SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 30 January 2019

doi: 10.2903/j.efsa.2019.5622

Re-evaluation of Quillaia extract (E 999) as a food additive and safety of the proposed extension of use

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Rainer Gürtler, Ursula Gundert-Remy, Trine Husøy, Wim Mennes, Agneta Oskarsson, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle, Polly Boon, Claude Lambré, Paul Tobback, Matthew Wright, Ana Maria Rincon, Camilla Smeraldi, Alexandra Tard and Peter Moldeus

Abstract

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion on Quillaia extract (E 999) when used as a food additive and the evaluation of the safety of its proposed extension of use as a food additive in flavourings. The Scientific Committee for Food (SCF) in 1978 established an acceptable daily intake (ADI) of 0–5 mg spray-dried extract/kg body weight (bw) per day for E 999. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established in its latest evaluation a group ADI of 0–1 mg/kg bw per day, expressed as quillaia saponins, for Quillaia extract for Type 1 and Type 2. The Panel considered it likely that intact Quillaia extract saponins are absorbed to a low extent, are hydrolysed in the gastrointestinal (GI) tract and that the aglycone is absorbed only to a limited extent. The Panel considered that the genotoxicity data available did not indicate a concern for genotoxicity. Taking into account the available toxicological database, various no observed adverse effect levels (NOAELs) relevant for the derivation of an ADI were identified. The Panel considered that the 2-year study in rats was the most robust and that the NOAEL of 1,500 mg Quillaia extract/kg bw per day could be used to derive the ADI for E 999. Considering that the adverse effects reported were due to the presence of saponins in the extract, that saponins were present in Quillaia extract Type 1 (around 20%) and using an uncertainty factor of 100, the Panel derived a ADI of 3 mg saponins/kg bw per day for E 999. None of the exposure estimates for the different population groups of the refined brand-loyal scenario exceeded the ADI of 3 mg saponins/kg bw per day. The proposed extension of use also would not result in an exceedance of this ADI for the refined scenario. The Panel proposed some recommendations for the European Commission to consider, in particular revising the EU specifications for E 999 in order to differentiate the extracts of Quillaia according to the saponins content and to include other parameters to better characterise the food additive.

Keywords: Quillaia extract, *Quillaja* extract, E 999, food additive, CAS No 68990-67-0

Requestor: European Commission

Question numbers: EFSA-Q-2011-00731; EFSA-Q-2014-00095

Correspondence: fip@efsa.europa.eu

Panel members: Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Rainer Gürtler, Ursula Gundert-Remy, Trine Husøy, Wim Mennes, Peter Moldeus, Agneta Oskarsson, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle and Maged Younes.

Acknowledgements: The Panel wishes to thanks the members of the former Working Group Application of the EFSA Panel on Food Additives and Nutrient Sources for the preparatory work on this scientific output and Fabiola Pizzo for the support provided to this scientific output. The FAF Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Gundert-Remy U, Husøy T, Mennes W, Oskarsson A, Shah R, Waalkens-Berendsen I, Wölfle D, Boon P, Lambré C, Tobback P, Wright M, Rincon AM, Smeraldi C, Tard A and Moldeus P, 2019. Scientific Opinion on the re-evaluation of Quillaia extract (E 999) as a food additive and safety of the proposed extension of use. EFSA Journal 2019;17(3):5622, 50 pp. https://doi.org/10.2903/j.efsa.2019.5622

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Quillaia extract (E 999) is obtained by aqueous extraction of the milled inner bark or wood of *Quillaja saponaria* Molina, or other *Quillaja* species, trees of the family Rosaceae. It contains a number of triterpenoid saponins consisting of glycosides of quillaic acid. Sugars – including glucose, galactose, arabinose, xylose, and rhamnose – are also present, along with tannin, calcium oxalate and other minor components.

Quillaia extract (E 999) was evaluated by the Scientific Committee for Food (SCF) in 1978 who established an acceptable daily intake (ADI) of 0–5 mg spray-dried extract/kg body weight (bw) per day based on two long-term studies in rats and mice.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established in its latest evaluation a group ADI of 0–1 mg/kg bw per day, expressed as quillaia saponins, for Quillaia extract for Type 1 and Type 2.

The Panel noted that existing EU specifications for E 999 do not describe any range for saponins content in the food additive. The JECFA specifications, however, differentiate two types of Quillaia extracts, Type 1 and Type 2, containing a different percentage of saponins and other parameters. The Panel considered that similar differentiation of the extracts of Quillaia should be presented in the EU specifications, including the percentage range for saponins, polyphenols (including tannins), protein, polysaccharides including fibre, reducing sugars, a maximum limit for calcium oxalate as well as microbiological specifications.

The Panel assumed that the test materials used in toxicological studies described as Quillaia extract approximated to Quillaia extract Type 1 as described in JECFA. However, from the information available, the Panel could not ascertain the comparability of test materials between different studies. The Panel noted that the material used in the toxicity studies may be compliant with the current EU specifications; however, it is only poorly characterised.

The Panel considered that the extent and the quality of the available data on the absorption, distribution, metabolism, excretion (ADME) of Quillaia extract saponins were very limited and that no conclusions could be drawn based on studies with Quillaia extracts. The Panel, therefore, considered the available data on gastrointestinal (GI) metabolism and ADME for structurally similar saponins. Based on read-across from these data, the Panel presumed that Quillaia extract saponins share a similar fate. Thus, the Panel considered it likely that intact Quillaia extract saponins are absorbed to a low extent; are hydrolysed in the GI tract and that the aglycone is absorbed only to a limited extent.

The Panel considered that, from the limited data available, the acute toxicity for Quillaia extract was low.

The Panel noted a no observed adverse effect level (NOAEL) of 0.6% Quillaia extract (approximately 400 mg/kg bw per day) from a 13-week study in rats in which relative organ weights were changed at the next higher dose of 1,200 mg/kg bw per day. The Panel also noted that no histopathological changes were observed at any dose.

The Panel considered that the data on the chemical composition of the mixture and the genotoxicity data available did not indicate a concern for genotoxicity.

The Panel identified a NOAEL of 750 mg Quillaia extract/kg bw per day from a 84-week study in mice and a NOAEL of 1,500 mg Quillaia extract/kg bw per day, the highest dose tested, from the 2-year study in rats. There was no indication for carcinogenicity. In the mouse study, slightly lower body weight gain and some organ weight changes in the high-dose group of 2,250 mg/kg bw per day Quillaia extract were reported but there were no histopathological changes.

No reproductive or developmental toxicity data were available to the Panel. However, in a chronic study in rats, no adverse effects were observed in the reproductive organs.

The Panel considered the gut epithelium - as a site of contact – a potential target for any effects of Quillaia extract. The Panel therefore focussed attention on any GI effects in the biological and toxicological studies. The Panel considered that although exposure to Quillaia extract resulted in changes in GI organ weights in several studies relevant to its use as a food additive, these changes were not accompanied by any consistent treatment- and/or dose-related histopathological changes.

Taking into account the available toxicological database, the Panel noted that various NOAELs relevant for the derivation of an ADI were identified:

- a NOAEL of 400 mg Quillaia extract /kg bw per day in a 13-week study in rats,
- a NOAEL of 750 mg Quillaia extract/ kg bw per day in an 84-week study in mice,
- a NOAEL of 1,500 mg Quillaia extract/kg bw per day (the highest dose tested) in a 2-year study in rats.

Overall, the Panel considered that the 2-year study in rats was the most robust and that the NOAEL of 1,500 mg Quillaia extract/kg bw per day could be used to derive the ADI for Quillaia extract (E 999). Considering that the adverse effects reported were due to the presence of saponins in the extract, that saponins were present in Quillaia extract Type 1 at around 20% (conservative scenario since Quillaia extract Type 1 may contain 20–26% of saponins) and using an uncertainty factor of 100, the Panel derived a ADI of 3 mg saponins/kg bw per day for the food additive Quillaia extract (E 999). Accordingly, the ADI for Quillaia extract (E 999) of 5 mg spray-dried extract/kg bw per day is withdrawn.

Quillaia extract (E 999) is currently authorised as a food additive for use in two food categories. The requested use as a food additive in flavourings relates to five other food categories. The exposure to this food additive was estimated based on maximum permitted levels (MPLs) and use levels, both for its current uses only and its current uses and intended extension of uses, based on the proposed maximum levels in final foods by the applicant (Section 3.4.1). As described in Section 3.4.1, overall the Panel estimated that the exposure to Quillaia extract (E 999) was overestimated in all scenarios.

The Panel noted that MPLs are expressed as anhydrous extract in Annex II to Regulation No 1333/2008. Therefore, exposure to Quillaia extract (E 999) was expressed in mg anhydrous extract of Quillaia extracts (E 999)/kg bw per day. Considering that the ADI is expressed in mg saponins, exposure was also converted to saponins, assuming that Quillaia extract (E 999) contains 70% saponins (Type 2) for the current authorised use levels and as proposed by the applicant, 20% saponins. Therefore, exposure using the current MPLs (*regulatory maximum level exposure assessment scenario*) ranged between < 0.1 mg saponins/kg bw per day at the mean and up to 6.7 mg saponins/kg bw per day at the 95th percentile. Using the refined exposure assessment scenario including the proposed extension of use, exposure ranged between < 0.1 mg saponins/kg bw per day at the mean and up to 0.7 mg saponins/kg bw per day at the 95th percentile.

To assess possible health risks related to the current use of Quillaia extract (E 999), the Panel selected the brand-loyal scenario, as the additive is used in FC 14.1.4 Flavoured drinks. None of the exposure estimates for the different population groups of the brand-loyal scenario exceeded the ADI of 3 mg saponins/kg bw per day. Also, the proposed extension of use as a food additive in flavourings did not result in an exceedance of this ADI at the refined exposure assessment scenario.

The applicant proposed the addition of sodium benzoate (E 211) to Annex III of Regulation (EC) No 1333/2008 as a preservative for use in liquid formulations of Quillaia extracts (E 999). The Panel noted that exposure to sodium benzoate (E 212) at the levels proposed to be used in the preparation of Quillaia extract (E 999) would add at the maximum 0.13% to the exposure to benzoic acid–benzoates (E 210–213) as food additives, considering their use according to Annex II to Regulation No 1333/2008 and additional exposure from food categories which may contain benzoic acid–benzoates due to carry-over, for which an exceedance of the ADI of 5 mg/kg bw per day, expressed as benzoic acid, was observed in the *non-brand-loyal scenario* for toddlers and children (EFSA ANS Panel, 2016).

The Panel recommended that the European Commission considers:

- Revising the EU specifications for Quillaia extracts (E 999) in order to differentiate extracts of Quillaia according to their saponins content (including a description of the principle of the method of analysis to quantify the content of saponins in line with the JECFA specifications), i.e. Type 1 and Type 2.
- Revising the EU specifications to include the percentage range for polyphenols (including tannins), protein, polysaccharides including fibre, reducing sugars, a maximum limit for calcium oxalate as well as microbiological specifications.
- Lowering the current limits for toxic elements (arsenic, lead and mercury) in the EU specifications for Quillaia extracts (E 999) in order to ensure that the food additive will not be a significant source of exposure to these toxic elements in food.
- Revising the maximum use levels for Quillaia extracts (E 999) established in Regulation (EC) No 1333/2008 to be expressed on saponin content.

Table of contents

1. Introduction

The present opinion document deals with the re-evaluation of Quillaia extract (E 999) when used as a food additive and the evaluation of the safety of its proposed extension of use as a food additive in flavourings.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background and Terms of Reference to the re-evaluation of the food additive Quillaia extract (E 999)

Background

Regulation (EC) No 1333/2008[1] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010.[2] This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[3] of 2001. The report "Food additives in Europe 2000[4]" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

Terms of Reference

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.1.2. Background and Terms of Reference to the evaluation of the safety of the extension of use of Quillaia extract (E 999)

Background

An application has been introduced for the authorisation of Quillaia extract (E 999) as a food additive in flavourings. This food additive (emulsifier, foaming agent) is intended to be added at different maximum levels [when added at the maximum proposed levels to flavourings, would give rise to maximum use levels] in the following food categories:

[1] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.
[2] Commission Regulation (EU) No 257/2010 of 25 march 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.
[3] COM(2001) 542 final.
[4] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, 560.

Food categories	Maximum use levels (mg/kg or mg/L)
5.2 Other confectionery including breath refreshening microsweets	18
5.3 Chewing gum	9
12.5 Soups and broths	10
14.1.5 Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusion and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	6
15.1 Potato-, cereal-, flour- or starch-based snacks	1

Quillaia extract (E 999) is currently an authorised food additive in the European Union under Annex II of Regulation (EC) No 1333/2008 for use in the food categories 14.1.4 Flavoured drinks and 14.2.3 Cider and perry, both at a maximum level of 200 mg/L.

The Scientific Committee for Food (SCF) assessed the information on the safety in use of Quillaia extract (E 999) as food additive (emulsifier, foaming agent) in soft drinks, and expressed its opinion on a report on Emulsifiers, Stabilizers, Thickeners and Gelling Agents dated from December 1978.[5] The same authority considered in detail the composition of that product, its pharmacological properties, the source of the material and the results of two long-term studies in the mouse and rat. For the spray-dried extract the Committee established an ADI of 5 mg/kg bw per day.

In addition, also the Joint FAO/WHO Expert Committee on Food Additives (JECFA) performed a safety evaluation of Quillaia extract in 2002[6] setting a 'temporary' ADI of 5 mg/kg bw per day, and in 2005[7] establishing a group ADI of 1 mg/kg bw per day for Type 1 and Type 2 extract expressed as quillaia saponins. The previously established ADI of 5 mg/kg bw per day for Type 1 extract was withdrawn.

Taking into account the outcome of the SCF and JECFA's assessments, the European Commission asks the European Food Safety Authority to consider the information submitted by the applicant and, in particular, to clarify its impact on the established ADI as well as to carry out a refined exposure assessment of Quillaia extract (E 999).

Terms of Reference

The European Commission asks the European Food Safety Authority to provide a scientific opinion on the safety of the proposed extension of use of Quillaia extract (E 999) as a food additive in 'flavourings' in the food categories specified in the dossier, in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

Interpretation of Terms of Reference

According to the applicant, as also mentioned in the JECFA specifications (JECFA, 2006c, 2014), sodium benzoate is used in liquid preparations of Quillaia extracts (E 999).

Currently, according to Annex III Part 2 'food additives other than carriers in food additives' of the Regulation (EC) No 1333/2008, sodium benzoate can only be used in colour preparations. The applicant proposed the addition of sodium benzoate (E 211) to Annex III of Regulation (EC) No 1333/2008 as a preservative for use in liquid formulations of Quillaia extracts (E 999) in line with the JECFA specifications (Documentation provided to EFSA n. 6).

Therefore, the Panel considered additional exposure to sodium benzoate (E 211) from the current authorised uses and the requested extension of use of Quillaia extracts (E 999).

1.2. Information on existing authorisations and evaluations

Quillaia extract (E 999) is authorised as a food additive in the EU in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and specifications have been defined in the Commission Regulation (EU) No 231/2012[8].

In the EU, Quillaia extract (E 999) was evaluated by the SCF in 1978 (SCF, 1978) who established an acceptable daily intake (ADI) of 0–5 mg spray-dried extract/kg body weight (bw) per day. In its

[5] http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_07.pdf
[6] http://www.inchem.org/documents/jecfa/jecmono/v48je03.htm
[7] http://whqlibdoc.who.int/trs/who_trs_934_eng.pdf
[8] Commission Regulation (EU) No 231/2012 of 9 march 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) no 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

opinion, the SCF specified that the toxicological evaluation considered for its opinion was carried out on a natural extract of Quillaia bark as specified in the British Pharmacopoeia 1973 (more information on the British Pharmacopoeia in 1973 available in Appendix A). The two major saponins present in Quillaia extract, as described in the SCF opinion, were quillaia sapogenin which has a triterpenoid structure and quillaic acid; these saponins were considered by the SCF to constitute about 10% of the extract. The ADI was based on two long-term studies in rats and mice [cited by the SCF as unpublished studies by Butterworth (1977a,b), later published as Phillips et al. (1979) and Drake et al. (1982) respectively].

Quillaia extract (E 999) was evaluated by JECFA in 1982, 1986, 2002, 2004 and 2006 (JECFA, 1982a, b, 1986, 2002a,b, 2004, 2005, 2006a,b). JECFA currently defines two types of Quillaia extract: Type 1 extract, INS No 999(i), obtained by means of aqueous extraction and characterised by a saponins content in the range of 20–26% and Type 2 extract, INS No 999(ii), prepared by ultrafiltration of Type 1 extract and with a saponins content of 65–90%. The latest evaluation by JECFA established a group ADI of 0–1 mg/kg bw per day for Type 1 and Type 2 extracts, expressed as quillaia saponins.

Quillaja saponaria Molina is included in the Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements (EFSA, online). The chemicals of interest for *Quillaja saponaria* Molina, as listed in the compendium, are triterpenoid saponins (quillaja saponins) and calcium oxalate (11%).

The Committee for Veterinary Medicinal Products (CVMP) of the European Medicines Agency (EMA) evaluated quillaia saponins as pharmacologically-active substances and concluded that there was no need to establish a maximum residue level (MRL) for these substances in food of animal origin (EMA, 1996).

Quillaja saponaria ext (CAS 68990-67-0) has been registered under the REACH Regulation 1907/2006[9] (ECHA, online).

The FSANZ established a group ADI of 0–1 mg quillaia saponins/kg bw to permit the use of Type 1 (unpurified) and Type 2 (saponin enriched) extracts (FSANZ, 2013).

2. Data and methodologies

2.1. Data

The Panel on Food Additives and Flavourings (FAF) was not provided with a newly submitted dossier for the re-evaluation of Quillaia extract (E 999) as a food additive. EFSA launched a public call for data[10] to collect information from interested parties.

An applicant has submitted a dossier in support of the application for the extension of use of Quillaia extract (E 999) as a food additive in flavourings which is also addressed in this opinion (see 'Documentation provided to EFSA n. 1').

Additional information was submitted by the applicant (jointly with other interested parties) to EFSA during the assessment process, in response to requests from EFSA sent on 10 March 2015, on 30 September 2015, on 3 June 2016, on 13 June 2018 and on 29 October 2018 (see 'Documentation provided to EFSA n. 6–10').

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to December 2018. Attempts were made to retrieve relevant original study reports on which previous evaluations or reviews were based, however these were not always available to the Panel.

Food consumption data used to estimate dietary exposure to Quillaia extract (E 999) were derived from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database[11]).

The Mintel's Global New Products Database (GNPD) was used to verify the use of Quillaia extract (E 999) in food and beverage products and food supplements within the EU's market. The Mintel's GNPD is an online database that contains the compulsory ingredient information present on the label of numerous products.

[9] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 396, 30.12.2006.

[10] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Published: 22 November 2009. Available from: http://www.efsa.europa.eu/en/dataclosed/call/ans091123

[11] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidance documents from the EFSA Scientific Committee.

The FAF Panel assessed the safety of Quillaia extract (E 999) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the SCF (2001) and taking into consideration the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) for studies in rodents or, in the case of other animal species, by JECFA (2000). In these cases, the daily intake was expressed as equivalent.

Dietary exposure to Quillaia extract (E 999) from its use as a food additive was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database with maximum permitted levels according to Annex II to Regulation (EC) No 1333/2008 and reported use levels submitted to EFSA following a call for data. The exposure was estimated according to different scenarios (see Section 3.4.1). Uncertainties in the exposure assessment were identified and discussed.

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

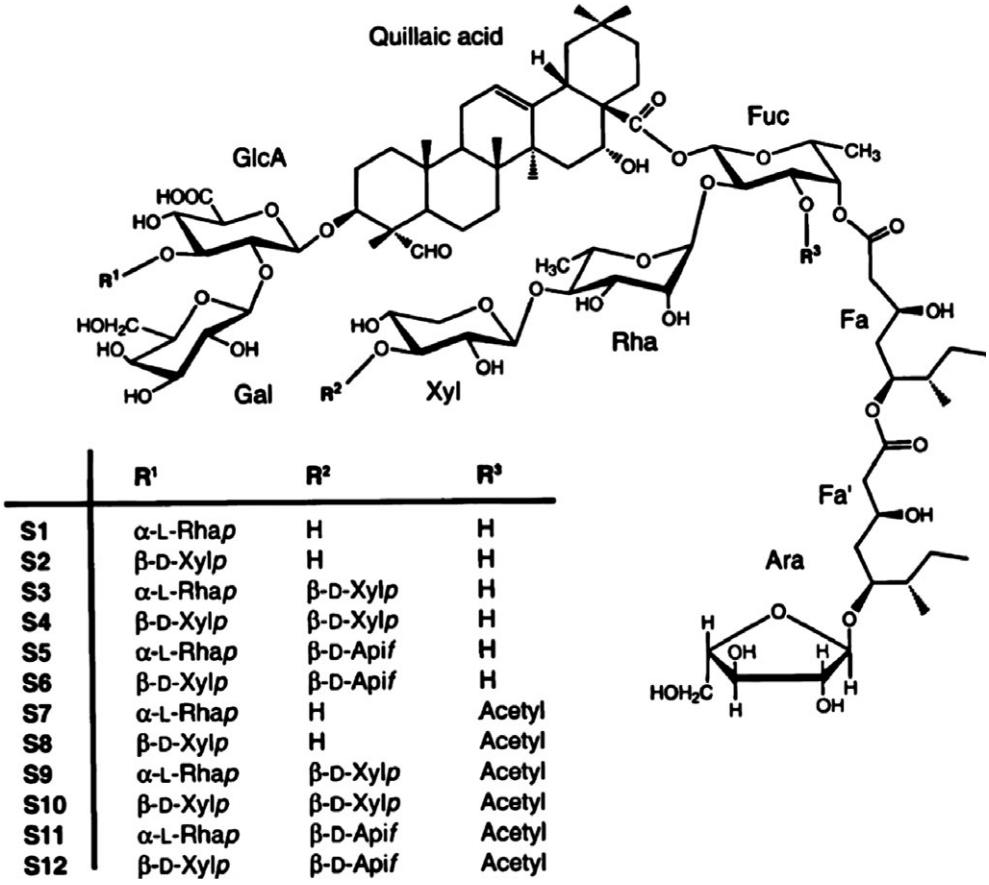
According to Commission Regulation (EU) No 231/2012, Quillaia extract (E 999) is obtained by aqueous extraction of *Quillaia saponaria* Molina, or other *Quillaia* species, trees of the family Rosaceae. It contains a number of triterpenoid saponins consisting of glycosides of quillaic acid. Sugars – including glucose, galactose, arabinose, xylose and rhamnose – are also present, along with tannin, calcium oxalate and other minor components. No chemical name, EC/EINECS Number, chemical formula or molecular weight are provided in the EU specifications.

Synonyms: Soapbark extract; Quillay bark extract; Panama bark extract; Quillai extract; Murillo bark extract; China bark extract.

Quillaja saponaria extract is registered with EC No 273-620-4 and CAS No 68990-67-0, the same CAS number cited for Quillaia extract Type 1 and Type 2 in the JECFA specifications (JECFA, 2006c, 2014).

The following trade names have been reported by the interested parties (Documentation provided to EFSA n. 6) for Quillaia extract (Type 1): Foamation™ Q200 (Ingredion), UPTAIA™ (Naturex), Saponin 5012/Saponin Powder HG (Kerry) and for Quillaia extract (Type 2): Q-Naturale™ (Ingredion); SAPNOV™ (Naturex).

The general structural formulae of quillaia saponins is shown in Figure 1.



	R¹	R²	R³
S1	α-L-Rha*p*	H	H
S2	β-D-Xyl*p*	H	H
S3	α-L-Rha*p*	β-D-Xyl*p*	H
S4	β-D-Xyl*p*	β-D-Xyl*p*	H
S5	α-L-Rha*p*	β-D-Api*f*	H
S6	β-D-Xyl*p*	β-D-Api*f*	H
S7	α-L-Rha*p*	H	Acetyl
S8	β-D-Xyl*p*	H	Acetyl
S9	α-L-Rha*p*	β-D-Xyl*p*	Acetyl
S10	β-D-Xyl*p*	β-D-Xyl*p*	Acetyl
S11	α-L-Rha*p*	β-D-Api*f*	Acetyl
S12	β-D-Xyl*p*	β-D-Api*f*	Acetyl

Gal: D-galactose; GlcA: D-glucoronic acid; Rha: D-rhamnose; Xyl: D-xylose; Ara: D-arabinose; Rhap: D-rhamnopyranose; Xylp: D-xylopyranose; Apif: apiofuranose.

Figure 1: General structure of quillaia saponins (quillaic acid as aglycone (sapogenin) component) (Güçlü-Ustündağ and Mazza, 2007)

3.1.2. Specifications

The specifications for Quillaia extract as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2006c, 2014) are listed in Table 1.

Table 1: Specifications for Quillaia extract (E 999) according to Commission Regulation (EU) No 231/2012 and JECFA (2006c, 2014)

	Commission Regulation (EU) No 231/2012	**JECFA (2006c) Type 1 [INS No 999(i)]**	**JECFA (2014) Type 2 [INS No 999(ii)]**
Definition	Quillaia extract is obtained by aqueous extraction of *Quillaia saponaria* Molina, or other *Quillaia* species, trees of the family Rosaceae. It contains a number of triterpenoid saponins consisting of glycosides of quillaic acid. Some sugars including glucose, galactose, arabinose, xylose, and rhamnose are also present, along with tannin, calcium oxalate and other minor components	Quillaia extract (Type 1) is obtained by aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaja saponaria* Molina (family Rosaceae). It contains triterpenoid saponins (quillaia saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are major components and some sugars and calcium oxalate will be present Quillaia extract (Type 1) is	Quillaia extract (Type 2) is obtained either by chromatographic separation or ultrafiltration of the aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaja saponaria* Molina (family Rosaceae). It contains triterpenoid saponins (quillaia saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are minor components. Some sugars and calcium

	Commission Regulation (EU) No 231/2012	JECFA (2006c) Type 1 [INS No 999(i)]	JECFA (2014) Type 2 [INS No 999(ii)]
		available commercially as liquid product or as spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol	oxalate will also be present Quillaia extract (Type 2) is available commercially as a liquid product or as a spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol
CAS Number		68990-67-0	
Formula weight		Monomeric saponins range from ca. 1,800 to ca. 2,300, consistent with a triterpene with 8–10 monosaccharide residues	
Assay			
Saponin content:		Not less than 20% and not more than 26% on the dried basis	Not less than 65% and not more than 90% on the dried basis
Description	Quillaia extract in the powder form is light brown with a pink tinge. It is also available as an aqueous solution	Red-brownish liquid or light brown powder with a pink tinge	Light red-brownish liquid or powder
Identification			
pH	Between 3.7 and 5.5 (4% solution)[(a)]	3.7–5.5 (4% solution)	
Solubility		Very soluble in water, insoluble in ethanol, acetone, methanol and butanol	
Foam		Dissolve 0.5 g of powder extract in 9.5 g of water or 1 mL of liquid extract in 9 ml of water. Add 1 mL of this mixture to 350 mL of water in a 1,000-mL graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam level (mL) after 30 min. Typical values are 150 mL of foam	Dissolve 0.5 g of the powder form in 9.5 mL of water or 1 mL of the liquid form in 9 ml of water. Add 1 mL of this solution to 350 mL of water in a 1,000-mL graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam volume (mL) after 30 min. Typical volumes are about 260 mL
Chromatography		The retention time of major peak of the sample corresponds to the major saponin peak (QS-18) of the standard	
Colour and turbidity		Powder form only: Dissolve 0.5 g in 9.5 g of water. The solution is not turbid Determine the absorbance of the solution against water at 520 nm. The absorbance is less than 1.2	Powder form only: Dissolve 0.5 g in 9.5 mL of water. The solution shall not be turbid. Determine the absorbance of the solution against water at 520 nm. The absorbance shall be less than 0.7
Purity			
Water	Not more than 6,0% (Karl Fischer method) (powder form only)	Powder form: not more than 6% (Karl Fischer Method)	
Loss on drying		Liquid form: 50 to 80% (2 g, 105°, 5 h)	Liquid form: 50 to 90% (2 g, 105°, 5 h)

	Commission Regulation (EU) No 231/2012	JECFA (2006c) Type 1 [INS No 999(i)]	JECFA (2014) Type 2 [INS No 999(ii)]
Ash		Not more than 14% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from loss on drying)	Not more than 5% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from Loss on drying)
Tannins		Not more than 8% on a dried basis	
Arsenic	Not more than 2 mg/kg		
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg	
Mercury	Not more than 1 mg/kg		

(a): According to recital 33) of Regulation (EC) 231/2012, the current specification relating to the pH range should be adjusted in order to bring it in line with JECFA.

The Panel noted that existing EU specifications do not describe any range for saponins content in the food additive. While the JECFA specifications differentiate two types of Quillaia extracts, Type 1 and Type 2, containing different percentages of saponins. The Panel also noted that the interested parties for the re-evaluation and the applicant for the extension of use as a food additive in flavourings have provided analytical data for liquid forms of Quillaia extract Type 1 and Type 2 (Documentation provided to EFSA n. 6, 8) and in addition to the parameters requested in the EU specifications, information on the percentage of saponins and tannins were determined, as well as percentage of ash (on a dried basis) that according to JECFA (2005) reflects the content of calcium oxalate. In addition, data on the percentage of protein and invert sugars were provided by the interested parties (Documentation provided to EFSA n. 6). The Panel considered that a similar differentiation of the Quillaia extracts should be presented in the EU specifications, e.g. as E 999(i) and E 999(ii), including the percentage range for saponins (including a description of the principle of the method of analysis to quantify the content of saponins in line with the JECFA specifications), polyphenols (including tannins), protein, polysaccharides including fibre, reducing sugars and a maximum limit for calcium oxalate.

The Panel noted that according to information from industry (Documentation provided to EFSA n. 6) the formula weight of 'monomeric saponins range from ca. 1,800 to ca. 2,800, consistent with a triterpene with 8–10 monosaccharide residues' while the formula weight in the JECFA specifications for Quillaia extract Type 1 and Type 2 is described as 'monomeric saponins range from ca. 1,800 to ca. 2,300'.

Information has been provided on the content of toxic elements in two different batches of Quillaia extract (Type 1) and of Quillaia extract (Type 2): lead (0.26–0.08 mg/kg), mercury (< 0.01 mg/kg) and arsenic (0.18–0.11 mg/kg) (Documentation provided to EFSA n. 6). The levels of lead, mercury, and arsenic analysed were all far below the levels as defined in the Commission Regulation (EU) No 231/2012, of 2, 2 and 1 mg/kg for arsenic, lead and mercury, respectively. Contamination at those levels could have a significant impact on exposure to these toxic elements, which are already close to the health-based guidance values or benchmark doses (lower confidence limits) established by EFSA (EFSA CONTAM Panel, 2009, 2010, 2012a,b, 2014).

The Panel noted that, according to the JECFA specifications, Quillaia extract Type 1 and Type 2 as liquid products are usually preserved with sodium benzoate. According to Annex III Part 2 'food additives other than carriers in food additives' of the Regulation (EC) No 1333/2008, sodium benzoate can only be used in colour preparations.

Because of the botanical origin, Quillaia extract can be subject to microbiological contamination. Interested parties have proposed microbiological specifications (Table 2). Additionally, according to the certificate of analysis, the presence of *Escherichia coli*, *Salmonella* and *S. aureus* is tested negative (Documentation provided to EFSA n. 6). The Panel agreed with the inclusion of microbiological parameters in the EU specifications for E 999 as proposed in Table 2, including also specifications for the absence of *E. coli*, *Salmonella* and *S. aureus*.

Table 2: Microbiological specifications for Quillaia extract (Types 1 and 2) as proposed by interested parties (Documentation provided to EFSA n. 6)

Parameter	Type 1 Limit (CFU/g)	Type 2 Limit (CFU/g)
Aerobic plate count	< 5,000	< 100
Yeast	< 100	< 10
Mould	< 100	< 10

One of the interested parties submitted a detailed report summarising soluble oxalates (oxalic acid) levels in five samples of Quillaia extract (Type 1) and three samples of Quillaia extract (Type 2). In all cases, the amount of oxalic acid in the liquid products was below the limit of quantification (LOQ) for the chromatographic method employed (i.e. 5 mg/L). In four out of the five samples of Quillaia extract (Type 1), the level was also below the limit if detection (LOD) (1 mg/L). The Panel considered that these levels are far below the typical level of oxalic acid identified in the EFSA compendium of botanicals (EFSA, online), i.e. 11% in the bark of the plant (110,000 mg/L).

In view of the botanical origin of Quillaia extract, limits for the presence of pesticides should be considered. According to analytical data provided by industry (Documentation provided to EFSA n. 6), no carbamates, organohalides and organophosphates pesticides were detected in the Quillaia extract (Types 1 and 2) batches tested. The Panel noted that MRLs for pesticides set under Regulation (EC) 396/2005 apply to *Quillaja saponaria* (Soapbark tree (bark)); *Quillaja saponaria* is listed in Part B of Annex I under the category of Herbal infusions from any other parts of the plant (product code 0639000). Thus, MRLs established for this commodity code equally apply to *Quillaja saponaria*. For processed products derived from Quillaia extract, the provisions of Article 20 are applicable, meaning that the changes in the levels of pesticide residues caused by processing need to be taken into account.

3.1.3. Manufacturing process

According to information provided by interested parties, the source material for the production of Quillaia extracts (Types 1 and 2) is the bark or wood of the tree *Quillaja saponaria* Molina (family Rosaceae), an evergreen tree native to China and South America (Documentation provided to EFSA n. 6).

Quillaia extract (Type 1) is obtained by the aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of the tree. This extraction process is followed by clarification, filtration, ultrafiltration, concentration and pasteurisation (Documentation provided to EFSA n. 6).

Quillaia extract (Type 2) is obtained by either chromatographic separation or ultrafiltration of the Type 1 extract, to remove most non-saponin solids, such as calcium oxalate, sugars, tannins and other polyphenols (Documentation provided to EFSA n. 6).

The Panel noted that, in recent literature, ultrasound-assisted extraction methods are described to improve the extraction yield of saponins from *Quillaja saponaria* Molina (Cares et al., 2010; Gaete-Garreton et al., 2011).

According to JECFA (2005), the traditional method for isolating Quillaia extracts for commercial use is through the milling of whole wood (including wood with bark and small branches) followed by water extraction at 70–80°C.

- To produce Quillaia extract (Type 1), the aqueous extract is stabilised by the addition of stabilising agents (e.g. PVP or egg albumin), followed by filtration with diatomaceous earth to remove compounds that tend to precipitate during storage (e.g. protein–polyphenol complexes). The commercial forms are spray-dried powders or concentrated liquids (normally 550 g/L solids) which may contain sodium benzoate (~ 0.5 g/L) or ethanol.
- To produce Quillaia extract (Type 2), Type 1 extract is further purified by chromatography or ultrafiltration to remove most non-saponin solids, such as calcium oxalate, sugars, tannins, and polyphenols. Quillaia extract (Type 2) may contain 65–90% saponins.

3.1.4. Methods of analysis in food

Based on information provided by interested parties, saponins in Quillaia extracts are analysed by reverse-phase high performance liquid chromatography (RP-HPLC) using the method described by San Martın and Briones (2000).

In the evaluation by FSANZ (2013), a method to determine the content of saponins in finished beverages using RP-HPLC is mentioned.

In literature, other methods to analyse saponins are described (Kensil and Marciano, 1991; Kensil et al., 1991; JECFA, 2005; Kite et al., 2007; Cheok et al., 2014).

3.1.5. Stability of the substance, and reaction and fate in food

Kartnig and Ri (1973) reported that the concentration of saponins from Quillaia bark in aqueous solutions may decrease by up to 8% during the first 14 days and by up to 9.3% by day 60 of storage. Levels then remain constant for at least the next four months.

In a study by Mitra and Dugan (2000), the authors studied the interaction between cholesterol and quillaia saponin, by measuring the effect of cholesterol on the surface and micellar properties of quillaia saponin solutions. Data show that, at well-defined critical micelle concentrations (cmc), cholesterol and saponin mixtures form micelles. The cmc for saponin solutions saturated with cholesterol was generally higher than that for saponin alone. The increase was dependent on the source of the saponin and on its composition. The addition of salt decreased the cmc values; the temperature dependence of these values was more complex. Surface adsorption studies showed that cholesterol preferentially adsorbs at the air/water interface, forming a closely packed monolayer. Saponin partially displaced the cholesterol at high saponin concentrations. Finally, the size, the intrinsic viscosity and the aggregation number of the cholesterol/saponin micelles are larger compared to those of saponin micelles alone; the radius of the micelles being between 20% and 40% larger at 25°C. According to the authors, the results indicated that cholesterol most likely solubilises within quillaia saponin micelles, and therefore, has a substantial impact on the micelle structure and the energetics of micelle formation.

3.2. Authorised uses and use levels

Maximum levels of Quillaia extract (E 999) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, Quillaia extract (E 999) is an authorised food additive in the EU at an MPL of 200 mg/L, calculated as anhydrous extract, in flavoured drinks, and in cider and perry (excluding *cidre bouché)* (Table 3).

Table 3: MPLs of Quillaia extract (E 999) in foods according to Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
14.1.4	Flavoured drinks		200[(a)]
14.2.3	Cider and perry	Excluding *cidre bouché*	200[(a)]

MPL: maximum permitted level.
(a): Calculated as anhydrous extract.

Quillaia extract (E 999) is not authorised according to Annex III to Regulation (EC) No 1333/2008.

3.2.1. Proposed extension of use as a food additive in flavourings

In addition to the re-evaluation of this food additive, the current opinion considers the proposed inclusion of Quillaia extract (E 999) (Type 1) in Annex III Part 4 'Food additives including carriers in food flavourings' of Regulation (EC) No 1333/2008. This extension of use is limited to the food categories and the corresponding proposed MPLs in the final food, as reported in Table 4.

Table 4: Proposed uses of Quillaia extract (E 999) as a food additive in flavourings according to information provided (Documentation provided to EFSA n. 10)

Food category number	Food category name	Proposed level in the final food[(a)] (mg/L or mg/kg as appropriate)
05.2	Other confectionery including breath refreshening microsweets	18
05.3	Chewing gum	9
12.5	Soups and broths	10
14.1.5	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusion and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	6
15.1	Potato-, cereal-, flour- or starch-based snacks	1

(a): As anhydrous extract.

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of Quillaia extract (E 999)

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call[12] for occurrence data (usage level and/or concentration data) on Quillaia extract (E 999). In response to this public call, updated information on the actual use levels of this additive in foods was made available to EFSA by industry. No analytical data were made available by the Member States.

Summarised data on reported use levels in foods provided by industry

FoodDrinkEurope (FDE) provided EFSA with data on use levels (n = 4) of Quillaia extract (E 999) in foods for the two food categories in which Quillaia extract (E 999) is authorised. The three use levels for FC 14.1.4. Flavoured drinks were for a niche product.

Appendix B provides data on the use levels of Quillaia extract (E 999) in foods as reported by FDE.

3.3.2. Summarised data extracted from the Mintel's Global New Products Database

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 2.9 million food and beverage products of which more than 1,100,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 20 out of its 28 member countries and Norway represented in the Mintel GNPD.[13]

For the purpose of this Scientific Opinion, the Mintel's GNPD[14] was used for checking the labelling of food and beverage products and food supplements for Quillaia extract (E 999) within the EU's food market as the database contains the compulsory ingredient information on the label.

According to the Mintel's GNPD, Quillaia extract (E 999) was labelled on 63 products belonging to six food subcategories: Carbonated Soft Drinks (n = 46), Beer (n = 9), Poultry Products (n = 3), Beverage Concentrates (n = 1), RTD (Iced) Tea (n = 1) and Coffee (n = 1). The products were published in this database between January 2013 and August 2018. The Panel noted that Quillaia extract (E 999) is labelled on a few products for which a new authorisation has been requested.

Appendix C lists the percentage of the food products labelled with Quillaia extract (E 999) out of the total number of food products per food subcategory for the six food subcategories. The percentages ranged from 0.1% or less in five food subcategories to 0.7% in Mintel's GNPD food subcategory

[12] https://www.efsa.europa.eu/en/data/call/160524
[13] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[14] http://www.gnpd.com/sinatra/home/ accessed on 18/10/2018.

'Carbonated Soft Drinks'. The average percentage of foods labelled to contain Quillaia extract (E 999) and belonging to food subcategories with at least one food labelled with the additive was 0.2%.

3.3.3. Food consumption data used for exposure assessment

EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a). Consumption surveys added in the Comprehensive database in 2015 were also taken into account in this assessment.[11]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups were used in the exposure assessment: infants, toddlers, children, adolescents, adults and the elderly. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 5).

Table 5: Population groups considered for the exposure estimates of Quillaia extract (E 999)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers[(a)]	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children[(b)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly[(b)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Netherlands, Sweden, UK

(a): The term 'toddlers' in the EFSA Comprehensive Database corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.
(b): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the Food Classification System (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories.

Food categories considered for the exposure assessment of Quillaia extract (E 999)

The food categories in which the use of Quillaia extract (E 999) is currently authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

For FC 14.2.3 Cider and perry, the exception 'excluding *cidre bouché*' could not be taken into account, and therefore the whole food category was considered in the different exposure scenarios in the exposure assessment (Appendix D).

The Panel noted that three of the four use levels referred to niche products (referring to FC 14.1.4 flavoured drinks). These levels are only considered in the refined exposure assessment if no other use levels are available for the food categories to which the niche products belong. As this was the case, these use levels were included in the refined assessment.

Furthermore, the Panel also considered the five food categories for which an extension of use as a food additive in flavourings was sought at the level of the proposed MPL in two additional exposure scenarios (Table 4).

3.4. Exposure estimates

3.4.1. Exposure to Quillaia extract (E 999) from its use as a food additive

The Panel estimated chronic exposure to Quillaia extract (E 999) for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure to Quillaia extract (E 999) was calculated by multiplying Quillaia extract (E 999) concentrations for each food category (Appendicies D and E) with their respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only one day per subject were excluded as they are considered as not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 5). Based on these distributions, the mean and 95th percentiles of exposure were calculated per survey and per population group. High percentile exposure was only calculated for those population groups where the sample size was sufficiently large to allow calculation of the 95th percentile of exposure (EFSA, 2011a). Therefore, in the present assessment, high levels of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not estimated.

Exposure assessment to Quillaia extract (E 999) was carried out by the FAF Panel based on four different sets of concentration data: (1) MPLs as set down in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*), (2) reported use levels (defined as the *refined exposure assessment scenario*), (3) regulatory maximum level exposure assessment together with the proposed maximum levels in the final food for the extension of use and (4) *brand-loyal refined estimated exposure scenario* together with the proposed maximum levels in the final food for the extension of use. These scenarios are discussed in detail below.

Regulatory maximum level exposure assessment scenarios for exposure to Quillaia extract (E 999) using the current MPL

The *regulatory maximum level exposure assessment scenario* of Quillaia extract (E 999) was based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008 and listed in Table 3.

The Panel considers the exposure estimates derived following this scenario as the most conservative since it is assumed that the consumer will be continuously (over a lifetime) exposed to Quillaia extract (E 999) present in food at MPL.

Refined exposure assessment scenario for exposure to Quillaia extract (E 999) using the reported use levels

The refined exposure assessment scenario is based on use levels reported by food industry. This exposure scenario can consider only food categories for which these data were available to the Panel.

Appendix D summarises the concentration levels of Quillaia extract (E 999) used in the refined exposure assessment scenario. Based on the available data set, the Panel calculated two refined exposure estimates based on two model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to Quillaia extract (E 999) present at the maximum reported use level for one food category. This exposure estimate was calculated as follows:

  - Combining food consumption with the maximum reported use level for the main contributing food category at the individual level.
  - Using the mean of the typical reported use level for the other food category.
- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to Quillaia extract (E 999) present at the mean reported use levels in food. This exposure estimate is calculated using the mean of the reported use levels for the two food categories.

Exposure scenarios including proposed extension of use of Quillaia extract (E 999) as a food additive in flavourings

To estimate the potential exposure to Quillaia extract (E 999) due to its proposed extension of use as a food additive in flavourings, two exposure scenarios were calculated. First, the *regulatory maximum level exposure assessment scenario* of Quillaia extract (E 999) was calculated as described above, including also the proposed maximum levels in the final food for the extension of use submitted by the applicant and listed in Table 4.

Second, a *refined estimated exposure scenario* was calculated based on maximum use levels reported by food industry and the proposed maximum levels in the final food for the extension of use submitted by the applicant.

Appendix E summarises the concentration levels of Quillaia extract (E 999) used in both scenarios.

Dietary exposure to Quillaia extract (E 999) from the current authorised uses

Table 6 summarises the estimated exposure to Quillaia extract (E 999) from its use as a food additive in six population groups (Table 5) according to the different exposure scenarios. Detailed results per population group and survey are presented in Appendix F.

Table 6: Summary of estimated exposure to Quillaia extract (E 999) from its use as a food additive in the regulatory maximum level exposure assessment scenario and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg Quillaia extracts (E 999) expressed as anhydrous extract/kg bw per day)

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario						
• Mean	0–0.5	0–2.7	0.2–3.0	0.3–2.3	0.1–1.0	< 0.1–0.3
• 95th percentile	0–3.2	0–9.6	1.0–7.6	1.2–5.3	0.5–3.4	0.2–1.2
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
• Mean	0–0.1	0–0.3	< 0.1–0.3	< 0.1–0.2	< 0.1–0.1	< 0.1–< 0.1
• 95th percentile	0–0.3	0–1.0	0.1–0.8	0.1–0.5	0.1–0.4	< 0.1–0.2
Non-brand-loyal scenario						
• Mean	0–< 0.1	0–0.2	< 0.1–0.3	< 0.1–0.2	< 0.1–0.1	< 0.1–< 0.1
• 95th percentile	0–0.3	0–0.8	0.1–0.6	0.1–0.5	< 0.1–0.3	< 0.1–0.1

bw: body weight.

In the *regulatory maximum level exposure assessment scenario*, mean exposure to Quillaia extract (E 999) from its use as a food additive ranged from 0 mg/kg bw per day in infants and toddlers to 3.0 mg/kg bw per day in children. The 95th percentile of exposure to Quillaia extract (E 999) ranged from 0 mg/kg bw per day in infants and toddlers to 9.6 mg/kg bw per day in toddlers.

In the *brand-loyal refined estimated exposure scenario*, mean exposure to Quillaia extract (E 999) from its use as a food additive ranged from 0 mg/kg bw per day in infants and toddlers to 0.3 mg/kg bw per day in toddlers and children. The high exposure to Quillaia extract (E 999) ranged from 0 mg/kg bw per day in infants and toddlers to 1.0 mg/kg bw per day in toddlers. In the *non-brand-loyal scenario*, mean exposure to Quillaia extract (E 999) from its use as a food additive ranged from 0 mg/kg bw per day in infants and toddlers to 0.3 mg/kg bw per day in children. The 95th percentile of exposure to Quillaia extract (E 999) ranged from 0 mg/kg bw per day in infants and toddlers to 0.8 mg/kg bw per day in toddlers.

The main contributing food category to the total mean exposure estimates for all population groups was flavoured drinks in all exposure scenarios (Appendix G).

Dietary exposure to Quillaia extract (E 999) including its proposed extension of use as a food additive in flavourings

Table 7 summarises the anticipated exposure to Quillaia extract (E 999) from its use as a food additive in six population groups (Table 5), including its proposed extension of use. Detailed results per population group and survey are presented in Appendix H. In these scenarios, the maximum levels proposed for the extension of use (Table 4) were taken into account together with the current MPLs (Table 3) or maximum use levels reported by industry (Appendix E).

Table 7: Summary of anticipated exposure to Quillaia extract (E 999) from its use as a food additive and including its proposed extension of use as a food additive in flavourings, in the regulatory maximum level exposure assessment scenario and in the refined exposure scenario, in six population groups (minimum–maximum across the dietary surveys in mg Quillaia extracts (E 999) expressed as anhydrous extract/kg bw per day)

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario + proposed extension of use						
• Mean	< 0.1–0.5	< 0.1–2.8	0.2–3.1	0.3–2.3	0.1–1.0	< 0.1–0.4
• High level (95th percentile)	0–3.2	< 0.1–9.6	1.1–7.6	1.2–5.4	0.5–3.4	0.2–1.2
Refined exposure assessment scenario + proposed extension of use						
• Mean	< 0.1–0.1	< 0.1–0.3	< 0.1–0.3	< 0.1–0.3	< 0.1–0.2	< 0.1–0.1
• High level (95th percentile)	0–0.3	< 0.1–1.0	0.1–0.8	0.1–0.6	0.1–0.4	< 0.1–0.3

bw: body weight.

In both scenarios, exposure estimates were almost identical to those considering only the current food categories in which Quillaia extracts (E 999) is authorised. Also the main food category (FC 14.1.4 Flavoured drinks) contributing to the exposure remained the same for most age groups, except for infants and the elderly in the *refined exposure assessment scenario*. In this exposure scenario, FC 12.5 Soups and broths contributed most to the exposure in infants and FC 14.1.5 Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products contributed most to the exposure in the elderly (Appendix I).

Uncertainty analysis

Uncertainties in the exposure assessment of Quillaia extract (E 999) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 8.

Table 8: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[a]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Methodology used to estimate high percentiles (95th) long-term (chronic) exposure based on data from food consumption surveys covering only a few days	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which Types of food the levels refer to	+/–
Uncertainty in possible national differences in use levels of food categories	+/–
Concentration data: use levels considered applicable to all foods within the entire food category, whereas on average 0.2% of the foods, belonging to a food category with foods labelled with additive, was labelled with the additive	+

Sources of uncertainties	Direction[(a)]
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception (1 FC for all scenarios)	+
Regulatory maximum level exposure assessment scenario (including also the extension of use): • exposure calculations based on the MPL according to Annex II to Regulation (EC) No 1333/2008 (and on the proposed maximum levels in the final food for its extension of use)	+
Refined exposure assessment scenarios (including also the extension of use): • exposure calculations based on the proposed maximum levels in the final food for extension of use • exposure calculations based on the maximum levels reported use from industries	+ +/–

FC: food category; MPL: maximum permitted level.
(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Quillaia extract (E 999) is currently authorised in two food categories. As use levels were reported by industries for both categories, all current authorisations were taken into account in the assessment (without taking into account the restriction for one of the two food categories (Section 3.3.3)).

The Panel noted that information from the Mintel GNPD (Appendix C) indicated that no cider or perry were labelled with Quillaia extract (E 999). Apart from flavoured drinks, in which the food additive is authorised, only a few other products that are not authorised to contain Quillaia extract (E 999) were labelled with the food additive (beer, poultry products, coffee and tea).

Overall, the Panel considered that the uncertainties summarised in Table 8 resulted in an overestimation of the exposure to Quillaia extract (E 999) from its use as a food additive according to Annex II in both the MPL and refined exposure scenario. This was also true for the two exposure scenarios considering the requested extension of use of Quillaia extract (E 999) as a food additive in flavourings in five food categories according to Annex III.

3.4.2. Exposure to Quillaia extract from other sources

The Panel noted that Quillaia extract is used in few preparations as a drug available in some EU Member States for use as an expectorant (Martindale, online) and this may be an additional source of exposure.

3.4.3. Exposure to sodium benzoate from Quillaia extract (E 999)

According to the applicant, as also mentioned in the JECFA specifications (JECFA, 2006c, 2014), sodium benzoate is used in liquid preparations of Quillaia extracts (E 999).

Currently, according to Annex III Part 2 'food additives other than carriers in food additives' of the Regulation (EC) No 1333/2008, sodium benzoate can only be used in colour preparations. The applicant proposed the addition of sodium benzoate (E 211) to Annex III of Regulation (EC) No 1333/2008 as a preservative for use in liquid formulations of Quillaia extracts (E 999) in line with the JECFA specifications (Documentation provided to EFSA n. 6).

The maximum level of sodium benzoate used in Quillaia extracts preparation was reported to be 1,000 mg/kg of the final preparation according to the applicant (Documentation provided to EFSA n. 6).

Based on the above information, the Panel estimated a high level of exposure to sodium benzoate for the *regulatory maximum level exposure assessment scenario* (for the current MPLs) and the *refined exposure assessment scenario* (using maximum reported use levels for the current uses and proposed maximum levels in the final food for the extension of uses). High level estimates (95th percentile) of sodium benzoate from the use of a preparation of Quillaia extracts (E 999) – containing sodium benzoate – were maximally 9.6 and 1.0 μg/kg bw per day, respectively for the two scenarios.

Exposure to benzoic acid–benzoates (E 210–213) from their authorised use according to Annex II (Regulation No 1333/2008) and via food categories which may contain benzoic acid–benzoates due to carry-over (for which data were available), was estimated to range between 0.8 mg/kg bw per day at the mean for adolescents and the elderly and 6.9 mg/kg bw per day at the 95th percentile for toddlers (EFSA ANS Panel, 2016). Based on these estimates and comparing the highest exposure estimate for benzoates (EFSA ANS Panel, 2016) to the lowest coming from Quillaia extract (current opinion) and

the lowest exposure estimate for benzoates (EFSA ANS Panel, 2016) to the highest coming from Quillaia extract (current opinion), the Panel calculated that the additional exposure to sodium benzoate (E 212) coming from the use of Quillaia extracts (E 999) would be in the range 0.01% to 0.13%.

3.5. Biological and toxicological data

The Panel assumed that the test materials used in toxicological studies described as Quillaia extract approximated to Quillaia extract Type 1 as described in JECFA specifications (2006c).

3.5.1. Absorption, distribution, metabolism and excretion

Fieger (1918) reported that quillaia saponins were not or only to a limited degree detected in urine from dogs (two animals) after administration.

Bäck (1918) reported that quillaia saponins were excreted in the faeces after oral administration to hens and dogs. Their presence was detected through their haemolytic action on erythrocytes isolated from a variety of species.

Ewart (1931) reported that saponins (not further specified) may be hydrolysed into sugars and sapogenins by diastase, animal trypsin and pancreatic juice. The author also reported that the saponins were resistant to dialysis, but that their absorption through the alimentary canal may be favoured by irritation or inflammation.

Hostettmann and Marston (1995) stated that triterpene saponins in general are poorly absorbed and either excreted unchanged or metabolised in the gut in animals (species not specified) after oral administration. Information on the fate of saponins in the gut (species not specified) is generally lacking but any breakdown of saponins is most likely caused by gut microorganisms, intestinal enzymes or gastric juice.

The Panel considered that the extent and the quality of the available data on the absorption, distribution, metabolism, excretion (ADME) of Quillaia extract saponins were very limited and that no conclusions could be drawn based on studies with Quillaia extracts.

Due to the limited data and quality of studies regarding the ADME of Quillaia saponins, the Panel therefore considered the available data on gastrointestinal (GI) metabolism and ADME for structurally similar saponins (Appendix J). These data are described in Appendices K and L, respectively. Based on read-across from these data, the Panel assumed that Quillaia extract saponins share a similar fate. Thus, the Panel considered it likely that intact Quillaia extract saponins are absorbed to a low extent; are hydrolysed in the GI tract and that the aglycon (sapogenin) is absorbed only to a limited extent.

3.5.2. Acute toxicity

JECFA (2006a) reported a study examining the acute oral toxicity of Quillaia extracts in rats. Sprague–Dawley rats (5 animals/sex per group) were given single oral doses of either Quillaia extract Type 1 (8.8% saponin content of the standardised test material corresponding to 20% saponin content on dry matter basis) or Type 2 (14% saponin content of the standardised test material corresponding to 72% saponin content on dry matter basis) ranging from 3,000 to 20,000 mg/kg bw and were observed for clinical signs of toxicity for the following 14 days. On the basis of the saponin content, JECFA reported LD_{50} values for the two extracts of approximately 900 mg saponin/kg bw.

In a subacute study over 21 days, C57BL/6 mice (10 animals/group, sex not specified) were administered Quillaia extract (from Garuda International, Inc and described as non-refined Quillaia extract containing 100% water-soluble Quillaia solids) in water by oral gavage at doses of 1, 5 and 10 mg per animal (Kirk et al., 2004). One group was given 10 mg Quillaia extract on days 0 and 7, while two other groups received 1 or 5 mg Quillaia extract per animal on days 0, 7 and 14. Mice in the 10 mg group had a hunched back, scuffed fur and lethargy and 40% died or were euthanised within the first week. One further animal died at day 12. Following the second dose on day 7, 40% of the mice died or were euthanised in the 5 mg group. One further animal died after the third dose in the 5 mg group. All mice in the 1 mg group survived the three-week trial without any gross signs of toxicity.

The Panel noted that from the limited data available, the acute toxicity for Quillaia extract was low.

3.5.3. Short-term and subchronic toxicity

Quillaia extract (source not further specified) was administered to young FDRL albino rats (5 animals/sex/group) in the diet at 0.05% Quillaia extract (equivalent to 45 mg/kg bw per day) for 12 weeks (Oser,

1966). Blood erythrocyte counts, erythrocyte fragility tests and haemoglobin determinations were performed at weeks 4, 8 and 12. Total and differential leucocyte counts, blood glucose and non-protein nitrogen were determined at week 12. At termination, the weights of the liver, kidneys, spleen, heart and adrenals were determined. Histological examinations were made of the liver, kidneys, adrenals, gonads, lymph nodes and bone marrow. Fifteen additional organs or tissues were examined microscopically in three rats of each sex. Growth response, behaviour and appearance of the animals were monitored. The authors reported that no adverse effects associated with the addition of Quillaia extract to the diet were observed.

Gaunt et al. (1974) treated weanling CFE strain rats (15 animals/sex per group) in the diet for 13 weeks with 0%, 0.6%, 2% or 4% Quillaia extract (spray-dried aqueous extract of Quillaia bark, where 15 parts of dried extract was equivalent to 100 parts of bark by weight) (description of the test material see Appendix M). In addition, groups of 5 male and 5 female rats were fed on diets containing 0%, 2% or 4% Quillaia extract for 2 or 6 weeks. The mean daily intakes of Quillaia extract were reported as 360, 1,180 and 2,470 mg/kg bw for males, and 440, 1,370 and 3,030 mg/kg bw for females for the dietary dose levels of 0.6%, 2% and 4%, respectively. A large series of organs were prepared for microscopic examination. No abnormalities of behaviour or condition were seen and there was no evidence for diarrhoea or other signs of GI irritation during the study. Food consumption was reduced in both sexes at all dose levels and the mean intakes over the whole experimental period were statistically significantly less than controls in mid- and high-dose females ($p < 0.01$). The body weights of the high-dose group were only significantly lower than those of the control up to day 78 in males and for the first 2 weeks in females. There were no differences in any haematological or urinary parameters between the Quillaia extract groups and the control groups. At autopsy, statistically significant organ weight changes included decreased relative liver weight (males at 2 and 4%, $p < 0.001$); decreased relative kidney weight (males at 2% and females at 4%, $p < 0.05$); increased relative stomach weight (males at 2.0% and 4.0% and females at 4%, $p < 0.01$); increased relative caecum weight (females at 4%, $p < 0.05$), increased relative brain weight (males at 4%, $p < 0.05$), and increased relative thyroid weight (males at 0.6% and females at 2%, $p < 0.05$). No histopathological changes attributable to Quillaia extract treatment were reported. The authors concluded that 'the no-untoward-effect level for Quillaia extract in this study was 0.6% Quillaia extract in the diet, equivalent to an intake of approximately 400 mg/kg bw per day'.

Jcl:Wistar rats (12 animals/sex per group) were given 1,200 mg/kg bw per day Quillaia extract (a 12% solution, equivalent to 100 mg sapogenin/kg bw per day) as positive control in deionised water by oral gavage for 90 days (Kawaguchi et al., 1994). The Panel noted that administration by gavage for 90 days of a bolus dose of the extract resulted in local toxicity as evidenced by inflammatory changes of the larynx, trachea and the forestomach, debility and death or killing for humane reason of 6 males and 2 females.

The Panel noted a no observed adverse effect level (NOAEL) of 0.6% Quillaia extract (approximately 400 mg/kg bw per day) from a 13-week study in rats in which relative organ weights were changed at the next higher dose of 1,200 mg/kg bw per day (Gaunt et al., 1974). The Panel also noted that no histopathological changes were observed at any dose.

3.5.4. Genotoxicity

Further to a request for additional information, the applicant and the interested parties for the food additive Quillaia extract (E 999) submitted results from new genotoxicity studies, conducted in accordance with the requirements of the EFSA Guidance on genotoxicity testing (EFSA Scientific Committee, 2011). According to the applicant, a Quillaia Extract Type 1 was selected based on the assumption that the material chosen would represent a worst case exposure scenario to both saponins and impurities (for more information on characterisation of the test material see Appendix N).

In vitro

A bacterial reverse mutation assay was conducted with a lot of Quillaia extract Type 1 (Appendix N) in accordance with OECD TG 471 and in compliance with good laboratory practice (GLP) (Documentation provided to EFSA n. 8.).

The Quillaia extract was tested *in vitro* for its ability to induce mutations in four strains of *Salmonella* Typhimurium (TA1535, TA1537, TA98 and TA100) and in one of *E. coli* (strain WP2 *uvrA*). A range-finder experiment was performed to determine the bacteriotoxic range of the test item under plate incorporation conditions in TA98 and WP2 *uvrA*, in the presence and absence of S9 mix (derived from livers of Aroclor 1254 treated rats). Mutation tests were then performed, using the plate

incorporation method and pre-incubation method, both in presence and absence of S9 mix. Bacteria were exposed to the test item dissolved in sterile water; the latter was also the negative control. Concentrations of Quillaia extract were corrected for saponin content and dosed up to 5 μL saponins/plate in a first experiment under plate incorporation conditions and in a second experiment under pre-incubation conditions, in all strains in the presence and absence of the S9 mix. All negative and positive controls fulfilled the acceptance criteria, demonstrating the sensitivity of the assay and metabolic activity of the S9 preparations. No precipitations or signs of toxicity were observed in any experiment. There were no dose-related or statistically significant increases in revertant numbers observed in any strain at any concentration of Quillaia extract compared with vehicle controls, in the presence or absence of S9 mix, under plate incorporation or pre-incubation conditions. Values at all concentrations of Quillaia extract (with all strains) were also within historical negative control ranges. Thus, Quillaia extract showed no mutagenic potential in the bacterial reverse mutation assay at concentrations up to 21.55 μL Quillaia extract/plate (5 μL saponins/plate) in the absence or presence of metabolic activation.

The same test material was used also in an *in vitro* mammalian cell micronucleus assay conducted in accordance with OECD TG 487 and in compliance with GLP (Documentation provided to EFSA n. 8). This test was carried out on TK6 human lymphoblast derived cells. A range-finder experiment was performed to determine the cytotoxic range of the test item for each treatment condition. In the main experiment, TK6 cells were treated with preparations of Quillaia extract, negative control (sterile water) or positive controls in both presence and absence of metabolic activation system (S9 mix, derived from livers of Aroclor 1254-treated rats). The treatment period was 3 h in the presence and absence of S9 mix. Harvesting was 44 h after initiation of treatment; continuous treatment (27 h) was not performed due to the positive result seen after the 3 h treatment in the presence of S9 mix. Treated cultures in the absence of S9 mix were selected for analysis based on the cytotoxicity limit (concentration that resulted in approximately 55% ± 5% cytotoxicity indicated by a reduction in relative increase in cell count (RICC) to 45% ± 5% compared with the negative controls). In the presence of S9 mix, cultures were selected for analysis based on the highest concentration which had an acceptable RICC. Concentrations of Quillaia extract were corrected for saponin content and those selected for analysis were 0.002, 0.01 and 0.012 μL saponins/mL in the presence of S9 mix and 0.002, 0.01, 0.012 and 0.014 μL saponins/mL in the absence of S9 mix. All negative and positive controls fulfilled the acceptance criteria, demonstrating the sensitivity of the assay and metabolic activity of the S9 preparations. The highest concentration analysed in the presence of S9 mix (0.012 μL saponins/mL) had a RICC of 72%. In the absence of S9 mix, the highest concentration analysed (0.014 μL saponins/mL) had a RICC of 36%. In the presence of S9 mix, there was a statistically significant increase in % micronucleated cells at 0.012 μL saponins/mL, compared to the negative control and the mean value was outside the historical control range. There was also a linear trend which was significant at 0.1%, indicating a dose response. There were no other statistically significant increases in micronucleated cells at any other concentration of Quillaia extract in the presence of S9 mix. There were statistically significant increases in % micronucleated cells at all concentrations in the absence of S9 mix. There was also a linear trend which was significant at 0.1%, indicating a dose response. However, as the mean values at all concentrations were within the historical negative control ranges, the results were considered to be equivocal. The authors of the study report concluded that the Quillaia extract tested in this study was either clastogenic or aneugenic when metabolically activated in the presence of S9 mix, while they considered the results equivocal in the absence of S9 mix. The Panel agreed with the conclusions from the authors.

In vivo

In accordance with the applicable SC Genotoxicity guidance (EFSA Scientific Committee, 2011) the positive and equivocal findings observed in the *in vitro* mammalian cell micronucleus assay were followed up with an *in vivo* mammalian erythrocyte micronucleus test performed in accordance with the OECD TG 474 and in compliance with GLP (Documentation provided to EFSA n. 8). The test was performed on rats (strain Crl:WI(Han)).

A range-finding experiment was performed to establish the highest dose of Quillaia extract that did not produce mortality or severe signs of clinical toxicity up to the maximum tolerated dose level (MTD). Groups of three males and three females were dosed by oral gavage at 500, 1,250 or 2,000 mg saponins/kg bw per day saponins (2,155, 5,387.5 or 8,620 mg Quillaia extract/kg bw per day) twice (approximately 24 h apart) at a dose volume of 10 mL/kg. The MTD was at least 2,000 mg saponins/kg bw per day in males and 1,250 mg saponins/kg bw per day in females. As there was no substantial (≥ 3-fold) difference in the MTD between males and females and only limited toxicity was observed in

the males, the main study was conducted in males only as a limit test. Blood samples were collected at 1, 2 and 4 h after the second dose for proof of target tissue (bone marrow) exposure.

For the main experiment, groups of six males were dosed by oral gavage with water (negative controls) or Quillaia extract at the maximum dose of 2,000 mg saponins/kg bw per day (8,620 mg Quillaia extract/kg bw per day) on two consecutive days, approximately 24 h apart. A positive control group, also of six males, was given a single oral gavage dose of cyclophosphamide (15 mg/kg bw per day). Two satellite groups with three males per group were dosed for checking target tissue exposure. Blood samples for micronucleus evaluation were taken approximately 48 h after the final dose. A minimum of 4,000 and a maximum of approximately 20,000 reticulocytes were scored by flow cytometry for the presence of micronuclei for each animal and the frequency of micronucleated reticulocytes was statistically analysed. Blood samples were also collected from animals of the satellite groups 4 h after the second dose, to demonstrate bone marrow exposure in peripheral blood.

To check target tissue exposure, plasma samples from animals from the dose-range finding study and from a satellite group of the main study were analysed for quillaic acid. This compound was chosen as a representative of all the saponins constituting Quillaia extract, due to its high concentration with respect to the other constituents in the test items. However, with the exception of one case, the concentration of quillaic acid in the dose-range finding study was below the limit of quantification. Therefore, only qualitative analysis was performed in the main study. According to the authors of the study report, after testing samples of plasma spiked with 1% of Quillaia extract and samples of blank plasma with and without internal standard, it was possible to confirm the presence of quillaic acid by detecting a peak eluting at 1.35 min in the test items.

The negative and positive control groups fulfilled the acceptance criteria, demonstrating the sensitivity of the assay. The mean frequency of micronucleated reticulocytes for animals treated with Quillaia extract was similar to that of the negative control group. The percentage of reticulocytes (% RET) for Quillaia extract treated animals was statistically significantly reduced (1.53 vs 3.87% in negative control animals, i.e. 60% reduction). While the Panel considered the plasma analysis data insufficient to demonstrate target tissue exposure, it considered that the reduction in the % RET confirmed test item exposure and toxicity to the target tissue (bone marrow).

Thus, there was no evidence of clastogenicity or aneugenicity following oral gavage administration of Quillaia extract up to the maximum dose level of 2,000 mg saponins/kg bw per day (8,620 mg Quillaia extract/kg bw per day) in male rats.

The Panel noted that the *in vivo* micronucleus assay was performed with a lot of Quillaia extract for which the shelf life of 18 months was already exceeded. While this may limit the reliability of the study to a certain extent, there is no indication that would justify assuming that the use of this lot resulted in a false-negative outcome of the study.

Accordingly, the Panel considered that the negative result of the *in vivo* micronucleus assay rules out the concern for structural and numerical chromosomal aberrations that resulted from the positive *in vitro* micronucleus test.

The Panel noted that this food additive is a mixture that is chemically only poorly characterised but that from the description of its chemical composition and the analytical data provided, there is no indication for the presence of genotoxic components in this mixture. Therefore, overall, the Panel concluded that the data available do not indicate a concern for genotoxicity.

3.5.5. Chronic toxicity and carcinogenicity

Mouse

In an 84-week study, TO mice (48 animals/sex per group) were fed a diet containing 0, 0.1, 0.5 or 1.5% Quillaia extract (description of the test material see Appendix J), equivalent to 0, 150, 750 and 2,250 mg/kg bw per day, respectively (Phillips et al., 1979). The condition and behaviour of the animals were frequently monitored and 16 males were weighed at weeks 0, 1, 4, 10, 14, 28, 40, 57 and 84. Blood samples were taken from 10 male and 10 female mice from the control group and the groups receiving 0.5% and 1.5% Quillaia extract at weeks 26, 54 and 84 for the determination of reticulocyte, erythrocyte and leucocyte counts, packed cell volume and haemoglobin concentration. At autopsy, any macroscopic abnormalities were recorded and the brain, heart, liver, kidneys, spleen, stomach, small intestine, caecum and testes were weighed. The weighed organs in addition to the salivary gland, thyroid, adrenal glands, lymph nodes, aorta, pancreas, pituitary, prostate, seminal vesicles, ovaries, uterus, urinary bladder, lungs, colon, rectum, spinal cord, skeletal muscle, eye, Harderian gland and other tissues that appeared abnormal were prepared for microscopic examination.

Histopathological examinations were performed on 45, 42, 41 and 43 males and on 44, 43, 43 and 46 females from the control, 0.1%, 0.5% and 1.5% groups, respectively. Survival rates and body weights were similar in treated and control animals and there were no adverse effects on condition or behaviour of the animals. There was a slightly lower body weight gain in high-dose males compared to controls (reported to be statistically significant only by the end of the study although a p-value was not given). The only statistically significant haematological change observed was in red blood cell parameters in that there was a decrease in erythrocyte counts at week 26 in high-dose females ($p < 0.01$) and in mid- and high-dose males ($p < 0.05$ and $p < 0.01$, respectively), and at week 84 in mid-dose males ($p < 0.01$). There was also an increased packed cell volume at week 84 in high-dose males ($p < 0.05$) and a decreased volume at week 84 in mid-dose females ($p < 0.05$). The relative brain and stomach weights were statistically significantly increased in high-dose males ($p < 0.05$); isolated significant changes in the weights of kidneys in males and in the small intestine in females were observed in the 1.5% Quillaia extract dose group. The incidences of both non-neoplastic and of neoplastic histopathological findings were similar in treated and control animals. The authors reported a 'no-untoward-effect level' for Quillaia extract of 0.5% (equivalent to an intake of approximately 750 mg/kg bw per day) based on the slightly lower body weight gain and some organ weight changes in the high-dose group (1.5%). The Panel noted that the effects reported were transient and non-dose dependent and agreed with the conclusion of the authors.

Rats

In a 2-year study (Drake et al., 1982), weanling Wistar-derived rats (48 animals/sex per group) were fed a diet containing 0%, 0.3%, 1.0% or 3.0% Quillaia extract (description of the test material see Appendix M). The mean intake of Quillaia extract was reported by the authors to be 120, 390 and 1,174 mg/kg bw per day in the 0.3%, 1.0% and 3.0% group, respectively, in males; and to be 47, 497 and 1,500 mg/kg bw per day in the 0.3%, 1.0% and 3.0% group, respectively, in females. Body weight and food and water consumption were measured approximately every second month. According to the authors, preference tests were run before the start of the 2-year study which showed that rats avoided the diet containing Quillaia extract. Urine was collected from 10 animals from the control group and 10 animals from the group given 3% Quillaia extract at weeks 13, 24 and 78. Haematological parameters (haemoglobin concentration, packed cell volume, erythrocyte and leucocyte counts) were determined after weeks 15, 25 and 52 in 10 male and 10 female rats from each group and after 108 weeks from all remaining animals. Serum was analysed for contents of urea, glucose, total protein and albumin, and glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactate dehydrogenase (LDH) activities. Autopsy was conducted on rats found in a moribund condition during the study or surviving to the end of the study. The brain, heart, liver, spleen, kidneys, stomach, small intestine, caecum, adrenal glands, gonads, pituitary and thyroid were weighed. The weighed organs in addition to the salivary glands, thymus, various lymph nodes, pancreas, aorta, nasal bones, lungs, trachea, oesophagus, colon, rectum, skeletal muscle, spinal cord, sciatic nerve, uterus, prostate, seminal vesicles, urinary bladder, mammary tissue, eye, Harderian gland and other tissues if they appeared abnormal, were examined for histopathological changes. Survival of male rats in the 1% group was lower than controls from week 73 (only statistically significant between weeks 85 and 91, $p < 0.01$ and $p < 0.05$, respectively). There was a trend towards lower survival among male rats in the 3% group (only statistically significant at weeks 97 and 99, $p < 0.05$ at week 99, no p-value reported for week 97). Lower body weights were observed in high-dose males (statistically significant between months 10 and 22 compared to control, with p values < 0.05 or < 0.01); however, the mean body weights in the high-dose group compared to the control differed between the range of 5 and 8%. Higher body weights were observed in low-dose females (statistically significant from weeks 4 to 25 compared to control, $p < 0.05$). In high-dose male rats, food consumption was consistently lower than that of the controls (not statistically significant) and water consumption was lower than in the controls from week 71 (not statistically significant). At week 78, the urine produced by high-dose male rats was of a higher specific gravity than that of the controls (statistically significant $p < 0.01$). The only statistically significant changes ($p < 0.05$) in red blood cell parameters were increased haemoglobin level at week 15 in mid-dose males and increased erythrocyte count in mid-dose females at week 108. Total leucocyte counts were statistically significantly higher in male rats given 1% or 3% Quillaia extract at week 15 ($p < 0.05$ and $p < 0.01$, respectively) and in males given 3% Quillaia extract at week 25 ($p < 0.01$), but were statistically significantly lower at week 108 in both sexes at 3% ($p < 0.05$). There were generally no significant alterations in the differential leucocyte counts (neutrophils, eosinophils, lymphocytes and monocytes). The relative liver

weight was decreased in males (statistically significant ($p < 0.05$) only at 1%) while it was increased in high-dose females (statistically significant, $p < 0.05$). The relative stomach and small intestine weights were increased in high-dose females (statistically significant, $p < 0.05$ and $p < 0.01$, respectively), the relative weights of both full and empty caecum were increased in low- ($p < 0.05$ and 0.001 respectively, at 0.3%) and high-dose females (($p < 0.01$ and $p < 0.001$, respectively, at 3%). In general, the incidences of non-neoplastic histopathological findings were similar in treated and control animals, except for an increased incidence of fibrosis of the heart (4/45 animals, $p < 0.05$) and of glandular dilatation of the glands of the gastric mucosa (9/42 animals, $p < 0.05$) in low-dose females. The only significant neoplastic finding was an increased incidence of thyroid adenoma in mid-dose females (5/45 animals, $p < 0.05$). The incidence was also increased in high-dose females although not statistically significantly (4/42 animals). The authors reported a 'no-untoward-effect level' for Quillaia extract of 3% (equivalent to an intake of approximately 1,500 mg/kg bw per day, the highest dose tested. The Panel agreed with the conclusions from the authors.

The Panel identified a NOAEL of 750 mg/kg bw per day Quillaia extract from a 84-week study in mice and a NOAEL of 1,500 mg/kg bw per day Quillaia extract, the highest dose tested, from the 2-year study in rats. The Panel noted that there was no indication for carcinogenicity.

3.5.6. Reproductive and developmental toxicity

No reproductive or developmental toxicity data were available to the Panel.

3.5.7. Effects on the GI tract

The Panel considered the gut epithelium – as a site of contact – a potential target for any effects of Quillaia extract. The Panel therefore focussed attention on any GI effects in the biological and toxicological studies discussed in previous sections.

In the subchronic toxicity feeding study in weanling CFE strain specific pathogen-free (SPF) rats (Gaunt et al., 1974), significant increases in relative stomach weights (in males in 2% and 4%, and females in 4% diet groups; $p < 0$ .01) and caecum weights (in females in 4% diet group; $p < 0.05$) were reported. The authors suggested that the increase in relative stomach weights may have been due to a local irritant effect, as quillaia saponins have previously been reported to cause irritation to the GI tract of hens and dogs (Bäck, 1918). Gross and microscopic examinations of these organs as well as the oesophagus, colon, and rectum were conducted on rats in the control and high-dose groups. No gross or histological abnormalities were observed upon necropsy, and the authors remarked that there were no occurrences of diarrhoea or evidence of any other signs of GI irritation.

In the 90-day oral gavage study of Kawaguchi et al. (1994), soft faeces and diarrhoea were reported in rats that died or were killed prior to the end of the study. The authors also reported that similar effects were also observed in the surviving animals that received Quillaia extract. The stomach and caecum (empty) were weighed at the end of the study. Samples of theses organs as well samples from the oesophagus, duodenum, jejunum, ileum, colon and rectum were prepared for histological examination. Significant increases in relative weights were reported for the caecum (males) and significant increases in both the absolute weight and weight relative to body weight were reported for the stomach in both sexes and the caecum in females. Histopathological examinations revealed inflammatory changes in the forestomach (not more than 2/6 males and 3/10 females for each observation), as well as the larynx (incidence not reported), trachea (incidence not reported) and lung (not more than 2/6 males and 0/10 females), and atrophy of the thymus (1/6 males). The Panel considered that many of these GI effects were associated with contact effects through its use at a high (emulsifying) concentration (12% of saponins) which is much higher than the concentration that E 999 is currently authorised as a food additive and therefore it was not relevant for hazard characterisation.

In the 84-week feeding study of Phillips et al. (1979), a significant increase in relative stomach weight was observed in high-dose males ($p < 0.05$; compared to male controls), and a significant increase in absolute small intestine weight was observed in high-dose females ($p < 0.05$; compared to sex-specific controls). Among other histopathological findings, chronic inflammation and degenerative changes were reported in the GI tract of 2 (of 42), 1 (of 41) and 3 (of 43) male mice in the low-, mid-, and high-dose groups for Quillaia extract, respectively, and in 1 (of 44) female in the control group; however, there were no significant differences in the incidences of the lesions between the treated and control groups with respect to histopathological abnormalities.

In the 2-year feeding study of Drake et al. (1982), the stomach, small intestine and cecum (empty and full) were weighed at sacrifice. Significant differences in absolute organ weights (compared to sex-specific controls) included increased stomach, small intestine and full caecum weight (in females provided diet containing 0.3% Quillaia extract; $p < 0.05$); increased empty caecum weight (in females provided diet containing 0.3% Quillaia extract; $p < 0.0001$); increased small intestine and full caecum weight (in females provided diet containing 3% Quillaia extract; $p < 0.01$); and increased empty caecum weight (in females provided diet containing 3% Quillaia extract; $p < 0.0001$). Significant differences in relative (to body weight) organ weights (compared to sex-specific controls) included increased full caecum weights (in females given diet containing 0.3% Quillaia extract; $p < 0.05$); increased empty caecum weight (in females given diet containing 0.3% Quillaia extract; $p < 0.0001$); increased stomach weight (in females given diet containing 3% Quillaia extract; $p < 0.05$); increased small intestine and full caecum weight (in females given diet containing 3% Quillaia extract; $p < 0.01$); and increased empty caecum weight (in females given diet containing 3% Quillaia extract; $p < 0.0001$). The authors did not attribute the changes in stomach, small intestine, and caecum (empty and full) weights to treatment as the changes were inconsistent, occurring only in the low- and high-dose groups, with no differences in the mid-dose group, and did not occur in the males. Histological examinations of samples of the stomach, small intestine, cecum, oesophagus, colon, and rectum were also conducted. The only significant difference in histopathological findings in the GI tract was a significant increase in dilation of gastric mucosal glands ($p < 0.05$) in females given diet containing 0.3% Quillaia extract; however, this effect was not attributed to treatment by the authors as there was no evidence of a dose–response relationship despite a 10-fold increase in dietary concentration at the high-dose level, and similar findings were not observed in the males. The authors noted that there was 'no evidence of irritation of the alimentary tract' following microscopic examination.

The Panel therefore considered that although exposure to Quillaia extract resulted in changes in GI organ weights in several studies relevant to its use as a food additive, these changes were not accompanied by any consistent treatment- and/or dose-related histopathological changes.

3.5.8. Hypersensitivity, allergenicity and food intolerance

Specific pathogen-free 4-week-old male ICR mice (9 animals/group) were given single doses of 0, 0.05, 0.5, 5 or 50 mg Quillaia extract (containing 45% of saponins)/kg bw by oral gavage (Naknukool et al., 2011). Twenty-four hours after administration, the chemotactic activity of peritoneal macrophages harvested from the mice was statistically significantly higher in all dosed animals compared to control. The phagocytosis activity of peritoneal macrophages was statistically significantly higher in all animals fed 0.5 or 5 mg Quillaia extract/kg compared to control. Chemotactic and phagocytosis activity of macrophages harvested from mice after 1, 2, 4 and 10 days of 0.5 mg Quillaia extract/kg was statistically significantly increased after day 1, 2 and 4, but not day 10.

Mice dosed with 0, 0.05, 0.5 or 5 mg Quillaia extract/kg were injected intraperitoneally 24 h later with an *E. coli* suspension. The survival rate of mice infected with *E. coli* showed that average survival time was 3.8 days for the 0.5 mg Quillaia extract/kg treated group vs. 2.5 days for the control group ($p < 0.05$).

Human studies

In the study by Naknukool and co-workers, eight healthy male volunteers (22–23 years of age) were given 0.5 mg Quillaia extract/kg bw per day in a drink for 7 days (Naknukool et al., 2011). Blood was sampled before intake of the drink and 24 h after last administration of the drink. Blood samples were analysed for macrophage activity, immunoglobulins E (IgE) and G (IgG), GOT, GPT, gamma-glutamyl transferase, C-reactive protein, interleukin-1-alpha (IL-1α) and tumour necrosis factor alpha (TNFα). The macrophages from the volunteers after Quillaia extract intake showed significantly increased chemotactic (43.9±7.3 vs. 7.5±1.3 cells/area) and phagocytosis activity (64.0±7.2 *vs.* 24.5±4.5%) than before Quillaia extract intake ($p < 0.05$) ($n = 6$). Levels of α1- and α2-globulin were elevated after Quillaia extract intake ($p < 0.05$) compared to before intake of Quillaia extract ($n = 8$). No other effects were observed in blood samples from the human volunteers. The authors concluded that Quillaia extract is a potent macrophage-stimulating agent that might be used to prevent pathogenic infection in humans. The Panel noted that no control group was included in this study and that reporting of results is biased because eight volunteers participated, whereas the results for macrophages were reported only for six volunteers.

Overall, the Panel noted that immunostimulatory effects of saponins from Quillaia extracts were already used in different species in veterinary vaccines owing to their role as an adjuvant (Rajput et al., 2007; Mendes et al., 2010; Ahlberg et al., 2017; Marciani, 2018). The mechanisms of action are poorly understood and these observations could not be used for the assessment of the food additive Quillaia extracts.

3.5.9. Studies with other emulsifiers

Quillaia extract (E 999) is described as an emulsifier and foaming agent (Documentation EFSA n. 6).

In several recent studies, some other emulsifiers have been reported to alter the gut microbiota, to promote gut inflammation, obesity and to impair glycaemic control (Swidsinski et al., 2009a,b; Renz et al., 2012; Merga et al., 2014; Cani and Everard, 2015; Chassaing et al., 2015; Romano-Keeler and Weitkamp, 2015; Lecomte et al., 2016; Chassaing et al., 2017; Nejrup et al., 2017; Shah et al., 2017; Jiang et al., 2018; Holder and Chassaing, 2018; Viennois and Chassaing, 2018). No such data are available for Quillaia extract .

3.6. Discussion

According to Commission Regulation (EU) No 231/2012, Quillaia extract (E 999) is obtained by aqueous extraction of *Quillaia saponaria* Molina, or other *Quillaia* species, trees of the family Rosaceae. It contains a number of triterpenoid saponins consisting of glycosides of quillaic acid. Sugars – including glucose, galactose, arabinose, xylose, and rhamnose – are also present, along with tannin, calcium oxalate and other minor components.

The Panel noted that existing EU specifications for E 999 do not describe any range for saponins content in the food additive. The JECFA specifications, however, differentiate two types of Quillaia extracts, Type 1 and Type 2, containing a different percentage of saponins and other parameters. The Panel considered that similar differentiation of the extracts of Quillaia should be presented in the EU specifications as E 999(i) and E 999(ii) including the percentage range for saponins, polyphenols (including tannins), protein, polysaccharides including fibre, reducing sugars, a maximum limit for calcium oxalate as well as microbiological specifications.

The Panel assumed that the test materials used in toxicological studies described as Quillaia extract approximated to Quillaia extract Type 1 as described in JECFA specifications (2006c). When available, details on the extracts used in toxicological studies are provided in Appendices M and N. However, from the information provided, the Panel could not ascertain the comparability of test materials between different studies. The Panel noted that the material used in the toxicity studies may be compliant with the current EU specifications; however, it is only poorly characterised.

The Panel considered that the extent and the quality of the available data on the ADME of Quillaia extract saponins were very limited and that no conclusions could be drawn based on studies with Quillaia extracts. The Panel, therefore, considered the available data on GI metabolism and ADME for structurally similar saponins. Based on read-across from these data, the Panel assumed that Quillaia extract saponins share a similar fate. Thus, the Panel considered it likely that intact Quillaia extract saponins are absorbed to a low extent; are hydrolysed in the GI tract and that the aglycone is absorbed only to a limited extent.

The CVMP of EMA (1996) noted that the inability of the haemolytic saponins to cross the gut mucosa has been attributed to the 'rapid elimination of permeabolised mucosal cells of the small intestine by the normal process of epithelial replacement', and the Committee suggested that 'the low oral toxicity of orally-administered saponins could then be explained by the large surface area of the GI tract of mammals in relation to the concentration of saponins'.

The Panel considered that, from the limited data available, the acute toxicity for Quillaia extract was low.

The Panel noted a NOAEL of 0.6% Quillaia extract (approximately 400 mg/kg bw per day) from a 13-week study in rats in which relative organ weights were changed at the next higher dose of 1,200 mg/kg bw per day (Gaunt et al., 1974). The Panel also noted that no histopathological changes were observed at any dose.

The Panel considered that the data on the chemical composition of the mixture and the genotoxicity data available did not indicate a concern for genotoxicity.

The Panel identified a NOAEL of 750 mg Quillaia extract/kg bw per day from a 84-week study in mice and a NOAEL of 1,500 mg Quillaia extract/kg bw per day, the highest dose tested, from the 2-year study in rats. There was no indication for carcinogenicity. In the mouse study, slightly lower body weight gain

and some organ weight changes in the high-dose group of 2,250 mg/kg bw per day Quillaia extract were reported but there were no histopatological changes.

No reproductive or developmental toxicity data were available to the Panel. However, in a chronic study in rats, no adverse effects were observed in the reproductive organs.

The Panel considered the gut epithelium - as a site of contact – a potential target for any effects of Quillaia extract. The Panel therefore focussed attention on any GI effects in the biological and toxicological studies. The Panel considered that although exposure to Quillaia extract resulted in changes in GI organ weights in several studies relevant to its use as a food additive, these changes were not accompanied by any consistent treatment- and/or dose-related histopathological changes.

Taking into account the available toxicological database, the Panel noted that various NOAELs relevant for the derivation of an ADI were identified:

- a NOAEL of 400 mg Quillaia extract/kg bw per day in a 13-week study in rats,
- a NOAEL of 750 mg Quillaia extract/kg bw per day in an 84-week study in mice,
- a NOAEL of 1,500 mg Quillaia extract/kg bw per day (the highest dose tested) in a 2-year study in rats.

Overall, the Panel considered that the 2-year study in rats was the most robust and that the NOAEL of 1,500 mg Quillaia extract/kg bw per day could be used to derive the ADI for Quillaia extract (E 999). Considering that the adverse effects reported were due to the presence of saponins in the extract, that saponins were present in Quillaia extract Type 1 at around 20% (conservative scenario since Quillaia extract Type 1 may contain 20–26% of saponins) and using an uncertainty factor of 100, the Panel derived a ADI of 3 mg saponins/kg bw per day for the food additive Quillaia extract (E 999).

Quillaia extract (E 999) is currently authorised in two food categories (Table 3). The extension of use as a food additive in flavourings relates to five other food categories (Table 4). The exposure to this additive was estimated based on MPLs and use levels, both for its current uses only and its current uses and intended extension of uses, based on the proposed maximum levels in final foods by the applicant (Section 3.4.1). As described in Section 3.4.1, overall the Panel estimated that the exposure to Quillaia extract (E 999) was overestimated in all scenarios.

The Panel noted that MPLs are expressed as anhydrous extract in Annex II to Regulation No 1333/2008. Therefore, exposure to Quillaia extract (E 999) was expressed in mg anhydrous extract of Quillaia extracts (E 999)/kg bw per day. Considering that the ADI is expressed in mg saponins, exposure was also converted to saponins, assuming that Quillaia extract (E 999) contains 70%[15] saponins (Type 2) for the current authorised use levels and as proposed by the applicant 20% saponins (Type 1) for the new proposed uses. Therefore, exposure using the current MPLs (*regulatory maximum level exposure assessment scenario*) ranged between < 0.1 mg saponins/kg bw per day at the mean up to 6.7 mg saponins/kg bw per day at the 95th percentile. Using the refined exposure assessment scenario including the proposed extension of use, exposure ranged between < 0.1 mg saponins/kg bw per day at the mean up to 0.7 mg saponins/kg bw per day at the 95th percentile (Appendix O).

To assess possible health risks related to the current use of Quillaia extract (E 999), the Panel selected the brand-loyal scenario as the additive is used in FC 14.1.4 Flavoured drinks. The exposure estimates of the scenario based solely on MPLs were not used for this purpose as they were recognised to be very conservative. None of the exposure estimates for the different population groups of the brand-loyal scenario exceeded the ADI of 3 mg saponins/kg bw per day. Also the proposed extension of use as a food additive in flavourings did not result in an exceedance of this ADI at the refined exposure assessment scenario.

The Panel noted that the refined exposure estimates are based on information provided on the reported level of use of Quillaia extract (E 999). If actual practice changes, this refined estimates may no longer be representative and should be updated.

The applicant proposed the addition of sodium benzoate (E 211) to Annex III of Regulation (EC) No 1333/2008 as a preservative for use in liquid formulations of Quillaia extracts (E 999). The Panel noted that exposure to sodium benzoate (E 212) at the levels proposed to be used in the preparation of Quillaia extract (E 999) would add at the maximum 0.13% to the exposure to benzoic acid–benzoates (E 210–213) as food additives, considering their use according to Annex II to Regulation No 1333/2008 and additional exposure from food categories which may contain benzoic acid–benzoates due to carry-over, for which an exceedance of the ADI of 5 mg/kg bw per day, expressed as benzoic acid, was observed in the *non-brand-loyal scenario* for toddlers and children (EFSA ANS Panel, 2016).

[15] From analytical data provided by interested parties (Documentation provided to EFSA n. 6)

4. Conclusions

The Panel concluded that any toxicity associated with Quillaia extract (E 999) is due to its constituent saponins and, therefore, an ADI based on saponins content can be derived. Accordingly, the ADI for Quillaia extract (E 999) of 5 mg spray-dried extract/kg bw per day is withdrawn.

Taking into account the toxicological data available, the Panel established an ADI of 3 mg saponins/kg bw per day for Quillaia extract (E 999) based on the NOAEL of 1,500 mg Quillaia extract/kg bw per day and the conservative assumption that it contained 20% saponins, and by applying an uncertainty factor of 100.

The exposure estimates for the different population groups of the brand-loyal scenario did not exceed the ADI of 3 mg saponins/kg bw per day at the reported use levels.

The Panel concluded that the proposed extension of use of Quillaia extract (E 999) as a food additive in flavourings at the proposed uses and use levels would also not result in an exceedance of the ADI at the refined exposure assessment scenario.

The proposed addition of sodium benzoate (E 211) to Annex III of Regulation (EC) No 1333/2008 as a preservative for use in liquid formulations of Quillaia extracts (E 999) at the proposed levels would add at the maximum 0.13% to the exposure to benzoic acid–benzoates (E 210–213) as food additives for which an exceedance of the ADI of 5 mg/kg bw per day, expressed as benzoic acid, was observed in the non-brand-loyal scenario for toddlers and children (EFSA ANS Panel, 2016).

5. Recommendations

The Panel recommended that the European Commission considers:

- Revising the EU specifications for Quillaia extracts (E 999) in order to differentiate extracts of Quillaia according to the saponins content (including a description of the principle of the method of analysis to quantify the content of saponins in line with the JECFA specifications), i.e. Type 1 and Type 2.
- Revising the EU specifications to include the percentage range for polyphenols (including tannins), protein, polysaccharides including fibre, reducing sugars, a maximum limit for calcium oxalate as well as microbiological specifications.
- Lowering the current limits for toxic elements (arsenic, lead and mercury) in the EU specifications for Quillaia extracts (E 999) in order to ensure that the food additive will not be a significant source of exposure to these toxic elements in food.
- Revising the maximum use levels for Quillaia extracts (E 999) established in Regulation (EC) No 1333/2008 to be expressed on saponin content.

Documentation provided to EFSA

1) Dossier "Additive E999, Quillaia extract, for use as a functional additive in a flavouring system". Submitted by International Flavors & Fragrances Inc, November 2013.
2) FDE (FoodDrinkEurope), 2017. Data on use levels of Quillaia extracts (E 999) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2017). Submitted to EFSA on 1 February 2017.
3) Kerry Ingredients & Flavours, 2012. Submission of data for the re-evaluation of Quillaia extract (E 999) following an EFSA call for data. Submitted by Kerry Ingredients & Flavours, August 2012.
4) National Starch Food Innovation, 2012. Response to the call for scientific data on Quillaia extracts (E 999). August 2012. Submitted by Intertek Cantox, August 2012.
5) Pre-evaluation document. DTU (National Food Institute) finalised on 1 march 2013.
6) Response to request for additional information by EFSA in March 2015. Submitted by Intertek Scientific &Regulatory Consultancy, August 2015.
7) Response to request for additional information by EFSA in September 2015. Submitted by Intertek Scientific &Regulatory Consultancy, February 2016.
8) Response to request for additional information by EFSA in June 2016. Submitted by Intertek Scientific &Regulatory Consultancy, April 2018.
9) Response to request for additional information by EFSA in June 2018. Submitted by Intertek Scientific &Regulatory Consultancy, August 2018.
10) Response to request for additional information by EFSA in June 2018. Submitted by Intertek Scientific &Regulatory Consultancy, November 2018.

References

Ahlberg V, Hjertner B, Wallgren P, Hellman S, Bengtsson KL and Fossum C, 2017. Innate immune responses induced by the saponin adjuvant Matrix-M in specific pathogen free pigs. Veterinary Research, 48, 30–43.

Bäck H, 1918. The excretion of saponin in the faeces. Biochem Zeisch, 86, 223–242.

Butterworth K, 1977a. The results of a long-term toxicity study of Quillaia extract. Unpublished information from BIBRA, as referred to by SCF (1978).

Butterworth K, 1977b. The results of an 84 week study in mice with Quillaia extract. Unpublished information from BIBRA, as referred to by SCF (1978).

Cani PD and Everard A, 2015. Keeping gut lining at bay: impact of emulsifiers. Trends in Endocrinology and Metabolism, 26, 273–274.

Cares MG, Vargas Y, Gaete L, Sainz J and Alarcon J, 2010. Ultrasonically assisted extraction of bioactive principles from Quillaja Saponaria Molina. Physics Procedia, 3, 169–178.

Chassaing B, Koren O, Goodrich JK, Poole AC, Srinivasan S, Ley RE and Gewirtz AT, 2015. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature, 519, 92–96.

Chassaing B, de Wiele TV, De Bodt J, Marzorati M and Gewirtz AT, 2017. Dietary emulsifiers directly alter human microbiota composition and gene expression ex vivo potentiating intestinal inflammation. Gut, 66, 1414–1427.

Cheok CY, Salman HAK and Sulaiman R, 2014. Extraction and quantification of saponins: a review. Food Research International, 59, 16–40.

Drake JJ, Butterworth KR, Gaunt IF, Hooson J, Evans JG and Gangolli SD, 1982. Long-term toxicity study Quillaia extract in rats. Food and Chemical Toxicology, 20, 15–23.

ECHA (European Chemical Agency), online. Available at: https://echa.europa.eu/brief-profile/-/briefprofile/100.066.909

EFSA (European Food Safety Authority), 2007. Scientific opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2007;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9 (3):1970, 27 pp. https://doi.org/10.2903/j.efsa.2011.1970

EFSA (European Food Safety Authority), online. Available online: https://www.efsa.europa.eu/en/microstrategy/botanical-summary-report

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760, 60 pp. https://doi.org/10.2903/j.efsa.2012.2760

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2016. Scientific Opinion on the re-evaluation of benzoic acid (E 210), sodium benzoate (E 211), potassium benzoate (E 212) and calcium benzoate (E 213) as food additives. EFSA Journal 2016;14(3):4433, 95 pp. https://doi.org/10.2903/j.efsa.2016.4433

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009. Scientific Opinion on arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. https://doi.org/10.2903/j.efsa.2009.1351

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2010. Scientific Opinion on lead in food. EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012a. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. https://doi.org/10.2903/j.efsa.2012.2985

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012b. Scientific Opinion on lead dietary exposure in the European population. EFSA Journal 2012;10(7):2831, 59 pp. https://doi.org/10.2903/j.efsa.2012.2831

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2014. Scientific Opinion on dietary exposure to inorganic arsenic in the European population. EFSA Journal 2014;12(3):3597, 68 pp. https://doi.org/10.2903/j.efsa.2014.3597

EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: general Principles. EFSA Journal 2009;7(7):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. https://doi.org/10.2903/j.efsa.2011.2379

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

EMA (European Medicines Agency), 1996. Committee for Veterinary Medicinal Products. Quillaia Saponins. Summary Report. EMEA/MRL/055/95-FINAL February 1996. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015820.pdf

Ewart AJ, 1931. The poisonous action of ingested sponins. Council for Scientific and Industrial Research. Bulletin No 50.
Fieger J, 1918. The excretion of saponins in the urine and their action on the blood after internal administration. Biochem Zeisth, 86, 244–297.
FSANZ (Food Standards Australia New Zealand), 2013. Risk and technical assessment report (at Approval)- Applications A1075. Quillaia Extract (Quillaja Extract) as a food additive (emulsifier). Available online: http://www.foodstandards.gov.au/code/applications/pages/applicationa1075quil5602.aspx
Gaete-Garreton L, Vargas-Hernandez Y, Cares-Pacheco M, Sainza J and Alarcon J, 2011. Ultrasonically enhanced extraction of bioactive principles from Quillaja Saponaria Molina. Ultrasonics, 51, 581–585.
Gaunt IF, Grasso P and Gangolli SD, 1974. Short-term toxicity of Quillaia extract in rats. Food Cosmetic Toxicology, 12, 641–650.
Gestetner B, Birk Y and Tencer Y, 1968. Soybean saponins: fate of ingested soybean saponins and the physiological aspect of their hemolytic activity. Journal of Agricultural and Food Chemistry, 16, 1031–1035.
Güçlü-Ustündağ O and Mazza G, 2007. Saponins: properties, applications and processing. Critical Reviews in Food Science and Nutrition, 47, 231–258.
Gutierrez J and Davis RE, 1962. Isolation of saponin-digesting bacteria from the rumen of bloating cattle on Ladino clover pasture. Journal of Animal Science, 21, 819–823.
Hasegawa H, 2004. Proof of the mysterious efficacy of ginseng: basic and clinical trials: metabolic activation of ginsenoside: deglycosylation by intestinal bacteria and esterification with fatty acid. Journal of Pharmacological Sciences, 95, 153–157.
Hattori M, Sakamoto T, Kobashi K and Namba T, 1983. Metabolism of glycyrrhizin by human intestinal flora. Planta Medica, 48, 38–42.
Hattori M, Sakamoto T and Yamagishi T, 1985. Metabolism of glycyrrhizin by human intestinal flora. II. Isolation and characterization of human intestinal bacteria capable of metabolizing glycyrrhizin and related compounds. Chemical & Pharmaceutical Bulletin, 33, 210–217.
He C, Li J, Xu N, Wang R, Li Z, Yang L and Wang Z, 2015. Pharmacokinetics, bioavailability, and metabolism of Notoginsenoside Fc in rats by liquid chromatography/electrospray ionization tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis, 109, 150–157.
Holder MK and Chassaing B, 2018. Impact of food additives on the gut-brain axis. Physiology & Behavior, 192, 173–176.
Hostettmann K and Marston A, 1995. Chemistry and pharmacology of natural products. Saponins. 5. Triterpene saponins- pharmacological and biological properties, 232–286.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1982a. Evaluation of certain food additives and contaminants. Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 683.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1982b. Quillaia extract. Toxicological evaluation of certain food additives and contaminants. Prepared by the twenty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Food Additives Series, 17.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1986. Evaluation of certain food additives and contaminants. Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 733.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2002a. Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 909, 14–18.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2002b. Safety evaluation of certain food additives and contaminants. Quillaia extracts. WHO Food Additives Series, 48.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2004. Evaluation of certain food additives and contaminants. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 922, 37–40.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005. Quillaia extracts Type 1 and Type 2. Chemical and Technical Assessment 65th JECFA. Available online: http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/65/quillaia.pdf.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006a. Evaluation of certain food additives. Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 934, 28–31.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006b. Quillaia extract Type 1 (addendum). Safety evaluation of certain food additives and contaminants. Prepared by the sixty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Food Additives Series, 56.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006c. Monograph 3. Online Edition "Combined compendium of food additive specifications". Available online: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2014. Monograph 16. Online Edition "Combined compendium of food additive specifications". Available online: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/

Jiang Z, Zhao M, Zhang H, Li Y, Liu M and Feng F, 2018. Antimicrobial emulsifier- glycerol monolaurate induces metabolic syndrome, gut microbiota dysbiosis and systemic low-grade inflammation in low-fat diet fed mice. Molecular Nutrition and Food Research, 1700547, 1–11.

Kartnig Th and Ri CY, 1973. Über sle Satibilltät von Saponinen in Drogenauszügen. Scientia Pharmaceutica, 41, 185–188.

Kawaguchi M, Kato T, Kamada S and Yahata A, 1994. Three-month oral repeated administration toxicity study of seed saponins of *Thea sinensis* L. (Ryokucha saponin) in rats. Food Chemical Toxicity, 32, 431–442.

Kensil CR and Marciano D, 1991. Saponin adjuvant. U.S. patent 5,057,540.

Kensil CR, Patel U, Lennick M and Marciano D, 1991. Separation and characterization of saponins with adjuvant activity from Quillaja saponaria Molina cortex. The Journal of Immunology, 146, 431–437.

Kim DH, Lee SW and Han MJ, 1999. Biotransformation of glycyrrhizin to 18β-glycyrrhetinic acid-3-*O*-β-D-glucuronide by *Streptococcus* LJ-22, a human intestinal bacterium. Biological & Pharmaceutical Bulletin, 22, 320–322.

Kim DH, Hong SW, Kim BT, Bae EA, Park HY and Han MJ, 2000. Biotransformation of glycyrrhizin by human intestinal bacteria and its relation to biological activities. Archives of Pharmacal Research, 23, 172–177.

Kirk DD, Rempel R, Pinkhasov J and Walmsley AM, 2004. Application of Quillaja saponaria extracts as oral adjuvants for plat-made vaccines. Expert Opinion on Biological Therapy, 4, 947–958.

Kite GC, Porter EA and Simmonds MSJ, 2007. Chromatographic behaviour of steroidal saponins studied by high-performance liquid chromatography–mass spectrometry. Journal of Chromatography A, 1148, 177–183.

Lecomte M, Couedelo Leslie, Meugnier E, Plaisancie P, Letisse M, Bérengère B, Gabert L, Penhoat A, Durand A, Pineau G, Joffre F, Géloën A, Vaysse C, Laugerette F and Michalski MC, 2016. Dietary emulsifiers from milk and soybean differently impact adiposity and inflammation in association with modulation of colonic goblet in high-fat fed mice. Molecular Nutrition and Food Research, 60, 606–620.

Li K, Ding L, Yang Z-L, Liu EH, Qi L-W, Li P and Hu Y-Z, 2010. Determination of asperosaponin VI in rat plasma by HPLC-ESI-MS and its application to preliminary pharmacokinetic studies. Biomedical Chromatography: BMC, 24, 550–555.

Luo J, Zhou C, Zhang W and Kong L, 2013. Pharmacokinetic study and metabolite identification of the bidesmosidic triterpenoid saponin BTS-1 in rat plasma. Acta Pharmaceutica Sinica B, 3, 174–179.

Marciani DJ, 2018. Elucidating the mechanisms of action of Saponin-Derived Adjuvants. Trends in Pharmacological Sciences, 39, 573–585.

Martindale, online. The Complete Drug Reference. Available online: http://www.medicinescomplete.com/mc/martindale [Accessed: 30 January 2019]

Mendes RE, Zafra R, Perez-Ecija RA, Buffoni L, Martinez-Moreno A, Tendler M and Perez J, 2010. Evaluation of local immune response to *Fasciola hepatica* experimental infection in the liver and hepatic lymph nodes of goats immunized with Sm14 vaccine antigen. Memórias do Instituto Oswaldo Cruz, 105, 698–705.

Merga Y, Campbell BJ and Rhodes JM, 2014. Mucosal barrier, bacteria and inflammatory bowel disease: possibilities for therapy. Digestive Diseases, 32, 475–483.

Mitra S and Dugan ST, 2000. Micellar properties of Quillaja Saponin. 2 Effects of solubilised cholesterol on solution properties. Colloids and Surfaces B: Biointerfaces, 17, 117–133.

Naknukool S, Horinouchi A and Hatta H, 2011. Stimulating macrophage activity in mice and humans by oral administration of Quillaja saponin. Bioscience, Biotechnology and Biochemistry, 75, 1889–1893.

Nejrup RG, Licht TR and Hellgren LI, 2017. Fatty acid composition and phospholipid types used in infant formulas modifies the establishment of human gut bacteria in germ-free mice. Scientific Reports, 7(3975), 1–11.

Okamura N, Miyauchi H, Choshi T, Ishizu T and Yagi A, 2003. Simultaneous determination of glycyrrhizin metabolites formed by the incubation of glycyrrhizin with rat feces by semi-micro high-performance liquid chromatography. Biological & Pharmaceutical Bulletin, 26, 658–661.

Oser BL, 1966. An evaluation of *Yucca mohavensis* as a source of food grade saponin. Food Cosmetic Toxicology, 4, 51–61.

Ouyang H, Guo Y, He M, Zhang J, Huang X, Zhou X, Jiang H, Feng Y and Yang S, 2015. A rapid and sensitive LC-MS/MS method for the determination of *Pulsatilla* saponin D in rat plasma and its application in a rat pharmacokinetic and bioavailability study. Biomedical Chromatography: BMC, 29, 373–378.

Phillips JC, Butterworth KR, Gaunt IF, Evans JG and Grasso P, 1979. Long-term toxicity study of Quillaia extract in mice. Food and Cosmetics Toxicology, 17, 23–27.

Qian T and Cai Z, 2010. Biotransformation of ginsenosides Rb1, Rg3 and Rh2 in rat gastrointestinal tracts. Chinese Medicine, 5, 19. https://doi.org/10.1186/1749-8546-5-19

Rajput ZI, Hu S-H, Xiao C-W and Arijo AG, 2007. Adjuvant effects of saponins on animal immune response. Journal of Zheijang University Science B, 8, 153–161.

Ren Y, Xu X, Zhang Q, Lu Y, Li X, Zhang L and Tian J, 2017. Isolation, characterization, and in rats plasma pharmacokinetic study of a new triterpenoid saponin from *Dianthus superbus*. Archives of Pharmacal Research, 40, 159–163.

Renz H, Brandtzaeg P and Hornef M, 2012. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. Nature Reviews-Immunology, 12, 9–23.
Romano-Keeler J and Weitkamp JH, 2015. Maternal influences on fetal microbial colonization and immune development. Pediatric Research, 77, 189–195.
Rubas W, Zezyk N and Grass GM, 1993. Comparison of the permeability characterization of human colonic epithelium (Caco-2) cell line to colon of rabbit, monkey and dog intestine and human drug absorption. Pharmaceutical Research, 10, 113–118.
San Martın R and Briones R, 2000. Quality control of commercial quillaja (Quillaja saponaria Molina) extracts by reverse phase HPLC. Journal of Science and Food Agriculture, 80, 2063–2068.
SCF (Scientific Committee for Food), 1978. Report of the Scientific Committee for Food on Emulsifiers, Stabilizers, Thickeners and Gelling agents. Opinion expressed 30 November 1978.
SCF (Scientific Committee for Food), 2001. Guidance on submissions for food additive evaluations by the Scientific Committee on Food. SCF/CS/ADD/GEN/26 Final. 12 July 2001.
Shah R, Kolanos R, DiNovi MJ, Mattia A and Kaneko KJ, 2017. Dietary exposures for the safety assessment of seven emulsifiers commonly added to foods in the United States and implications for safety. Food Additives and Contaminants: Part A, 34, 905–917.
Swidsinski A, Loening-Baucke V and Herber A, 2009a. Mucosal flora in Crohn's disease and ulcerative colitis - an overview. Journal of Physiology and Pharmacology, 60(supplement 6), 61–71.
Swidsinski A, Ung V, Sydora BC, Loening-Baucke V, Doerffel Y, Verstraelen H and Fedorak RN, 2009b. Bacterial overgrowth and inflammation of small intestine after carboxymethylcellulose ingestion in genetically susceptible mice. Inflammatory Bowel Diseases, 15, 359–364.
Takeda S, Ishihara K, Wakui Y, Amagaya S, Maruno M, Akao T and Kobashi K, 1996. Bioavailability study of glycyrrhetic acid after oral administration of glycyrrhizin in rats; Relevance to the intestinal bacterial hydrolysis. The Journal of Pharmacy and Pharmacology, 48, 902–905.
TemaNord (Nordic Council of Ministers), 2002. Quillaia Extract (E 999). Food Additives in Europe 2000 - Status of safety assessments of food additives presently permitted in the EU, 642–643.
Varma MV, Gardner I, Steyn SJ, Nkansah P, Rotter CJ, Whitney-Pickett C, Zhang H, Di L, Cram M, Fenner KS and El-Kattan AF, 2012. pH-Dependent solubility and permeability criteria for provisional biopharmaceutics classification (BCS and BDDCS) in early drug discovery. Molecular Pharmaceutics, 9, 1199–1212.
Viennois E and Chassaing B, 2018. First victim, later aggressor: how the intestinal microbiota drives the pro-inflammatory effects of dietary emulsifiers? Gut Microbes, 9, 289–291.
Volpe DA, 2011. Drug-permeability and transporter assays in Caco-2 and MDCK cell lines. Future Medicinal Chemistry, 3, 2063–2077.
Wan JY, Liu P, Wang HY, Qi LW, Wang CZ, Li P and Yuan CS, 2013. Biotransformation and metabolic profile of American ginseng saponins with human intestinal microflora by liquid chromatography quadrupole time-of-flight mass spectrometry. Journal of Chromatography. A, 1286, 83–92.
Wang Z, Nishioka M, Kurosaki Y, Nakayama T and Kimura T, 1995. Gastrointestinal absorption characteristics of glycyrrhizin from glycyrrhiza extract. Biological & Pharmaceutical Bulletin, 18, 1238–1241.
Wang HY, Qi LW, Wang CZ and Li P, 2011. Bioactivity enhancement of herbal supplements by intestinal microbiota focusing on ginsenosides. The American Journal of Chinese Medicine, 39, 1103–1115.
Wu X, Liu L, Zhang M, Wu D, Wang Y, Sun Y, Fawcett JP, Gu J and Zhang J, 2010. Simultaneous analysis of isomers of escin saponins in human plasma by liquid chromatography-tandem mass spectrometry: application to a pharmacokinetic study after oral administration. Journal of Chromatography B, 878, 861–867.
Wu X-J, Zhang M-L, Cui X-Y, Fawcett JP and Gu J-K, 2014. Comparative pharmacokinetics and the bioavailability of escin Ib and isoescin Ib following the administration of escin, pure escin Ib and isoescin Ib in rats. Journal of Ethnopharmacology, 151, 839–845.
Wu X-J, Zhang M-L, Cui X-Y, Gao F, He Q, Li X-J, Zhang J-W, Fawcett JP and Gu J-K, 2012. Comparative pharmacokinetics and bioavailability of escin Ia and isoescin Ia after administration of escin and of pure escin Ia and isoescin Ia in rat. Journal of Ethnopharmacology, 139, 201–206.
Yang XW, Zhao J, Cui JR and Guo W, 2004. [Studies on the biotransformation of escin Ia by human intestinal bacteria and the anti-tumor activities of desacylescin I]. Beijing Da Xue Xue Bao. Yi Xue Ban. Journal of Peking University. Health Sciences 36, 31–35 [Chinese – English abstract only]. Also Cited In: Wu et al., 2012 [As: Journal of Peking University (Health Sciences).
Yoo HH, Lee SK, Lim SY, Kim Y, Kang MJ, Kim EJ, Park YH, Im G-J, Lee BY and Kim D-H, 2008. LC-MS/MS method for determination of hederacolchiside E, a neuroactive saponin from Pulsatilla koreana extract in rat plasma for pharmacokinetic study. Journal of Pharmaceutical and Biomedical Analysis, 48, 1425–1429.
Yoshikoshi M, Kahara T, Yoshiki Y, Ito M, Furukawa Y, Okubo K and Amarowicz R, 1995. Metabolism and nonabsorption of soybean hypocotyl saponins in the rat model. Acta Alimentaria, 24, 355–364.
Yu K, Chen F and Li C, 2012. Absorption, disposition, and pharmacokinetics of saponins from Chinese medicinal herbs: what do we know and what do we need to know more? Current Drug Metabolism, 13, 577–598.
Zhao H, Liu H, Ma Z, Wang Y, Li Y-L, Ye W-C and Wu B, 2015. Metabolite profiling of anhuienoside C by rat intestinal bacteria using the LC-MS metabolomic approach 5-ring. Xenobiotica; The Fate of Foreign Compounds in Biological Systems, 45, 189–196.

Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
AUC	area under the plasma concentration-time curve
BMI	body mass index
bw	body weight
C_{max}	maximum concentration in plasma
CAS	Chemical Abstracts Service
CMC	critical micelle concentration
CONTAM	EFSA Panel on Contaminants
CVMP	Committee for Veterinary Medicinal Products
ECHA	European Chemical Agency
EINECS	European Inventory of Existing Commercial Chemical Substances
EMA/EMEA	European Medicines Agency
FAF	EFSA Panel on Food Additives and Flavourings
FC	food category
FCS	food categorisation system
FDE	Food Drink Europe
FSANZ	Food Standards Australia New Zealand
GI	gastrointestinal
GLP	good laboratory practice
GNPD	Global New Products Database
GOT	glutamic oxaloacetic transaminase
GPT	serum glutamic pyruvic transaminase
IgE	immunoglobulin E
IgG	immunoglobulin G
IL-1α	interleukin-1-alpha
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC–MS/MS	liquid chromatography tandem mass spectrometry
LD_{50}	lethal dose, median
LDH	lactate dehydrogenase
LLOQ	lower limit of quantification
LOD	limit of detection
LOQ	limit of quantification
MPL	maximum permitted level
MRL	maximum residue level
MRT	mean residence time
MTD	maximum tolerated dose level
NGFc	Notoginsenoside Fc
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PPD	protopanaxadiol
PPT	protopanaxatriol
QS	*quantum satis*
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RET	reticulocytes
RICC	reduction in relative increase in cell count
RP-HPLC	reverse-phase high performance liquid chromatography
SCF	Scientific Committee on Food
SPF	specific pathogen-free
$t_{1/2}$	half-life
TemaNord	is a publishing series for results of the often research-based work that working groups or projects under Nordic Council of Ministers have put in motion
TNFα	tumour necrosis factor alpha
UPLC-Q/TOF-MS	ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry
WHO	World Health Organization

Appendix A – Specifications of the 1973 British Pharmacopoeia

The latest evaluation performed by the SCF (1978) was based on toxicological studies carried out with a natural extract of Quillaia bark as specified in the 1973 edition of the British Pharmacopoeia, as reproduced in Table 2.

Table A.1: Specifications for Quillaiæ cortex (Quillaia Bark) and Powdered Quillaia as reported in British Pharmacopoeia (1973)

Quillaia	**Quillaiæ cortex; Quillaia Bark**
Quillaia is the dried inner part of the bark of *Quillaja saponaria* Molina, and of other species of *Quillaja*	
Description:	Odourless; dust strongly sternutatory; taste, acrid and astringent
Macroscopical	Pieces flat, up to about 1 metre long, 10–20 cm broad and 3 to 6 to 10 mm thick. Outer surface brownish-white or pale reddish-brown, longitudinally striated or coarsely reticulated, with occasional black-brown patches of adherent outer bark; inner surface yellowish-white, smooth and very hard; fracture splintery and laminated, the broken surface showing numerous large prisms of calcium oxalate as glistening points. Smoothed transversely cut surface appearing chequered, with delicate radial lines representing medullary rays and tangential lines formed by alternating tangential bands of fibrous and non-fibrous phloem containing fibres
Microscopical.	Outer bark, when present, consisting of reddish-brown cork cells alternating with bands of brown parenchyma containing numerous groups of phloem fibres and large prisms of calcium oxalate. Inner bark consisting of alternating bands of tortuous fibres irregularly enlarged at intervals about 500 to 1,000 μm long and 20 to 50 μm wide and of mixed sieve tissue with parenchyma. Medullary rays mostly 3 to 4, but sometimes up to 6 cells wide, with occasional pitted, subrectangular sclereids adjacent to the bundles of phloem fibres. Starch grains 5 to 10 to 20 μm in diameter, and prisms of calcium oxalate usually 50 to 170 μm long and up to 30 μm wide present in the parenchymatous cells
Acid-insoluble ash	Not more than 1.0%, page A88
Alcohol (45%)-soluble extractive	Not less than 22.0%, page A89
Foreign organic matter	Not more than 2.0%, page A99
Powdered Quillaia	
Complies with the requirements for Acid-insoluble ash, Alcohol (45%)-soluble extractive, and Foreign organic matter stated under Quillaia and with the following requirement	
Description:	Pale buff with a pink tinge. Diagnostic structures: phloem fibres, in bundles; occasional sclereids; calcium oxalate prisms, often in fragments; starch grains and cork cells in small amount. When shaken with water a copious persistent froth is produced
Action and use	Emulsifying agent

Appendix B – Summary of reported use levels (mg/kg or mg/L as appropriate) of Quillaia extracts (E 999) provided by industry

Appendix C – Number and percentage of food products labelled with Quillaia extracts (E 999) out of the total number of food products present in the Mintel GNPD per food subcategory between 2013 and 2018

Appendix D – Concentration levels of Quillaia extracts (E 999) used in the exposure assessment scenarios (mg/kg or mL/kg as appropriate)

Appendix E – Concentration levels of Quillaia extracts (E 999) used in the exposure assessment scenarios including the extensions of use (mg/kg or mL/kg as appropriate)

Appendix F – Summary of total estimated exposure of Quillaia extracts (E 999) from its use as a food additive for the regulatory maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

Appendix G – Main food categories contributing to exposure to Quillaia extracts (E 999) using the regulatory maximum level exposure assessment scenario and the refined exposure assessment scenarios (> 5% to the total mean exposure)

Appendix H – Summary of total estimated exposure of Quillaia extracts (E 999) from its use as a food additive including the proposed extensions of use, for the regulatory maximum level exposure scenario and the maximum exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

Appendix I – Main food categories contributing to exposure to Quillaia extracts (E 999) from its use as a food additive including the proposed extensions of use, for the regulatory maximum level exposure scenario and the maximum exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

Appendicies B–I can be found in the online version of this output ('Supporting information' section)

Appendix J – Identity of the substances proposed for read-across for ADME data; gastrointestinal metabolism

Data from other saponins, structurally related to Quillaia saponins, have been used for the assessment.

A number of other triterpenoid saponins have an aglycone backbone similar to quillaic acid: for the purpose of this opinion, read-across from saponins – having oleanolic acid instead of quillaic acid (see Figure J.1) – was considered relevant for ADME data (Table J.1).

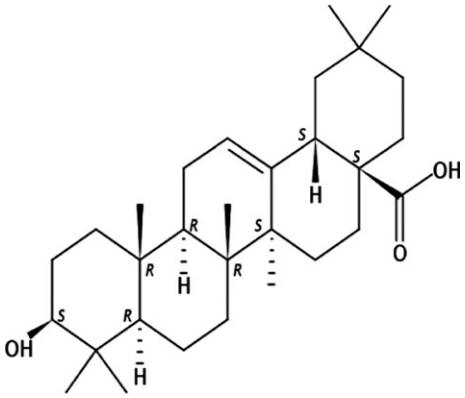
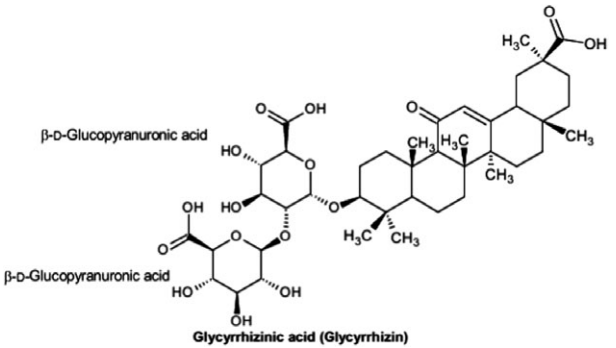
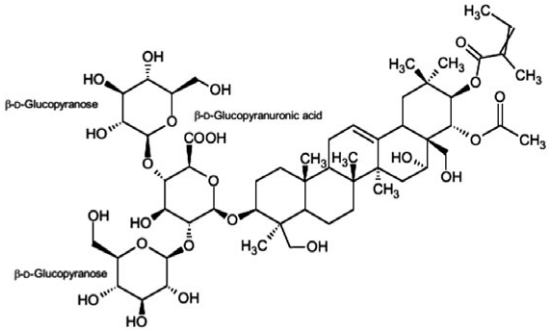
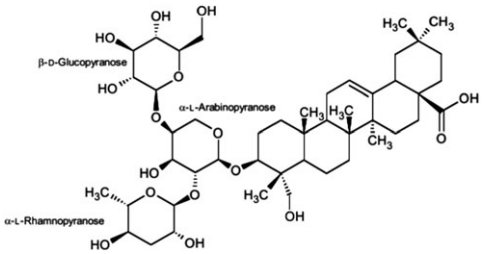
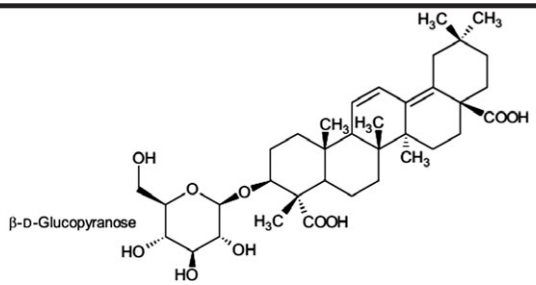
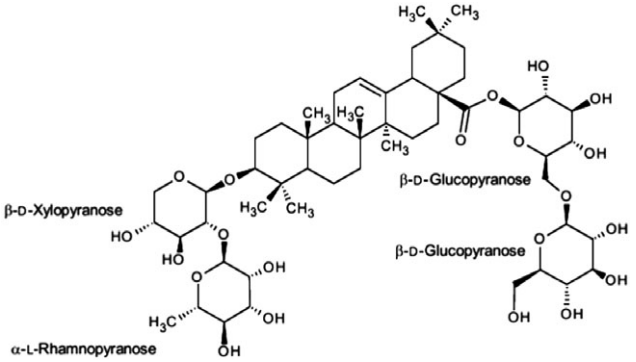


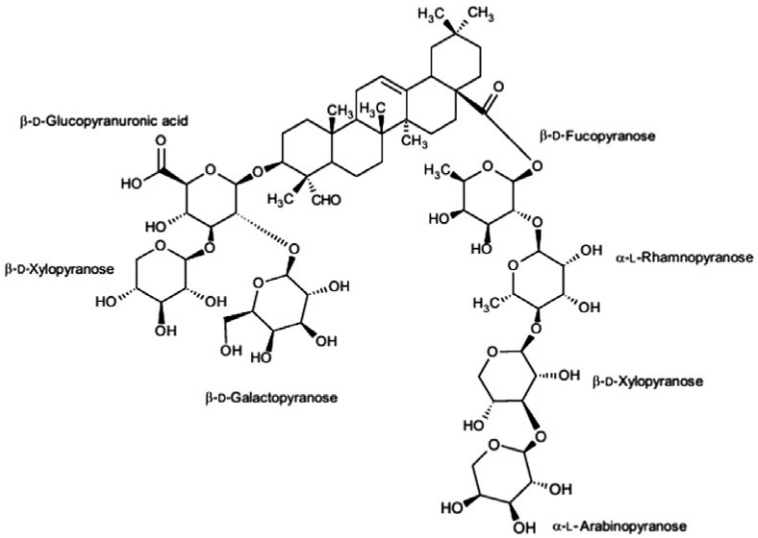
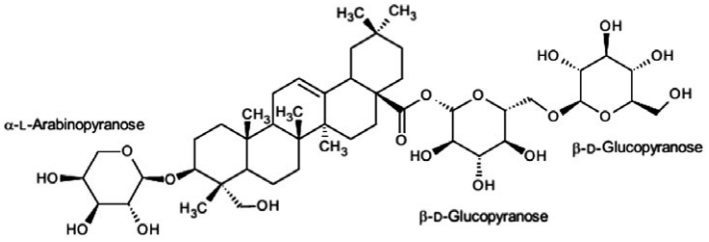
Figure J.1: Structural formula of oleanolic acid

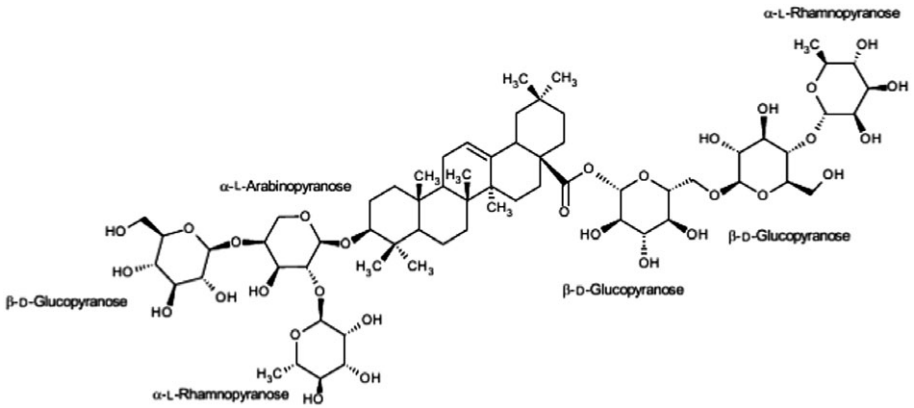
Other saponins (i.e. gingseng saponins, soybean saponins) were also considered relevant for the read-across on ADME data due to the fact that they bear side chain attachments similar to the quillaia saponins, despite having a 4-ring backbone structure instead of a 5-ring structure.

Table J.1: Saponins considered for read-across

Name	Botanical source	Structure
Glycyrrhizin	Liquorice	
β-Aescin (β-escin)	*Aesculus hippocastanum*	
Pulsatilla saponin D	*Pulsatilla chinensis*	

Name	Botanical source	Structure
DS-1	*Dianthus superbus*	
Anhuienoside C	*Anhemone flaccida* Fr. Schmidt (rhizome) (traditional Chinese medicine 'Di Wu')	

Name	Botanical source	Structure
BTS-1	*Gypsophila oldhamiana* (roots)	
Asperosaponin VI (akebia saponin D)	*Dipsacus asper* Wal	

Name	Botanical source	Structure
Hederacolchiside E	*Pulsatilla koreana*	

Appendix K – Gastrointestinal metabolism of saponins

In silico

The apical to basal permeability coefficient (Papp,A-B) of anhuienoside C was reported to be 3.04×10^{-6} cm/s (Volpe, 2011). This value was below the threshold value for poorly absorbed drugs (1×10^{-5} cm/s) (Rubas et al., 1993) and thus indicative of a poorly absorbed compound (Varma et al., 2012).

In vitro studies

Gutierrez and Davis (1962) isolated colonies of saponins-digesting bacteria from the rumen fluid obtained from yearling steers that were permitted to graze on lush, pre-bloom Ladino clover (*Trifolium repens*) for two, one and a half-hour period and also experienced bloating. The authors quantified the amount of 'slime' harvested from inoculations of the bacteria, 0.5% yeast extract, 0.5% trypticase broth, and 2 g of alfalfa saponins after a 24-h incubation period. The 'slime harvest' ranged from 0.146 to 0.884 g (compared to 0.080 g for an uninoculated control), and analysis revealed that the slime was primarily composed of residual sapogenins.

The CVMP of the EMA (formerly EMEA) noted that hydrolysis of saponins to sapogenins and sugars has been demonstrated *in vitro* (EMA, 1996). Although no study details were provided, two publications (Gutierrez and Davis, 1962; Gestetner et al., 1968) were cited in the Maximum Residue Limit Expert Report submitted to the EMEA for Quillaia saponins.

Okamura et al. (2003) incubated glycyrrhizin with fresh faeces from male Wistar rats and reported that the primary metabolite retrieved was glycyrrhetic acid. In addition, trace amounts of 3-α-hydroxyglycyrrhetic acid and 3-dehydroglycyrrhetic acid were formed when incubated under anaerobic conditions while under aerobic conditions, increasing amounts of 3-dehydroglycyrrhetic acid were produced over the 48-h incubation period.

Kim et al. (1999, 2000) isolated microbial glucuronidases from various intestinal bacteria and demonstrated that some glucuronidases remove both glucuronide moieties from glycyrrhizin, while others remove only one. Data from other *in vitro* studies also indicate that human intestinal bacteria isolated from faeces are capable of metabolising glycyrrhizinic acid to glycyrrhetic acid, as well as to 3-epi-glycyrrhetic acid and 3-dehydroglycyrrhetic acid (Hattori et al., 1983). In the same study, it was also demonstrated that human intestinal bacteria can metabolise glycyrrhetic acid to 3-epi-glycyrrhetic acid and the parent compound. A 'fair number' of bacteria from the human gastrointestinal flora were reported to have β-glucuronidase activity, and thus, the capability of metabolising glycyrrhizinic acid to glycyrrhetic acid (Hattori et al., 1985). A fewer number of bacteria were capable of reducing 3-dehydroglycyrrhizinic acid to glycyrrhizinic acid or 3-epi-glycyrrhizinic acid.

Escin Ia also is reported to be extensively metabolised by the intestinal microbiota and specifically by *Lactobacillus brevis* (Yang et al., 2004).

Anhuienoside C, a triterpenoid saponin from the rhizome of *Anemone flaccida* Fr. Schmidt (known as 'Di Wu' in Chinese), was demonstrated to be stable in fasted state simulating gastric fluid for at least 3 h and was sequentially deglycosylated into 4 metabolites by the intestinal microflora from male Sprague–Dawley rats (Zhao et al., 2015). The structures of the metabolites were determined using ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF-MS) and confirmed using nuclear magnetic resonance spectroscopy. The final metabolite was the aglycone oleanolic acid, whose structure also was confirmed using its reference standard. According to the authors, oral bioavailability of anhuienoside C was primarily limited by its bacterial metabolism.

Deglycosylation and oxygenation were considered primary and secondary metabolic pathways, respectively (Hasegawa, 2004; Qian and Cai, 2010; Wang et al., 2011). In the deglycosylation reactions, intestinal bacteria cleave the oligosaccharides connected to the C-3 or C-20 hydroxyl group of the aglycone in a step-wise manner from the terminal sugar. Using an UPLC-Q/TOF-MS technique, notoginsenoside Fc (NGFc), a protopanaxadiol (PPD)-Type saponin, was proposed to go through a series of deglycosylation reactions, forming eight metabolites, which were identified in the faeces of male Sprague–Dawley rats following a single oral dose of 100 mg/kg (He et al., 2015).

Human gastrointestinal microflora also is capable of metabolising the ginsenosides (Yu et al., 2012). *In vitro* studies indicated that the sugar side chains from ginsenosides Rb1, Rb2, Rc, Rd, Re and Rg1 were removed following anaerobic incubation with human fecal microflora. Incubation of American ginseng (*Panax quinquefolius* L.) root extract with human intestinal microflora obtained from the fresh faeces of a healthy male resulted in the identification of 25 metabolites that were either not detected

or present only in trace amounts in the original extract (Wan et al., 2013). Major metabolic pathways of the PPD and protopanaxatriol (PPT) ginsenosides included the removal of C-3 sugar moieties, removal of the C-20 sugar moieties, and dehydration, while the major metabolic pathways of the minor oleanane ginseng saponins were removal of sugar moieties from the C-3 and/or C-28 positions. Thus, deglycosylation by sequential cleavage of the sugar moieties was the primary metabolic pathway of saponins from American ginseng extract.

To elucidate further the metabolism of saponins in the digestive tract, Gestetner et al. (1968) excised the small intestine, cecum and colon of rats, mice and chicks that had been fed a standard diet lacking soybean flour for 14 days and incubated the various sections with soybean saponins extract at the corresponding pH levels for 3 h at 37°C. Chromatographic analysis of the digests indicated that only saponins were present in the small intestine, whereas both saponins and sapogenins were present in the cecum and colon. As summarised above, Gestetner et al. (1968) then demonstrated that the microorganisms from the cecum and colon were responsible for the metabolism of saponins to sapogenins and suggested that the enzyme responsible was a glycosidase(s) with a low degree of specificity due to the liberation of several sugars from soybean and alfalfa saponins.

In vivo studies

Glycirrhizin was metabolised to glycyrrhetic acid by the gastrointestinal microflora through the removal of the two molecules of glucuronic acid attached at carbon 3 following the oral administration of 10 mg/kg bw glycyrrhizin (i.e. glycyrrhizinic acid) to male Wistar and Sprague–Dawley rats (Takeda et al., 1996).

The low bioavailability of escin saponins was suggested by Wu et al. (2012) to be caused by first-pass metabolism in the gastrointestinal tract.

Ginseng contains a complex mixture of ginsenosides, with greater than 150 ginsenosides having been identified in various *Panax* species (Yu et al., 2012). Among these, the PPD-Type ginsenosides Rb1, Rb2, Rc and Rd and the PPT-type ginsenosides Re and Rg1 comprise greater than 90% of the total ginsenoside content. Ginseng ginsenosides are metabolised extensively in the gastrointestinal tract of animals and humans by the intestinal microflora following oral administration (Hasegawa, 2004; Qi et al., 2011; Wang et al., 2011; Yu et al., 2012; Wan et al., 2013).

Appendix L – ADME of related saponins

In vivo studies

In the Gestetner et al. (1968) study, groups of 10 male albino mice, 3 male rats and 3 male Leghorn chicks were fed diets comprising 20% heated soybean flour for 10 days, and their digestive tracts were excised for the determination of soybean saponin and sapogenin contents. Soybean saponins were detected (detection limit 40 μg) in the small intestine, but not in the caecum, colon, or blood of the mice, rats, and chicks that had consumed diets comprising 20% heated soybean flour for 10 days. Sapogenins (LOD 4 μg) were detected in the caecum and colon, but not in the small intestine or blood. The lack of detection of saponins and sapogenins in the blood indicates that neither the soya bean saponins nor their hydrolysis products were absorbed from the digestive tract of mice, rats, or chicks, within the LODs. The authors noted that the lack of detection of saponins in the cecum or colon did not necessarily indicate that they were fully metabolised to sapogenins, as they may have still been present but below the limit of detection due to their initially low concentration.

Yoshikoshi et al. (1995) came to a similar conclusion, as neither saponins nor their aglycones were detected in the blood (detection limit 0.01 μM) of Wistar rats that had consumed a diet containing 10% soybean hypocotyls for 2 weeks. Although not quantified, low levels of soybean saponins and greater levels of their aglycones were detected in faeces using thin layer chromatography. The authors suggested that these results indicated that the majority of soybean saponins were hydrolysed to their aglycones in the gastrointestinal tract.

The oral bioavailability of the escin saponins, glycyrrhizin, pulsatilla saponin D and DS-1 from *Dianthus superbus* has been investigated in rats (Wang et al., 1995; Wu et al., 2012; 2014; Ren et al., 2017; Ouyang et al., 2015). The escin saponins had oral bioavailabilities ranging from 0.16% to 1.97% of the administered dose (Wu et al., 2012, 2014). The oral bioavailability of glycyrrhizin following oral administration of a glycyrrhiza extract was 1.7%, whereas the oral bioavailability of glycyrrhizin following administration of the pure saponins was 4.0% (Wang et al., 1995). Thus, the bioavailability of glycyrrhizin was less when consumed as a complex mixture (i.e. the extract) than when consumed as a purified compound. The oral bioavailability of pulsatilla saponin D was slightly higher, at 2.83% (Ouyang et al., 2015), whereas the oral bioavailability of DS-1 was quite low, at 0.92% (Ren et al., 2014). Ren et al. (2014) suggested that the low oral bioavailability of DS-1 may be due to its large molecular mass, high hydrogen-bonding capacity, and poor water solubility.

Pharmacokinetic parameters were also investigated for asperosaponin VI, BTS-1 and hederacolchiside E (Yoo et al., 2008; Li et al., 2010; Luo et al., 2013). Although the oral bioavailabilities were not determined, the area under the plasma concentration–time curve (AUC) values were quite low, ranging from 0.56 to 138.3 μg*h/mL, and thus, it is expected that their corresponding oral bioavailabilities also will be low. Double peaks were observed in the plasma-concentration time curves for asperosaponin VI, which suggested that it may undergo enterohepatic recirculation (Li et al., 2010).

The pharmacokinetics of escin saponins were investigated in a single study involving 10 healthy male volunteers (Wu et al., 2010). Although the bioavailability of the isomers was not determined, the AUC values were low, ranging from 1.8 to 22.4 ng*h/mL, which suggests that the escin saponins have low oral bioavailability in humans. Similar to what was observed in rats, multiple peaks were observed in the AUC for the escin saponins in some subjects, suggesting that either tissue redistribution or enterohepatic recycling was occurring. A summary of the pharmacokinetic data from this study is presented in Table L.1.

Table L.1: Pharmacokinetic parameters for escin saponins (from Wu et al., 2010) (Documentation provided to EFSA n. 7)

Saponin	Population	Dose tested (mg)[(a)]	C_{max} (ng/mL)	$t_{1/2}$ (h)	MRT (h)	Apparent clearance (ml/min)	$AUC_{0-\infty}$ (ng*h/mL)	Bioavailability (F, %)	Assay information	Reference
Escin Ia	10 healthy males (20–30 years old; BMI = 20–24)	18.6	0.77 ± 0.64	8.5 ± 3.2	NR	1,020 ± 410	5.7 ± 3.9	ND	LC–MS/MS Validated according to U.S. FDA, 2001 LLOQ: 33 pg/mL	Wu et al. (2010)
Escin Ib		11.4	0.38 ± 0.26	4.7 ± 3.0	NR	2,900 ± 1,360	1.8 ± 0.9	ND		
Isoescin Ia		16.8	1.82 ± 1.60	13.7 ± 3.8	NR	964 ± 426	22.4 ± 13.6	ND		
Isoescin Ib		7.2	0.74 ± 0.73	8.4 ± 2.5	NR	3,890 ± 1,940	5.8 ± 3.8	ND		

BMI: body mass index; C_{max}: maximum concentration in plasma; LC–MS/MS: liquid chromatography tandem mass spectrometry; LLOQ: lower limit of quantification; MRT: mean residence time; ND: not determined; NR: not reported; $t_{1/2}$: half-life; AUC: area under the plasma concentration–time curve.
(a): From 60 mg total escins.

Appendix M – Characterisation of the test material used in toxicological studies evaluated by the SCF and JECFA

The current ADI of 5 mg/kg bw per day (expressed as dry extract 15% w/w) was set by the SCF in 1978 on the basis of two long-term studies in rats and mice.

The study in mice Butterworth (1977a, as referred to by the SCF (1978)) was later published by Phillips et al. (1979). The study in rats Butterworth (1977b, as referred to by the SCF (1978)) was later published as Drake et al. (1982).

Test material from the same supplier was possibly used also by Gaunt et al. (1974) in a short-term toxicity study in rats.

The characteristics of the test material used in these three studies, as described in the publications, are reported in Table M.1.

Table M.1: Description of the test material used in the toxicological studies by Gaunt et al. (1974), Phillips et al. (1979) and Drake et al. (1982)

	Gaunt et al. (1974)	**Phillips et al. (1979; cited by SCF as Butterworth, unpublished, 1977a)**	**Drake et al. (1982; cited by SCF as Butterworth, unpublished, 1977b)**
Description	Spray-dried aqueous extract prepared in such a way that 100 parts by weight of bark yielded approximately 15 parts of extract. In addition, the sample contained 5% lactose added to the extract before drying	Spray-dried aqueous extract prepared in such a manner that 100 parts by weight of bark yielded approximately 15 parts of extract before drying	Spray-dried aqueous extract prepared in such a way that 100 parts by weight of bark yielded approximately 15–18 parts of powdered extract
Supplier:	Food Industries Ltd, Birkenhead, Cheshire	Food Industries Ltd, Bromborough Port, Wirral, Merseyside	Food Industries Ltd, Bromborough Port, Merseyside
Moisture:	Less than 10%	Less than 10%	Less than 10%
Ash (at 550°C):	Less than 10%	Less than 10%	Less than 10%
Conformity:	Not stated; however, JECFA (2002b) considered the extract to meet specifications for Quillaia extract according to the Emulsifiers and Stabilisers in Food Regulation 1975	Requirements of The Emulsifiers and Stabilizers in Food Regulation 1975 (Statutory Instrument 1975, 1486)	UK Emulsifiers and Stabilisers in Food Regulation 1975 (Statutory Instrument 1975, no. 1486) and to that in the British Pharmacopoea (1973)

Appendix N – Characterisation of the test material used in the newly generated genotoxicity tests

Upon request from EFSA, additional studies performed in accordance with the EFSA SC opinion on genotoxicity testing strategies (EFSA Scientific Committee, 2011) were submitted to EFSA for evaluation (See Section 3.5.4).

The material used for these studies was characterised as presented in Table N.1.

Table N.1: Description of the test material used in the genotoxicity tests submitted in response to a request from EFSA (Documentation provided to EFSA n. 8)

Test	Specification	Results
Appearance	Dark brown liquid	Dark brown liquid
Absorbance	≤1.5 (10% w/w, 520 nm)	1,075
pH	3.7–4.1 (direct)	3.8
Soluble solids (° Brix)	46.0–52.0 (Hydrometer)	48.0
Saponin in solids (%)	20.0–26.0 (UPLC)	23.18
Saponin in product	9.0–13.0%	10.5
Loss on drying (%)	≤ 55%	53.83
Tannins in solids (%)	≤ 8.0%	1.28
Ash (% w/w) on dry solids	≤ 14%	4.50
Foam (mL)	60–120 mL	90
Specific gravity	1.21–1.24 g/cm^3	1.22
Sedimentation	2,000 ppm	Pass
Invert sugar (%)	≤ 40.0%	13.9
Lead (mg/kg)	≤ 2 ppm	0.12
Arsenic (mg/kg)	≤ 2 ppm	0.14
Mercury (mg/kg)	≤ 1 ppm	< 0.05
Yeast (CFU/g)	≤ 100	< 10
Mould (CFU/g)	≤ 100	< 10
Aerobic plate count (CFU/g)	≤ 1,000	< 10
E. coli	Negative	Negative
Salmonella	Negative	Negative
S. aureus	Negative	Negative

UPLC: ultra-performance liquid chromatography; CFU: colony forming unit.

Appendix O – Summary of anticipated exposure to Quillaia extract (E 999) from its use as a food additive in the regulatory maximum level exposure assessment scenario for the current authorised uses and in the refined exposure scenario including the proposed extension of use, in six population groups (minimum–maximum across the dietary surveys expressed as mg saponins/kg bw per day)

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario						
• Mean	0–0.4	0–1.9	0.1–2.1	0.2–1.6	0.1–0.7	< 0.1–0.2
• High level (95th percentile)	0–2.2	0–6.7	0.7–5.3	0.8–3.7	0.4–2.4	0.1–0.8
Refined exposure assessment scenario + proposed extension of use						
• Mean	< 0.1–< 0.1	< 0.1–0.2	< 0.1–0.2	< 0.1–0.2	< 0.1–0.1	< 0.1–< 0.1
• High level (95th percentile)	0–0.2	< 0.1–0.7	0.1–0.5	0.1–0.4	< 0.1–0.3	< 0.1–0.2

bw: body weight.