated biphenyls (PCBs) was < 0.50 μg/kg. Mycotoxins (aflatoxins B1, B2, G1 and G2 and ochratoxin A) were below their respective limit of quantification (LOQ).[14,15] None of the data on chemical impurities raised concerns.

Analysis of the microbial contamination of five batches of quebracho extract indicated that *Salmonella* spp. was absent in 25 g, total mesophilic count, *Clostridium perfringens*, total coliforms, *E. coli* and Enterobacteriaceae were < 10 colony forming unit (CFU)/g, yeast and moulds < 100 CFU.

3.2.3. Shelf-life

The typical shelf-life of the additive 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).[16] However, no data supporting this statement were provided.

3.2.4. Conditions of use

Red quebracho extract is intended to be added to feed for all animal species without withdrawal time. The maximum proposed use level in complete feed and in water for drinking for certain target species are reported in Table 2.

[13] Technical dossier/Supplementary information February 2019/Literature search_quebracho_extract.
[14] Technical dossier/Supplementary information February 2019/Annex_VII. LOD: 0.1 mg/kg for mercury and cadmium, 1 mg/kg for lead and arsenic; LOQ for dioxins in the range < 0.1 to < 0.5 ng WHO PCDD/F-TEQ (World Health Organization polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) toxic equivalents)/kg; LOQ for mycotoxins: < 1 μg/kg for aflatoxins B1, B2, G1 and G2.
[15] Technical dossier/Supplementary information February 2019/Annex_III. LOQ for ochratoxin A: < 0.25 μg/kg.
[16] Technical dossier/Section II.

Table 2: Conditions of use for red quebracho extract (*Schinopsis balansae* Engl. and *Schinopsis lorentzii* (Griseb.) Engl.): maximum proposed use levels in feed and in water for drinking for certain target species

	Use level in feed (mg/kg feed)	Use level in water (mg/kg)
Chickens for fattening	400	225
Laying hens	600	300
Turkeys for fattening	540	270
Piglets	720	300
Pigs for fattening	860	350
Sow lactating	1,050	375
Veal calves (milk replacer)	1,680	560
Cattle for fattening	1,580	320
Dairy cows	1,030	105
Sheep/goat	1,580	530
Horse	1,580	440
Rabbit	630	125
Salmon	1,810	–(a)
Dogs	1,900	635
Cats	1,580	530
Ornamental fish	3,000	–(a)

(a): The additive is not intended for use in water for drinking for fish.

3.3. Safety

The assessment of safety is based on the use levels proposed by the applicant (Table 2).

No studies specifically focused on the absorption, distribution, metabolism and excretion (ADME) of the red quebracho extract under assessment were carried out. The applicant submitted a literature search on the ADME of condensed tannins, which is summarised in the following section.

The applicant also submitted a toxicological dataset including *in vitro* genotoxicity tests with a test item closely related with the additive under assessment and a 90-day oral toxicity study in rat performed with the additive under assessment. The studies are described below.

3.3.1. Absorption, distribution, metabolism and excretion

Condensed tannins (CT; synonym: proanthocyanidins) of the red quebracho extract are composed of dimers (10.7%), trimers (38.2%), tetramers (25.3%), pentamers (11.3%), hexamers (8.3%) and heptamers (1.2%), constituted by a unit of catechin bonded to one or more units of fisetinidol (see Section 3.2.1, Figure 1). The degree of polymerisation will modulate their absorption and metabolism at the gastrointestinal level. It is generally assumed that in the small intestine, only monomers, dimers, and eventually trimers may be absorbed to some extent, while larger oligomers and polymers remain in the lumen and are excreted via the faeces (Cires et al., 2017). Additionally, it is assumed that the type of bridges between the constituent units and their chemical features influence the stability and biotransformation of CT in the gastrointestinal tract.

The applicant submitted some studies performed in experimental animals and in target species, where some information on the ADME of CT is given. A review reporting experimental studies was also submitted (Cires et al., 2017), from which additional information was retrieved and is briefly summarised.

The absorption, metabolism and excretion of procyanidin B2 (PB2: epicatechin-(4β-8)-epicatechin) was studied in rats after oral administration of 50 mg/kg body weight (bw) of the compound (Baba et al., 2002, as referenced in Cires et al., 2017). Blood samples were collected at several times up to 5 h and the urine of 18 h after administration. Plasma and urine samples were analysed by liquid chromatography–mass spectrometry (LC–MS). PB2, epicatechin and 3'-*O*-methyl-epicatechin were detected in plasma after sulfatase hydrolysis and peaked at 30–60 min. The three compounds were excreted in urine, in conjugated forms. The sum of the three compounds in the urine excreted within

18 h was 0.48% of the administered dose, showing that the dimer is absorbed, metabolised and excreted.

The bioavailability of procyanidin dimer B3, trimer C2 and polymer (composed of catechin units only) isolated from willow tree catkins was investigated in rat and compared with that of the monomer catechin (Gonthier et al., 2003, as referenced in Cires et al., 2017). Groups of rats received in their diet 1 g of each compound/kg dry feed for 5 days and urine of 24 h was analysed by liquid chromatography-electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS) after enzymatic hydrolysis for the presence of metabolites. No procyanidins were detected in urine of rats fed with dimer, trimer and polymer while rats fed with catechin excreted 25% the dose as catechin and 3′-*O*-methyl-catechin. Sixteen metabolites of microbial origin were identified as phenylvaleric, phenylpropionic, phenylacetic and benzoic acid derivatives in urine of all groups in a decreasing level (10.6% for monomer, 6.5% for dimer, 0.7% for trimer and 0.5% for polymer). The study shows that the degree of polymerisation affects both the absorption of the condensed tannins and their microbial degradation in the colon to phenolic compounds, before absorption. The FEEDAP Panel notes that the CT evaluated in these studies are chemically different from those under assessment, having catechin as the starter unit in common; however, the data show that if some degradation happens in the red quebracho extract and free catechin is liberated, it is absorbed, biotransformed and excreted.

Data from in *vitro* degradation of CT by microbiota are not consistent. ^{14}C-Labelled purified proanthocyanidin polymers (condensed tannins, free of any catechin monomers, dimers and trimers, average polymerisation degree of 6) were incubated with human colonic microbiota in anoxic conditions. Polymers were almost totally degraded after 48 h of incubation. Phenylacetic, phenylpropionic and phenylvaleric acids, monohydroxylated mainly in the meta or para position, were identified by gas chromatography–mass spectrometry (GC–MS) (Déprez et al., 2000, as referenced in Cires et al., 2017). Using a rumen simulation technique (RUSITEC), oxen rumen was incubated for 40 h with or without purified tannins of red quebracho (spray-dried quebracho extract (SDQT), Trask Chem Corporation (Marietta, GA, USA)) or of *Dichostachys cinerea* leaves (mixture of condensed tannins and hydrolysable tannins). No degradation of both condensed and hydrolysable tannins was observed (Makkar et al., 1995). The inconsistency of data can be attributed to the different nature of the units and type of linkages in the CT studied.

Studies carried out in target animals also show some evidence of poor absorption of CT in the gut and their metabolism by microbiota. Three experiments were performed with condensed tannins to evaluate their gastrointestinal digestion in sheep (Terrill et al., 1994, as referenced in Cires et al., 2017): (i) incubation of purified CT extracted from *Lotus pedunculatus* in different parts of the digestive tract of sheep that gave a recovery of 80 and 58% in rumen, 51 and 49% in abomasum, 65 and 46% in duodenum and 43 and 39% in ileum at 0 and 4 h of incubation, respectively; (ii) administration of ^{14}C-CT *per abomasum* fistulae for 6.5 h; almost all of the labelled compound was recovered in digesta (92%), none being detected in blood and only residual radioactivity in liver; (iii) feeding sheep with *Lotus pedunculatus* and analysing CT in rumen, abomasal and ileal digesta and in faeces; CT levels were very low in all digesta samples and only 15% were recovered in faeces. These unexpected results were attributed by the authors to the reaction of the tannins with proteins in the gastrointestinal tract being these complexes not detected by the analytical methods used. The results obtained with labelled tannins administered in abomasum showed no evidence of absorption.

Quebracho condensed tannin extract containing 760 g CT/kg (specific composition unknown) was administered via ruminal cannulae to ewes at 0, 35 or 70 g/kg feed for 51 days. Faeces were collected daily from day 43 to 50 and analysis revealed 30% recovery of the CT administered doses. The authors also mentioned the difficulties of obtaining reliable results because of analytical drawbacks (Hervás et al., 2004).

A total of 16 Comisana lambs, weaned at 45 days of age, were grouped to two dietary treatments of eight animals each: a control group fed a diet without quebracho tannins and the other fed with the same diet supplemented with 6.4% (DM basis) quebracho tannins (from *S. lorentzii*) (López-Andrés et al., 2013). All lambs had access to the diets until 1 h before being slaughtered, at day 70. Blood and liver samples were collected for analysis. Phenolic compounds were purified and isolated from feed and animal tissues by solid phase extraction (SPE) and analysed by LC–MS. Fisetin (fisetinidol, see Figure 1b), dimers consisting of one fisetinidol unit plus one catechin unit, trimers consisting of two fisetinidol units plus one catechin unit, and tetramer consisting of three fisetinidol units plus one catechin unit were identified in feed sample extracts. No derived quebracho compounds were identified (LOD not given) in plasma and liver extracts of lambs fed with quebracho extract. CT from quebracho seem not to be degraded or absorbed in the gastrointestinal tract.

Pigs were fed with grape seed extract (MegaNatural Gold grape seed extract containing 91.9% of total phenolics (w/w) as gallic acids equivalent) (1% w/w) daily for 6 days. Faeces were analysed by LC–MS. Intact dimer-pentamer compounds were present in faeces on days 3 and 6 of the experiment, at the same concentration, but absent 48 h post-feeding. Several metabolites were identified as phenolic acids probably resulting from microbiota metabolism, being 4-hydroxyphenylvaleric acid the most abundant, followed by 3-hydroxybenzoic acid (Choy et al., 2014, as referenced in Cires et al., 2017). The study showed that the condensed tannins present in extract were found intact in faeces of pigs, rapidly excreted and partially metabolised by microbiota.

Groups of chicks were given via capsule different extracts prepared from seeds of Sorghum, containing labelled condensed tannins (identification of polymers not given) or non-tannin compounds; after 8 h, the animals were killed and blood, tissues and excreta collected for analysis. No radioactivity was detected in plasma and tissue samples of chicks given the condensed tannin fraction, being all recovered in excreta and gastrointestinal tract; animals given the non-tannin extracts showed radioactivity in their tissues and organs (Jimenez-Ramsey et al., 1994, as referenced in Cires et al., 2017).

The data above described indicate that absorption of condensed tannins depends on the degree of polymerisation, decreasing from dimers to polymers, and that they tend to accumulate in gastrointestinal tract, being partly metabolised in colon by microbiota before being absorbed and/or excreted in faeces. For some catechin dimers and trimers, there is evidence of degradation to monomers. After absorption they are conjugated to sulfate, glucuronide, and methylated derivatives being excreted through urine. No appropriate ADME studies in target species were carried out with the CT of red quebracho extract under assessment. The scarce data available point to negligible absorption and great stability in the gut of profisetinidins, the condensed tannins of red quebracho extract, which is attributed to the absence of the 5-OH group in the fisetinidol units compared to the catechin units, making them very stable (resistant) to degradation (López-Andrés et al., 2013).

3.3.2. Toxicological studies

3.3.2.1. Genotoxicity

A test item similar in composition to the additive under assessment was tested for the induction of gene mutations in bacteria. The only difference between the two substances is related to [redacted].[17] This difference is considered not to have any impact on genotoxicity profile of the substance. The study was carried out in *Salmonella* Typhimurium strains TA1535, TA1537 TA98, and TA100 and in *Escherichia coli* strain WP2 uvrA[18] The experimental protocol was performed in accordance with OECD Test Guideline (TG) 471 (OECD, 1997) and following Good Laboratory Practice (GLP). Five concentrations ranging from 0.016 to 1.6 mg/plate were tested both in the absence and presence of metabolic activation. The top concentration was limited by cytotoxicity, as detected in a preliminary cytotoxicity test. No significant increase in revertant colony numbers was observed at any tested concentration in any tester strain with or without S9-mix. Therefore, the test item did not induce gene mutations in bacteria under the experimental conditions applied in this study.

The same test item was used in an *in vitro* micronucleus (MN) assay to evaluate the induction of chromosomal damage in human lymphocytes.[19] The study was conducted in accordance with OECD TG 487 (OECD, 2016) and following GLP. Positive and negative controls were within the historical control ranges of the test laboratory. Comparable MN frequencies were observed after treatment with 0.5, 1 and 2 mg/mL of the test item and the solvent control. Thus, the test item was negative for the induction of structural and numerical chromosome aberrations in human lymphocytes.

3.3.2.2. Repeated dose toxicity study

Groups of 12 Sprague–Dawley rats of each sex received 'red quebracho extract' (the additive under assessment) in the diet at concentrations of 0 (control), 30, 40 and 50 g/kg diet for 90 consecutive days. The study was conducted in compliance with GLP and with OECD TG 408.[20] Two satellite groups

[17] Technical dossier/Supplementary information May 2021/2021_05_05_SIn reply_BDG08_Quebracho extract.
[18] Technical dossier/Supplementary information November 2020/Annex_VI_AMES_CONFID.
[19] Technical dossier/Supplementary information/September 2022/Annex_MN_CONFID.
[20] Technical dossier/Supplementary information July 2020/Annex_VII.

(recovery controls and high dose recovery, 6 rats/sex per group) were offered normal diet for 30 days (recovery period).

All animals survived the treatment period, and no treatment-related clinical signs were observed. At all doses tested there were higher blood levels of total protein, albumin, globulin and urea in both sexes compared to controls. However, the values were within normal ranges for SD-rats and the differences were not present after the recovery period. As these differences were not accompanied by histopathological changes in the liver or kidney, they were not considered adverse. The no observed adverse effect level (NOAEL) for 'red quebracho extract' in the study was 50 g/kg feed (corresponding to 3,571 and 3,696 mg/kg bw per day in males and females, respectively[21]), the highest dose tested.

3.3.2.3. Conclusions on toxicology

The red quebracho extract under assessment is not considered to be genotoxic since no induction of gene mutation or chromosomal damage was observed in *in vitro* tests.

No adverse effects were observed in the 90-day study in rats up to the highest dose tested, suggesting a NOAEL for 'red quebracho extract' of 50 g/kg feed (corresponding to 3,600 mg/kg bw per day), the highest dose tested. Considering all the available evidence, the FEEDAP Panel retains a NOAEL of 3,600 mg/kg bw per day for red quebracho extract based on the study in rats.

3.3.3. Safety for the target species

The applicant submitted additional studies from the literature on the effects of the administration of red quebracho tannins similar to the additive under assessment to food-producing animals.

Lambs were fed with a commercial red quebracho tannin source (containing 75% condensed tannins, prepared from *Schinopsis* spp.) mixed with alfalfa hay at levels of 0, 10, 20 and 30 g/kg DM diet for 56 days. The resulting levels of condensed tannins were 0, 7.5, 15 and 22.5 g/kg DM. Increased weight gain and reduced feed to gain ratio were observed in all supplemented groups compared to controls, however the difference was statistically significant only in the 20 g/kg DM group. No adverse effects were observed up to the highest level of red quebracho extract in feed (Al-Dobaib, 2009).

Similar effects on performance parameters (increased weight gain and reduced feed to gain ratio) were observed in rabbits fed for 42 days with commercial pelleted food containing 10 and 30 g/kg diet of red quebracho extract (prepared from *S. balansae* and *S. lorentzii*, content of condensed tannins not given) compared to animals receiving the control diet. Adverse effects were not observed up to the highest level of red quebracho extract in food (Dalle Zotte and Cossu, 2009).

No signs of toxicity were observed in common carp (*Cyprianus carpio*) when soybean and fish meal-based diet were supplemented with red quebracho tannins (content of condensed tannins not given) at a level of 20 g/kg diet for up to 84 days. Red quebracho tannins did not affect feed intake, body weight gain, average metabolic growth rate and oxygen consumption during the experimental period (84 days). The carp grew from an initial body weight of about 30 g to a final body weight of 150 g, and the carcass composition was also not affected by red quebracho tannins (Becker and Makkar, 1999).

Two studies by the same authors investigated the effects of intraruminal administration of red quebracho tannins in sheep. In the first study, four groups of four sheep were dosed intraruminally with 0, 0.5, 1.5 and 3.0 g red quebracho tannins extract/kg bw per day (containing 76% condensed tannins, prepared from *Schinopsis* spp.) for 21 days. The group treated with the highest dose (3 g/kg bw per day, corresponding to 150 g red quebracho/kg feed) showed signs of intoxication, including depression of feed intake, body weight loss, weakness, lesions in the digestive tract and changes in plasma biochemistry. These animals were killed after 10 days to avoid suffering (Hervás et al., 2003). No adverse effects were seen in the other groups up to 1.5 g/kg bw per day corresponding to 75 g red quebracho extract/kg feed. In the second study, no signs of intoxication by condensed tannins were observed in fistulated sheep fed lucerne hay and dosed for a period of 51 days with 0, 35 and 70 g/kg red quebracho tannin extract (containing 76% condensed tannins, prepared from *Schinopsis* spp.), corresponding to 0, 0.7 and 1.4 g/kg bw per day, respectively (Hervás et al., 2004).

All the studies in the target species with red quebracho extracts similar in composition to the additive under assessment consistently indicate that concentrations up to 20–75 g red quebracho

[21] Average exposures of quebracho extract in male and female rats are calculated considering data from groups fed with 50 g quebracho extract/kg (including the "high dose recovery group").

extract/kg feed do not cause adverse effects, whereas signs of intoxication were observed in sheep dosed intraruminally with red quebracho extract at 150 g/kg feed. However, considering the limitations in the study design and the differences in the test items, these studies were considered as supporting evidence.

A subchronic toxicity study performed in rats with 'red quebracho extract' was made available, from which the FEEDAP Panel identified a NOAEL of 3,600 mg/kg bw per day as a suitable reference point to assess the safety of the extract under assessment.

Applying an uncertainty factor (UF) of 100 to the NOAEL the safe daily dose for the target species was derived following the EFSA Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), and thus the maximum safe feed concentration was calculated (Table 3). Since glucuronidation of degradation products of flavan-3-ol dimers and trimers is an important metabolic pathway facilitating the excretion of these compounds (Section 3.3.1), the calculation of safe concentrations in cats feed needs an additional UF of 5. This factor is due to the unusually low capacity for glucuronidation in cats (Court and Greenblatt, 1997; Lautz et al., 2021).

Table 3: Maximum safe concentration in feed for different target animals for red quebracho extract

	Body weight (kg)	Feed intake (g DM/day)	Daily feed intake (g DM/kg bw)	Maximum safe concentration (mg/kg feed)(1)
Chicken for fattening	2	158	79	401
Laying hen	2	106	53	598
Turkey for fattening	3	176	59	540
Piglet	20	880	44	720
Pigs for fattening	60	2,200	37	864
Sow lactating	175	5,280	30	1,128
Veal calf (milk replacer)	100	1,890	19	1,800
Cattle for fattening	400	8,000	20	1,584
Dairy cow	650	20,000	31	1,030
Sheep/goat	60	1,200	20	1,584
Horse	400	8,000	20	1,584
Rabbit	2	100	50	634
Salmon	0.12	2.1	18	1,810
Dog	15	250	17	1,901
Cat(2)	3	60	20	317
Ornamental fish	0.012	0.054	5	7,040

DM: dry matter; bw: body weight.
(1): Complete feed containing 88% DM, milk replacer 94.5% DM.
(2): The uncertainty factor for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation.

The FEEDAP Panel concludes that red quebracho extract added to the feed of all animal species is safe at the maximum proposed use levels of 400 mg/kg for chickens for fattening and other growing poultry, 600 mg/kg for laying hens and other laying/breeding birds kept for egg production/ reproduction, 540 mg/kg for turkey for fattening, 720 mg/kg for piglets and other growing Suidae, 860 mg/kg for pigs for fattening, 1,050 mg/kg for sows, 1,680 mg/kg for veal calves (milk replacer), 1,580 for cattle for fattening and other growing ruminants, 1,030 mg/kg for dairy cows and other dairy ruminants, 1,580 mg/kg for sheep, goats and horses, 630 mg/kg for rabbits, 1,810 mg/kg for salmonids and other fin fish, 1,900 mg/kg for dogs and 3,000 mg/kg for ornamental fish. For cats, the calculated safe concentration in feed 317 mg/kg complete feed. For other minor species not included in Table 3, the additive is considered safe at 317 mg/kg complete feed.

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

3.3.3.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that red quebracho extract under assessment is safe up to the maximum proposed use levels in complete feed of 400 mg/kg for chickens for fattening and other growing poultry, 600 mg/kg for laying hens and other laying/breeding birds kept for egg production/

reproduction, 540 mg/kg for turkey for fattening, 720 mg/kg for piglets and other growing *Suidae*, 860 mg/kg for pigs for fattening, 1,050 mg/kg for sows, 1,680 mg/kg for veal calves (milk replacer), 1,580 for cattle for fattening and other growing ruminants, 1,030 mg/kg for dairy cows and other dairy ruminants, 1,580 mg/kg for sheep, goats, horses, 630 mg/kg for rabbits, 1,810 mg/kg for salmonids and other fin fish, 1,900 mg/kg for dogs and 3,000 mg/kg for ornamental fish. For cats, the calculated safe concentration in feed 317 mg/kg complete feed. For all the other minor species, the additive is considered safe at 317 mg/kg complete feed.

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

3.3.4. Safety for the consumer

European consumers are not known to be naturally exposed to the condensed red quebracho tannins. However, due to natural occurrence in food and to uses in food supplements, consumers are exposed to other sources of condensed tannins (proanthocyanidins), such as grape skin and grape seed proanthocyanidins. The latter are built from similar monomeric units of catechin or epicatechin derivatives as condensed red quebracho tannins, which are oligomers consisting mainly of fisetinidol units besides catechin units and robinetinidol units (EFSA FEEDAP Panel, 2016).

No data on residues in products of animal origin were made available for the constituents of 'red quebracho extract'. However, considering the ADME of the condensed tannins and their degradation products, which are either excreted via the faeces, or when absorbed, show a rapid conjugation and elimination (see Section 3.3.1), a relevant increase of the uptake of these compounds by humans consuming products of animals exposed to the additive is not expected.

Consequently, no safety concern would be expected for the consumer from the use of red quebracho extract up to the highest safe use level in feed.

3.3.5. Safety for the user

The eye irritation potential of 'reed quebracho extract' was tested in a valid study performed following the principles of GLP and according to OECD TG 405,[22] which showed that it is not an eye irritant.

No specific studies investigating skin irritation and skin sensitisation potential of the additive were submitted.

The extract under assessment is not an eye irritant but in the absence of data, no conclusion can be drawn on its potential to be a skin irritant and a dermal and respiratory sensitiser.

3.3.6. Safety for the environment

The addition of naturally occurring substances that will not result in a substantial increase of the concentration in the environment are exempt from further assessment (EFSA FEEDAP Panel, 2019). This exemption applies to botanical preparations from plants native to Europe. However, *S. balansae and S. lorentzii* are not native to Europe. Therefore, the safety for the environment is assessed based on the individual components of the extract.

Condensed tannins, such as those included in red quebracho extract, are naturally occurring in feed materials and in the environment. Therefore, it is highly unlikely that its use as a feed additive would increase its concentration in the environment to any measurable extent; therefore, no risk to the safety of the environment is foreseen.

3.4. Efficacy

Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) includes an entry for 'quebracho' with a description which includes both white (*Aspidosperma quebracho-blanca*) and red (*S. lorentzii*) quebracho tree species. This is followed by an entry 'quebracho bark extract' which makes reference to the previous entry as the source of the raw material and so covers extracts of both red and white quebracho. The Flavor Extract Manufacturers Association (FEMA) also recognises 'quebracho bark extract' as a flavour, referencing the same two tree species as Fenaroli but using a single reference number 2972. Although, the description used in Fenaroli's Handbook and by FEMA does not distinguish between extracts of bark from red and white quebracho, both are recognised as flavours.

[22] Technical dossier/Supplementary information February 2019/Annex_IX_SIn_Quebracho_extract_AcuteCorrosion_CONF.

Since red quebracho and its extracts are recognised to flavour food and their function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

4. Conclusions

The use of red quebracho extract from *S. balansae* Engl. and *S. lorentzii* (Griseb.) Engl., is safe up to the maximum proposed use levels of 400 mg/kg for chickens for fattening and other growing poultry, 600 mg/kg for laying hens and other laying/breeding birds kept for egg production/ reproduction, 540 mg/kg for turkey for fattening, 720 mg/kg for piglets and other growing Suidae, 860 mg/kg for pigs for fattening, 1,050 mg/kg for sows, 1,680 mg/kg for veal calves (milk replacer), 1,580 for cattle for fattening and other growing ruminants, 1,030 mg/kg for dairy cows and other dairy ruminants, 1,580 mg/kg for sheep, goats, horses, 630 mg/kg for rabbits, 1,810 mg/kg for salmonids and other fin fish, 1,900 mg/kg for dogs and 3,000 mg/kg for ornamental fish. For cats, the calculated safe concentration in feed 317 mg/kg complete feed. For all the other minor species, the additive is considered safe at 317 mg/kg complete feed. The Panel considers that the use of the additive in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

No concerns for consumer safety were identified following the use of the additive at the maximum proposed use level in animal feed.

The extract under assessment is not an eye irritant but in the absence of data, no conclusion can be drawn on its potential to be a skin irritant and a dermal and respiratory sensitiser.

The use of red quebracho extract under the proposed conditions of use in feed is not expected to pose a risk for the environment.

Since quebracho and its preparations are recognised to flavour food and its 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. Chemically defined flavourings from Botanical Group 08 – Sapindales 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
17/06/2016	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". Discussion on the ongoing work regarding the pilot dossiers BDG08 and BDG 09
27/04/2017	Trilateral meeting organised by the European Commission with EFSA and the applicant FEFANA on the assessment of botanical flavourings: characterisation, substances of toxicological concern present in the botanical extracts, feedback on the pilot dossiers
19/03/2018	Application validated by EFSA – Start of the scientific assessment
20/06/2018	Comments received from Member States
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: Amyris oil, Cashew oil, Neroli bigarade oil, Petitgrain bigarade absolute, Mandarin terpenes, Grapefruit oil expressed, Grapefruit extract (sb), Grapefruit extract.
03/05/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization, safety for the target species, safety for the consumer, safety for the user, safety for the environment*
14/02/2019	Reception of supplementary information from the applicant (partial submission)
01/07/2020	Reception of supplementary information from the applicant (partial submission)
17/03/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives

Date	Event
09/11/2021	The application was split and the original EFSA-Q-2010-01517 remained associated to the preparation included in the present assessment.
01/09/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
22/11/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
BDG	botanically defined group
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CFU	colony forming unit
CT	condensed tannins
DM	dry matter
EEIG	European economic interest grouping
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavor 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
FL-No	FLAVIS number
GC	gas chromatography
GC-FID	gas chromatography with flame ionisation detector
GC–MS	gas chromatography–mass spectrometry
GLP	Good Laboratory Practice
ISO	International Organization for Standardization
LOD	limit of detection
LOQ	limit of quantification
LC–MS	liquid chromatography-mass spectrometry
LC-ESI-MS–MS	liquid chromatography-electrospray-*tandem* mass spectrometry
MN	micronucleus (assay)
MALDI-TOF	matrix-assisted laser desorption ionisation time of flight
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCBs	polychlorobiphenyls
PCDD	polychlorinated dibenzo-*p*-dioxins
PCDF	polychlorinated dibenzofurans
RUSITEC	rumen simulation technique
SC	EFSA Scientific Committee
SDQT	spray-dried quebracho extract
SPE	solid phase extraction
TG	Testing Guideline
UF	uncertainty factor

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for buchu leaves oil, olibanum extract (wb), lime oil, petigrain bigarade oil, bitter orange extract of the whole fruit, lemon oil expressed, lemon oil distilled (residual fraction), lemon oil distilled (volatile fraction), orange oil cold pressed, orange terpenless (concentrated 4 times), orange terpenless (concentrated 10 times), orange terpenless (folded), orange terpenes, mandarin oil and quebracho extract (wb) from botanically defined flavourings Group (BDG 08) – Sapindales

In the current grouped application an authorisation is sought under Articles 4(1) and 10(2) for *buchu leaves oil, olibanum extract (wb), lime oil, petigrain bigarade oil, bitter orange extract of the whole fruit, lemon oil expressed, lemon oil distilled (residual fraction), lemon oil distilled (volatile fraction), orange oil cold pressed, orange terpenless (concentrated 4 times), orange terpenless (concentrated 10 times), orange terpenless (folded), orange terpenes, mandarin oil and quebracho extract (wb)* from *botanically defined flavourings group 08 (BDG 08)1*, under the category/functional group 2(b) 'sensory additives'/'flavouring compounds', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for all animal species. For each preparation the Applicant indicated the corresponding phytochemical marker(s) and the corresponding range of content. The *feed additives* are intended to be incorporated into *feedingstuffs* or drinking *water* directly or through flavouring *premixtures* with no proposed minimum or maximum levels. However, the Applicant suggested the typical maximum inclusion level of the *feed additives* of 25 mg/kg *feedingstuffs*.

For the quantification of the phytochemical markers *d-limonene* and *d,l-isomenthone* in *buchu leaves oil* and *d-limonene* in *orange terpenless (concentrated 10 times)* oil, the Applicant submitted a method using gas chromatography coupled with flame ionisation detection (GC-FID) based on the generic standard ISO 11024. The quantification is performed by using the normalisation approach for the estimation of the area percentage of individual components. The Applicant tested the method, following an experimental design proposed by the EURL, and obtained satisfactory performance characteristics.

For the quantification of the phytochemical markers *11-keto-β-boswellic acid* and *3-O-acetyl-11-keto-β-boswellic acid* in *olibanum extract (wb)*, the Applicant submitted a method using high performance liquid chromatography (HPLC) with spectrophotometric (UV) detection at 250 nm described in the European Pharmacopeia monograph for Indian Frankincense (Olibanum indicum). The quantification of *11-keto-β-boswellic acid* and *3-O-acetyl-11-keto-β-boswellic acid* is performed by means of specific expressions and is indicated as percentage content (absolute value). The Applicant, using the HPLC-UV method, analysed 5 batches of the *feed additive* obtaining results within the proposed specifications.

For the quantification of the phytochemical marker *d-limonene* in *lime oil* the Applicant submitted a GC-FID method based on the corresponding standard ISO 3519:2005 for the characterisation of the "oil of lime distilled, Mexican type (Citrus aurantifolia [Christm.] Swingle)". The quantification is performed using the normalisation approach for the estimation of the area percentage of individual components. The Applicant presented a chromatogram and the specific analytical procedure for the analysis of *d-limonene* in *lime oil*.

For the quantification of the phytochemical markers *linalyl acetate* and *linalool* in *petigrain bigarade oil* the Applicant submitted a GC-FID method based on the corresponding standard ISO 8901:2003 for "Oil of bitter orange petitgrain, cultivated (Citrus aurantium L.)". The quantification is performed using the normalisation approach for the estimation of the area percentage of individual components. The Applicant presented a chromatogram and the specific analytical procedure for the analysis of *linalyl acetate* and *linalool* in *petigrain bigarade oil*.

For the quantification of the phytochemical marker *naringin* in *bitter orange extract of the whole fruit* the Applicant submitted a single-laboratory validated and further verified method based on HPLC-UV (284 nm). The method has been developed for the determination of total flavonoids (including *naringin* alone) in a mixture of citrus flavonoids. The quantification of *naringin* is performed using the normalisation approach for the estimation of the area percentage of individual components. The Applicant provided validation and verification studies demonstrating the applicability of the method for

the analysis of pure *naringin*. Furthermore, *naringin* has been satisfactory quantified in the *feed additive* by the proposed method in 5 different lots of *bitter orange extract of the whole fruit*.

For the quantification of the phytochemical marker *d-limonene* in *lemon oil expressed, lemon oil distilled (residual fraction)* and *lemon oil distilled (volatile fraction)* the Applicant submitted a GC-FID method based on the corresponding standard ISO 855:2003 for "Oil of lemon (Citrus limon (L.) Burm. f.), obtained by expression". The quantification is performed using the normalisation approach for the estimation of the area percentage of individual components. The Applicant presented a chromatogram and the specific analytical procedure for the analysis of *d-limonene* in *lemon oil expressed, lemon oil distilled (residual fraction)* and *lemon oil distilled (volatile fraction)*.

For the quantification of the phytochemical marker *d-limonene* in *orange oil cold pressed*, *orange terpenless (concentrated 4 times)* oil, *orange terpenless (folded)* oil and *orange terpenes* oil the Applicant submitted a GC-FID method based on the corresponding standard ISO 3140:2019 for "Essential oil of sweet orange expressed (Citrus sinensis (L.))". The quantification is performed using the normalisation approach for the estimation of the area percentage of individual components. The Applicant presented a chromatogram and the specific analytical procedure for the analysis of *d-limonene* in *orange oil cold pressed*, *orange terpenless (concentrated 4 times)* oil, *orange terpenless (folded)* oil and *orange terpenes* oil.

For the quantification of the phytochemical marker *d-limonene* in *mandarin oil* the Applicant submitted a GC-FID method based on the corresponding standard ISO 3528:2012 for "Essential oil of mandarin, Italian type (Citrus reticulate Blanco)". The quantification is performed using the normalisation approach for the estimation of the area percentage of individual components. For *mandarin oil*, the Applicant presented a chromatogram and the specific analytical procedure for the analysis of the *d-limonene* in *mandarin oil*.

For the quantification of the phytochemical marker *tannins* in *quebracho extract (wb)* the Applicant submitted the method ISO 14088:2020 "Leather - Chemical tests - Quantitative analysis of tanning agents by filter method". The method proposed is suitable for the determination of tanning agents in all vegetable tanning products and it is based on indirect gravimetric analysis of tanning agents with fixing of the absorbent compounds in low chromed hide powder. The quantification of *tannins* in *quebracho extract (wb)* is performed by means of specific expressions and is indicated as percentage content (absolute value). Furthermore, the Applicant provided satisfactory results for the analysis of *tannins* in 3 batches of *quebracho extract (wb)*.

The accurate quantification of the *feed additives* in *premixtures* and *feedingstuffs* is not achievable experimentally and the Applicant did not provide experimental data to determine the *feed additives* in *water*. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify the *feed additives* in *premixtures*, *feedingstuffs* and *water*.

Based on the information above, the EURL recommends for official control: (i) the GC-FID method based on the generic standard ISO 11024 for the quantification of d-limonene and d,l-isomenthone in buchu leaves oil and d-limonene in orange terpenless (concentrated 10 times) oil; (ii) the HPLC-UV method described in the European Pharmacopeia monograph "Indian Frankincense (Olibanum indicum)" for the quantification of 11-keto-β-boswellic acid and 3-O-acetyl-11-keto-β-boswellic acid in olibanum extract (wb); (iii) the GC-FID method based on the standard ISO 3519:2005 for the quantification of d-limonene in lime oil; (iv) the GC-FID method based on the standard ISO 8901:2003 for the quantification of linalyl acetate and linalool in petigrain bigarade oil; (v) the HPLC-UV single-laboratory validated and further verified method for the quantification of naringin in bitter orange extract of the whole fruit; (vi) the GC-FID method based on the standard ISO 855:2003 for the quantification of d-limonene in lemon oil expressed, lemon oil distilled (residual fraction) and lemon oil distilled (volatile fraction); (vii) the GC-FID method based on the standard ISO 3140:2019 for the quantification of d-limonene in orange oil cold pressed, orange terpenless (concentrated 4 times) oil, orange terpenless (folded) oil and orange terpenes oil; (viii) the GC-FID method based on the standard ISO 3528:2012 for the quantification of d-limonene in mandarin oil; and (ix) the indirect gravimetric analysis of tanning agents with fixing of the absorbent compounds in low chromed hide powder described in ISO 14088:2020 for the quantification of tannins in quebracho extract (wb).

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.