NATUREX
part of Givaudan


07 January 2020

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

Dear Dr. Gaynor:

**Re: GRAS Notice for Quillaia Extract Type 2**

In accordance with 21 CFR §170 Subpart E consisting of §§ 170.203 through 170.285, Naturex SA [250 rue Pierre Bayle, BP 81218 - 84911, Avignon, Cedex 9, France], as the notifier, is submitting one hard copy and one electronic copy (on CD) of the GRAS Notice for quillaia extract type 2, containing all data and information supporting the company's conclusion that quillaia extract type 2 is GRAS on the basis of scientific procedures, for use in specified conventional food and beverage products across multiple categories; these food uses of quillaia extract type 2 are therefore not subject to the premarket approval requirements of the *Federal Food, Drug and Cosmetic Act*. Information setting forth the basis for Naturex's GRAS conclusion, as well as a consensus opinion of an independent panel of experts, also are enclosed for review by the agency.

I certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Symantec Endpoint Protection 12.1.4.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Yours sincerely

[redacted]

**Coralie Chakour**
Regulatory Affairs Manager
Naturex SA

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# GRAS NOTICE FOR THE USE OF QUILLAIA EXTRACT TYPE 2 IN FOOD

**SUBMITTED TO:**
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

**SUBMITTED BY:**
Naturex SA
250 rue Pierre Bayle
BP 81218 - 84911
Avignon, Cedex 9
France

**DATE:**
07 January 2020

# GRAS Notice for the Use of Quillaia Extract Type 2 in Food

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# GRAS Notice for the Use of Quillaia Extract Type 2 in Food

## Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Naturex SA (Naturex) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended uses of quillaia extract type 2, as manufactured by Naturex, in various conventional food and beverage products as described in Part 1.3 below, are not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Naturex's view that these notified uses of quillaia extract type 2 are Generally Recognized as Safe (GRAS). In addition, as a responsible official of Naturex, the undersigned hereby certifies that all data and information presented in this notice represents a complete and balanced submission that is representative of the generally available literature. Naturex considered all unfavorable as well as favorable information that is publicly available and/or known to Naturex and that is pertinent to the evaluation of the safety and GRAS status of quillaia extract type 2 as an ingredient for addition to food and beverage products, as described herein.

Signed,

07 January 2020

Coralie Chakour
Regulatory Affairs Manager
Naturex SA
coralie.chakour@givaudan.com

Date

### 1.1 Name and Address of Notifier

Naturex SA
250 rue Pierre Bayle
BP 81218 - 84911
Avignon, Cedex 9
France

### 1.2 Common Name of Notified Substance

Quillaia extract type 2

## 1.3 Conditions of Use

Quillaia extract type 2 is intended for use as an emulsifier in the following food groups, as summarized in Table 1.3-1: alcoholic beverages, beverages and beverage bases, chewing gum, coffee and tea, condiments and relishes, confections and frostings, dairy product analogues, fats and oils, frozen dairy desserts, fruit and water ices, hard candy, jams and jellies, soft candy, and dietary supplements (for a technological purpose). These applications include direct use of quillaia extract, as well as carry over from use of quillaia extract type 2 in food flavors, colors, cloudy agents, and other ingredients in the proposed food uses. These proposed uses are additive to current food uses of quillaia extract that have Flavor and Extract Manufacturers Association of the United States (FEMA) GRAS status (FEMA No. 2973) or that have been concluded to be GRAS by the American Beverage Association (ABA) (see Part 3.1 and Table 3.1-1).

**Table 1.3-1 Summary of the Proposed Food-Uses and Use-Levels for Quillaia Extract Type 2 in the U.S.**

| Food Category (21 CFR §170.3) | Proposed Food-Uses | Quillaia Extract Type 2 Use-Level, as Saponins (mg/100 g)[a] | Quillaia Extract Type 2 Use-Level, (mg/100 g)[b] |
|---|---|---|---|
| Beverages, Alcoholic | Cocktail Drinks[c] | 19.5 | 30 |
| | Distilled Liquors[c] | 19.5 | 30 |
| Beverages and Beverage Bases | Energy Drinks[c] | 19.5 | 30 |
| Chewing Gum | Chewing Gum | 39.0 | 60 |
| Coffee and Tea | Specialty Coffee Drinks (Lattes, Cappuccinos, Mochas)[c] | 19.5 | 30 |
| Condiments and Relishes | Mustard | 120 | 184.6 |
| Confections and Frostings | Frostings, Icings | 120 | 184.6 |
| | Coatings | 120 | 184.6 |
| Dairy Product Analogs | Coffee Whiteners[c] | 19.5 | 30 |
| | Non-Dairy Milk and Cream | 45 | 69 |
| Fats and Oils | Fat-Based Sauces | 90 | 138.5 |
| | Mayonnaise and Mayonnaise-Type Dressings | 120 | 184.6 |
| | Salad Dressings | 90 | 138.5 |
| Frozen Dairy Desserts | Ice Cream * | 26 | 40 |
| | Other Frozen Milk Desserts | 65 | 100 |
| Fruit and Water Ices | Edible Ices, Sherbet, and Sorbet | 60 | 92.3 |
| Hard Candy | Hard Candy | 39 | 60 |
| Jams and Jellies | Jams, Jellies, Preserves, and Marmalades[c] | 19.5 | 30 |
| Soft Candy | Nougat and Toffees | 20 | 30.8 |
| | Gummies | 20 | 30.8 |
| | Soft Candy | 20 | 30.8 |
| Dietary Supplements | Solid dietary supplements | 600; 3 mg/500 mg serving[d] | 923; 4.6 mg/500 mg serving |
| | Liquid dietary supplements | 600; 30 mg/5 g serving[d] (~1 tsp) | 923; 46 mg/5 g serving |
| | Botanical supplements, powdered | 1,300; 6.5 mg/500 mg serving[d] | 2,000; 10 mg/500 mg serving |

**Table 1.3-1 Summary of the Proposed Food-Uses and Use-Levels for Quillaia Extract Type 2 in the U.S.**

| Food Category (21 CFR §170.3) | Proposed Food-Uses | Quillaia Extract Type 2 Use-Level, as Saponins (mg/100 g)[a] | Quillaia Extract Type 2 Use-Level, (mg/100 g)[b] |
|---|---|---|---|

CFR = Code of Federal Regulations; tsp = teaspoon; U.S. = United States.

[a] Use-levels 'as saponins' (reference component) were used in the intake assessment described in Part 3.2.2, considering both current and proposed use-levels. When there was overlap in the 'current' (as Table 3.1-1) and 'proposed' uses, the proposed use-levels were utilized. In cases where a single food-use within a food category was proposed at a higher use-level than current uses, the proposed use-level was used for the identified food-use and the current use-level was used for all remaining food-uses within the food category.

[b] Use-levels converted from a saponin basis (reference component) to the ingredient itself using a minimum specification of 65%. These values were used in the intake assessment described in Part 3.2.3, considering proposed use-levels only.

[c] Use of quillaia extract is present at specified use-level in final food through carry-over, including food flavors, colors, cloudy agent, or other ingredients.

[d] Values for an average serving were based on typical products in which quillaia extract are proposed for use.

* Quillaia is intended for use in unstandardized products when standards of identity do not permit its addition.

## 1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a)(b) of the Code of Federal Regulations (CFR) (U.S. FDA, 2019), Naturex has concluded that the intended uses of quillaia extract type 2 as described herein are GRAS on the basis of scientific procedures.

## 1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. FDA upon request, or will be available for review and copying at reasonable times at the offices of:

Naturex SA
250 rue Pierre Bayle
BP 81218 - 84911
Avignon, Cedex 9
France

Should the FDA have any questions or additional information requests regarding this Notification, Naturex will supply these data and information upon request.

## 1.6 Freedom of Information Act, 5 U.S.C. 552

It is Naturex's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempted from the Freedom of Information Act, 5 U.S.C. 552.

# Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

## 2.1 Identity

### 2.1.1 Common or Usual Names and Synonyms

| | |
|---|---|
| Botanical Name: | *Quillaja saponaria* Molina (family *Rosaceae*) |
| Common Name: | Quillaia extract type 2 |
| Synonyms: | Quillaja extract type 2, Soapbark extract, Quillay bark extract, Bois de Panama, Panama bark extract, Quillai extract |
| Commercial Name: | Sapnov™; Uptaia™ |

### 2.1.2 Chemical Abstract Service (CAS) Number

68990-67-0

### 2.1.3 Other Identification Numbers

E999, FEMA GRAS No. 2973, INS No. 999(ii)

### 2.1.4 Botanical Source

| | |
|---|---|
| Botanical Source: | *Quillaja saponaria* Molina (family *Rosaceae*) |
| Part of plant used: | Wood and/or bark |
| Known toxicants: | The toxicity of quillaia extracts has been attributed to the quillaia saponins on the basis that the median lethal dose ($LD_{50}$) values for the type 1 and 2 extracts were about the same when expressed on a saponin basis (JECFA, 2006a).<br><br>All quillaia extract test articles in the studies discussed herein were converted to a saponin basis, taking into consideration that the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the test material in the toxicology studies was representative of a type 1 extract and the lower limit of 20% saponins in the type 1 extract (JECFA, 2006b).<br><br>Dietary intake estimates for quillaia extract type 2 were assessed on a saponin basis and included estimates of intake from current permitted uses of quillaia extract (see Part 3.2). |

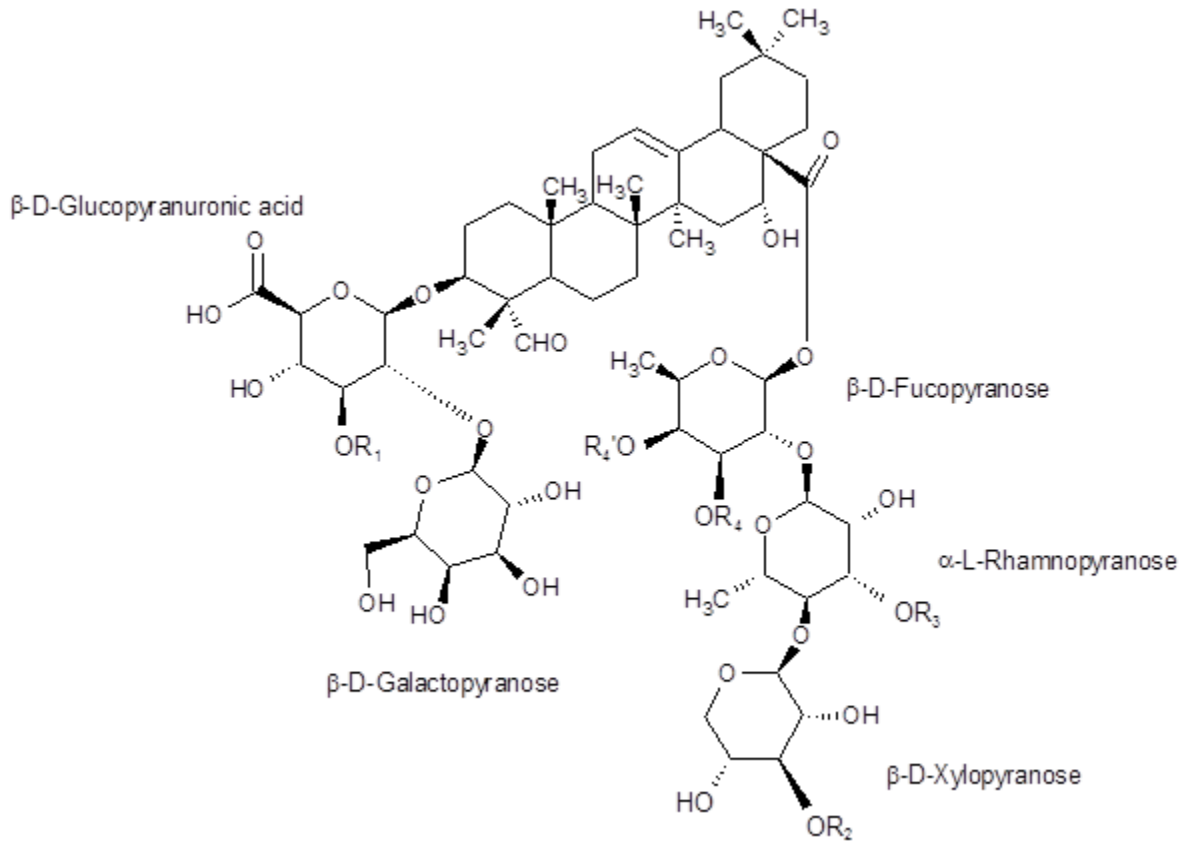
### 2.1.5 Chemical and Physical Characteristics

Quillaia extract type 2 is manufactured as a clear brown or brown liquid or powder that is very soluble in water and insoluble in standard solvents (*e.g.*, ethanol, acetone, methanol, and butanol). The primary component of quillaia extract type 2 is quillaia saponins, which consist of a hydrophobic fat-soluble triterpene structure with water-soluble carbohydrate chains. The molecular weight of monomeric saponins is approximately 1,800 to 2,300 g/mol, which is consistent with a triterpene containing 8 to 10 monosaccharide units, such as those found in quillaia extract type 2.

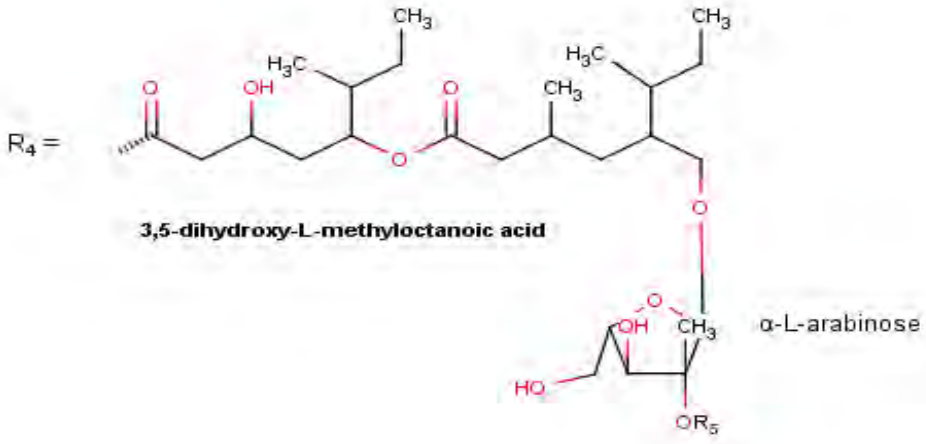
### 2.1.6 Chemical Structure of Quillaia Saponins

Quillaia saponins have 2- to 5-unit sugar chains attached at C-3 and C-28 of the aglycone and an 18-carbon acyl chain attached to the fucose first sugar unit at C-28 position in the majority of the saponins (see Figure 2.1.6-1 below). A summary of the molecular structure of the 4 major quillaia saponins is provided in Table 2.1.6-1 below.

**Figure 2.1.6-1 Chemical Structure of Quillaia Saponins**




**Quillaia Saponins**




**18-Carbon Acyl Sidechain of Quillaia Saponins**

**Table 2.1.6-1 Molecular Structures of Quillaia Saponins**

| Saponin | R1 | R2 | R3 | R4 | R4' | R5 | Molecular Weight |
|---|---|---|---|---|---|---|---|
| QS 7 | ND | ND | ND | ND | ND | ND | 1,862 |
| QS 17 | β-D-xylose | β-D-Apicose | β-D-glucose | 18-Carbon Acyl Sidechain | H | α-L-rhamnose | ND |
| QS 18 | β-D-xylose | β-D-Apicose | β-D-glucose | 18-Carbon Acyl Sidechain | H | H | 2,150 |
| QS 21 | β-D-xylose | β-D-Apicose | H | 18-Carbon Acyl Sidechain | H | H | 1,988 |

ND = not determined.
Source: FAO (2005).

## 2.2 Manufacturing

### 2.2.1 Raw Materials, Processing Aids, and Additives

Quillaia is extracted from wood and/or bark of the Chilean tree *Quillaja saponaria* Molina (family *Rosaceae*), a large evergreen tree with shiny, leathery leaves and thick bark that is harvested in a sustainable manner. The source wood and/or bark is not classified as a genetically modified organism (GMO). The material does not contain any ingredients or processing aids that might have been derived from GMOs. The organoleptic quality is confirmed by assessing the aspect, color, and flavor of the quillaja wood and/or bark, and the identity is confirmed using thin layer chromatography. To ensure the material is fit for human consumption, Naturex has also established limits for heavy metals (lead <3 ppm, arsenic <3 ppm, cadmium <1 ppm, and mercury <0.1 ppm) and foreign matter (<2%, consistent with the European Pharmacopeia) in the wood and bark. Pesticide residues in the quillaja wood and bark must also conform to the limits established in Regulation (EC) No. 396/2005 (EC, 2005) and the United States Pharmacopeia (USP, 2018).

Naturex manufactures quillaia extract type 2 in both powder and liquid forms and the processing aids and food additives used to manufacture quillaia extract type 2 liquid and powder vary depending on the requirements of Naturex's customers and the target market (*e.g.*, vegan, organic, *etc.*). Regardless, all food contact articles, processing aids, and additives used in the manufacture of quillaia extract type 2 are food-grade and approved for their intended use in accordance with an appropriate federal regulation, effective food contact notification, or have previously been concluded to be GRAS. Examples of the processing aids and additives used in the production of quillaia extract type 2 include, but are not limited to, phosphoric acid, citric acid, hydrochloric acid, pectinase, bentonite, perlite, polyvinylpyrrolidone, diatomaceous earth, pea protein, bovine gelatin, and sodium benzoate.

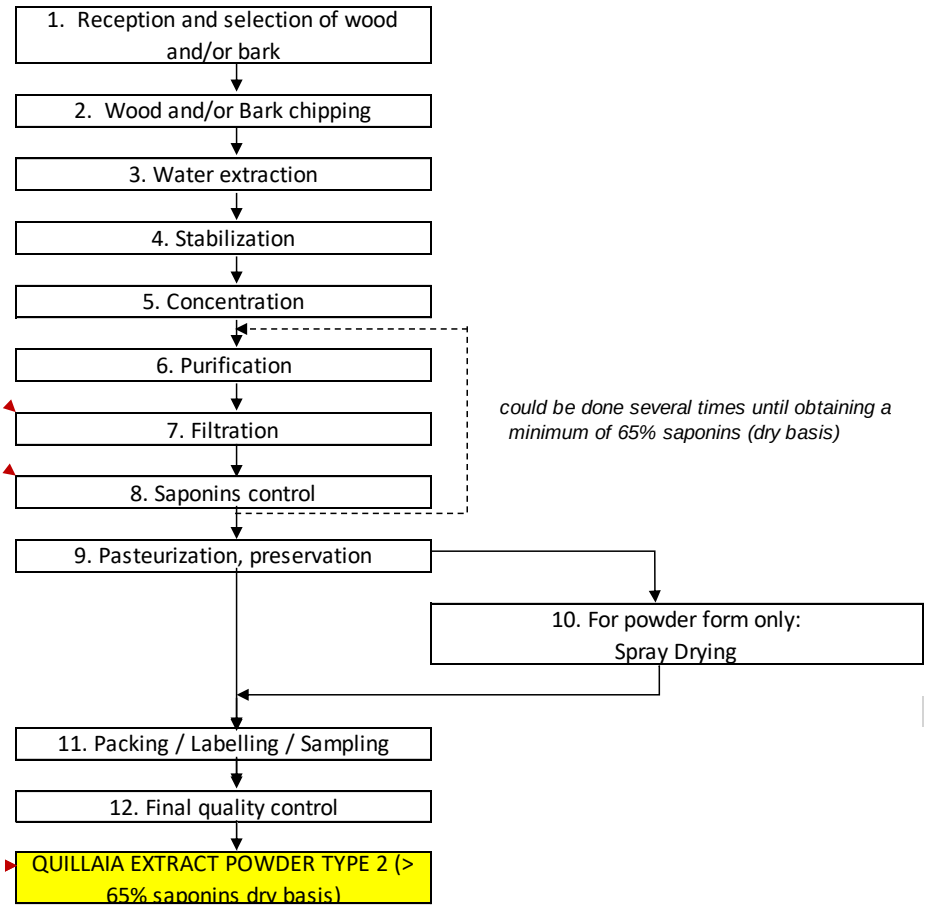
### 2.2.2 Manufacturing Process

Quillaia extract type 2 is manufactured following a Food Safety Assurance Plan based on the Hazard Analysis and Critical Control Point (HACCP) system and in accordance with the principles of current Good Manufacturing Practice (cGMP).

To produce quillaia extract type 2, *Quillaia saponaria* wood and/or bark is milled to wood/bark chips, which are then extracted with the addition of hot water. The extract is stabilized using a food-grade acid (thus reducing pH to less than 4.0) and a pectinase enzyme preparation may or may not be used. Following stabilization, the extract is concentrated by evaporation. The extract is then purified and filtered using food-grade clarifying agents and/or filtration aids until a minimum content of 65% saponins on a dry basis is

achieved. The saponin content in the extract is determined by high-performance liquid chromatography (HPLC). Once the required saponin levels are achieved, the extract is pasteurized. For liquid products, the liquid extract can be formulated with 0.1 to 0.2% sodium benzoate as a preservative. For powder products, the liquid extract is spray-dried with an inlet air temperature of <150°C. Samples of the final quillaia extract type 2 product are retained for final quality control analyses, consisting of general aspect, analytical quality, and microbiological quality before the product is released. See Figure 2.2.2-1 below for a schematic overview of the manufacturing process for quillaia extract type 2.

**Figure 2.2.2-1 Schematic Overview of the Manufacturing Process for Quillaia Extract Type 2**



Potential hazards during each step of the manufacturing process are documented along with their methods of control and acceptable limits as a part of the HACCP system and maintain compliance with cGMP. Upon assessment of the potential hazards of quillaia extract type 2 manufacturing, the pasteurization step was identified as the only critical control point. The critical limit for quillaia extract type 2 identified in the HACCP plan and the justification for its selection are provided in Table 2.2.2-1 below.

**Table 2.2.2-1 Critical Control Point in the Manufacture of Quillaia Extract Type 2**

| Critical Control Point | Limit | Justification |
|---|---|---|
| Pasteurization | ≥72°C for ≥20 minutes | *Salmonella* growth is inhibited at 46.2°C. Pasteurization at ≥72°C for ≥20 minutes will result in the elimination of pathogens such as *Salmonella*. |

## 2.3 Product Specifications and Batch Analyses

### 2.3.1 Specifications

Naturex has established physical, chemical, and microbiological limits for quillaia extract type 2 liquid and powder that are consistent with those established by JECFA (2014) to ensure the products are of food-grade quality. The physical, chemical, and microbiological specifications for quillaia extract type 2 liquid and powder established by Naturex are presented in Table 2.3.1-1 along with the specifications provided by JECFA for comparison. The specifications presented in Table 2.3.1-1 are applicable to all quillaia extract type 2 products, regardless of the specific processing aids used in their manufacture.

**Table 2.3.1-1 Physical, Chemical, and Microbiological Specifications for Quillaia Extract Type 2**

| Specification Parameter | Specification | | | Method |
|---|---|---|---|---|
| | JECFA Quillaia Extract Type 2[a] | Naturex (liquid products) | Naturex (powder products) | |
| ***Identity*** | | | | |
| Appearance | Light red-brownish liquid or powder | Clear brown or brown liquid | Clear brown or brown powder | Visual |
| Flavor | NE | Bittersweet | Bittersweet | Sensory testing |
| Brix | NE | >20 | NA | Brix meter |
| Color/absorbance | NE[b] | 2 max absorbance of a 10° Brix @ 520 nm (50% w/w) solution | 2 max absorbance of a 10% w/w @ 520 nm | Spectrometry UV |
| ***Chemical Specifications*** | | | | |
| pH | 3.7 to 5.5 (4% solution) | 3.7 to 4.2 | 3.7 to 4.3 | pH meter |
| Water/loss on drying (%)[c] | 50 to 90 | 50 to 90 | NA | I.R. Balance (CQ-MO-018) |
| Moisture content (%) | NMT 6 | NA | <6.00 | I.R. Balance (CQ-MO-018) |
| Total solid content (%)[c] | NE | Report | NA | I.R. Balance (CQ-MO-018) |
| Ash (% dwb) | NMT 5 | See control limits | See control limits | See control limits |
| Saponins (% dwb) | 65 to 90 | 65 to 75 | >65 | HPLC |
| Tannins (% dwb) | NMT 8 | See control limits | See control limits | See control limits |

**Table 2.3.1-1 Physical, Chemical, and Microbiological Specifications for Quillaia Extract Type 2**

| Specification Parameter | Specification | | | Method |
|---|---|---|---|---|
| | JECFA Quillaia Extract Type 2[a] | Naturex (liquid products) | Naturex (powder products) | |
| ***Heavy Metals*** | | | | |
| Lead (mg/kg) | NMT 2 | See control limits | See control limits | See control limits |
| ***Microbiological Specifications*** | | | | |
| Aerobic plate count (cfu/g) | NE | <100 | <100 | CQ-MO-231; NF EN ISO 4833 |
| Yeast (cfu/g) | NE | <10 | <10 | CQ-MO-244; NF V 08-059 |
| Mold (cfu/g) | NE | <10 | <10 | CQ-MO-244; NF V 08-059 |

cfu = colony-forming units; dwb = dry weight basis; HPLC = high-performance liquid chromatography; I.R. = infrared; JECFA = Joint Expert Committee for Food Additives; NA = not applicable; NE = not established; NMT = not more than; UV = ultraviolet.
[a] JECFA (2014).
[b] Limit not established for liquid forms.
[c] Internal measure for calculation of parameters with limits on a dry weight basis.

Additional product control plan limits were established to ensure the final products meet all specifications established by JECFA for quillaia extract type 2 (JECFA, 2014). The product control plan includes compositional limits and limits for lead (see Table 2.3.1-2 below).

**Table 2.3.1-2 Product Control Limits for Quillaia Extract Type 2 Liquid and Powder**

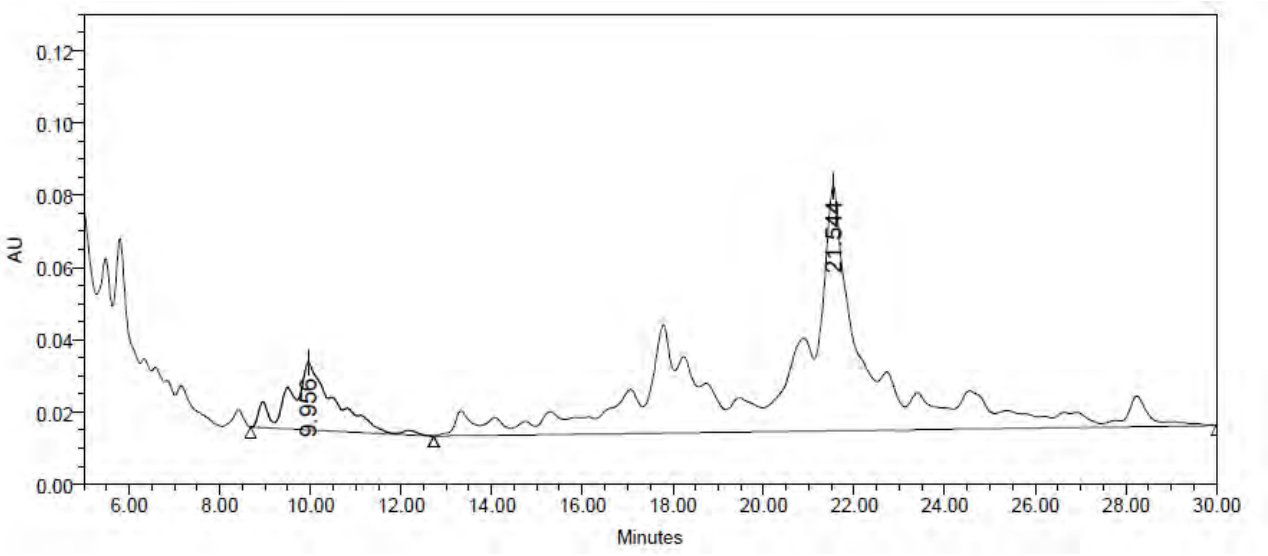
| Specification Parameter | Specification | | Method |
|---|---|---|---|
| | JECFA Quillaia Extract Type 2[a] | Naturex | |
| ***Compositional Limits*** | | | |
| Ash (% dwb) | NMT 5 | ≤5 | Oven |
| Tannins (% dwb) | NMT 8 | ≤8 | AOAC 17 Ed. 999.11 (external laboratory) |
| ***Heavy Metals Limits*** | | | |
| Lead (mg/kg) | NMT 2 | ≤2 | ICP |

dwb = dry weight basis; ICP = inductively coupled plasma; JECFA = Joint Expert Committee for Food Additives; NMT = not more than.
[a] JECFA (2014).

## 2.3.2 Batch Analysis

The saponins in quillaia extract type 2 are separated and their total quantity calculated using reverse phase HPLC. Six non-consecutive lots of quillaia extract type 2 liquid were analyzed in triplicate using the HPLC method, and the results demonstrate that the chromatographic profiles are consistent with the chromatographic standard in the JECFA specifications (JECFA, 2014). A representative chromatogram is provided in Figure 2.3.2-1. The HPLC chromatogram also is representative of quillaia extract type 2 powder, as quillaia extract type 2 powder is obtained by adding a final drying step to the liquid extract after purification, concentration, and pasteurization (see Part 2.2.2). The peak eluting at 9.956 minutes corresponds to the saponin QS-7, while the major peak eluting at 21.544 minutes corresponds to the major saponin QS-18. The reported saponin content for each lot in Table 2.3.2-1 below was calculated from the mean value of the triplicate results (as-is) for each lot and expressed on a dry weight basis. The saponin content of the quillaia extract type 2 powder products (see Table 2.3.2-2) were calculated using the same method.

**Figure 2.3.2-1 Chromatogram of Quillaia Extract Type at 220 nm (Lot No.: Q110/002/A18)**




Analysis of 6 non-consecutive lots of quillaia extract type 2 liquid and 4 lots of quillaia extract type 2 powder demonstrates that the manufacturing process as described in Part 2.2 produces a consistent product that meets specifications. A summary of the physical, chemical, and microbiological analyses for the 6 lots of quillaia extract type 2 liquid and the 4 lots of quillaia extract type 2 powder is presented in Tables 2.3.2-1 and 2.3.2-2, respectively.

**Table 2.3.2-1 Summary of Batch Analysis Results for 6 Lots of Quillaia Extract Type 2 Liquid**

| Specification | Limit | Manufacturing Lot | | | | | |
|---|---|---|---|---|---|---|---|
| ***Identity*** | | | | | | | |
| Appearance | Clear brown liquid | Conforms | Conforms | Conforms | Conforms | Conforms | Conforms |
| Flavor | Bittersweet | Conforms | Conforms | Conforms | Conforms | Conforms | Conforms |
| Brix | >20 | 23.5 | 23.4 | 23.5 | 23.6 | 23.4 | 24.8 |
| Color/Absorbance | 2 max absorbance of a 10° Brix (50% w/w) solution | 1.704 | 1.55 | 1.57 | 1.694 | 1.83 | 0.923 |
| ***Chemical Specifications*** | | | | | | | |
| pH | 3.7 to 4.2 | 4.12 | 3.8 | 4.14 | 4.14 | 4.08 | 3.91 |
| Total solid content (%)[a] | Report[b] | 21.713 | 21.553 | 21.172 | 22.438 | 22.496 | 23.376 |
| Water/loss on drying (%)[a] | 50 to 90 | 78.287 | 78.447 | 78.828 | 77.562 | 77.504 | 76.624 |
| Saponins (% dwb) | 65 to 75 | 70.262 | 72.449 | 74.05 | 70.41 | 74.43 | 73.828 |

**Table 2.3.2-1 Summary of Batch Analysis Results for 6 Lots of Quillaia Extract Type 2 Liquid**

| Specification Parameter | Limit | Manufacturing Lot | | | | | |
|---|---|---|---|---|---|---|---|
| | | Q059/003/ D17 | Q059/005/ D17 | Q059/007/ D17 | Q110/002/ A18 | Q110/004/ A18 | Q310/002/ A17 |
| ***Microbiological Specifications*** | | | | | | | |
| Aerobic plate count (cfu/g) | <100 | Conforms | Conforms | Conforms | <10 | <10 | <100 |
| Yeast (cfu/g) | <10 | Conforms | Conforms | Conforms | <10 | <10 | <10 |
| Mold (cfu/g) | <10 | Conforms | Conforms | Conforms | <10 | <10 | <10 |

cfu = colony-forming units; dwb = dry weight basis.

[a] Internal measure for calculation of parameters with limits on a dry weight basis. Result not reported on Certificate of Analysis.

[b] No limit established; however, total solid content is required to calculate the parameters that are reported on a dry weight basis.

**Table 2.3.2-2 Summary of Batch Analysis Results for 4 Lots of Quillaia Extract Type 2 Powder**

| Specification Parameter | Limit | Manufacturing Lot | | | |
|---|---|---|---|---|---|
| | | Q126/001/A19 | Q240/004/D18 | Q243/006/A18 | Q155/003/D19 |
| ***Identity*** | | | | | |
| Appearance | Powder | Conforms | Conforms | Conforms | Conforms |
| Flavor | Bittersweet | Conforms | Conforms | Conforms | Conforms |
| Color/Absorbance | 2 max absorbance of a 10% w/w @ 520 nm | 1.60 | 1.6 | 1.607 | 1.60 |
| ***Chemical Specifications*** | | | | | |
| pH | 3.7 to 4.3 | 3.7 | 3.95 | 3.73 | 3.73 |
| Moisture content (%)[a] | <6.00 | 4.66 | 3.93 | 4.664 | 4.66 |
| Saponins (% dwb) | >65 | 65.21 | 72.70 | 65.21 | 65.21 |
| ***Microbiological Specifications*** | | | | | |
| Aerobic plate count (cfu/g) | <100 | <100 | <100 | <100 | <100 |
| Yeast (cfu/g) | <10 | <10 | <10 | <10 | <10 |
| Mold (cfu/g) | <10 | <10 | <10 | <10 | <10 |

cfu = colony-forming units; dwb = dry weight basis.

[a] Internal measure for calculation of parameters with limits on a dry weight basis. Result not reported on Certificate of Analysis.

The 6 non-consecutive lots of quillaia extract type 2 liquid also were analyzed for the control plan parameters. The results (see Table 2.3.2-3) demonstrate that the manufacturing process as described in Part 2.2 produces a consistent product that meets all control plan limits. The analytical results for the liquid product also are representative of the powder product, as quillaia extract type 2 powder is obtained by adding a final drying step to the liquid extract after purification, concentration, and pasteurization (see Part 2.2.2).

**Table 2.3.2-3 Summary of Control Plan Analytical Results for 6 Lots of Quillaia Extract Type 2 Liquid**

| Specification Parameter | Limit | Manufacturing Lot | | | | | |
|---|---|---|---|---|---|---|---|
| | | Q059/003/ D17 | Q059/005/ D17 | Q059/007/ D17 | Q110/002/ A18 | Q110/004/ A18 | Q310/002/ A17 |
| ***Compositional Limits*** | | | | | | | |
| Ash (% dwb) | ≤5 | 3.59 | 3.94 | 2.93 | 4.59 | 4.45 | 4.57 |
| Tannins (% dwb) | ≤8 | NT | NT | NT | 1.30 | 1.05 | 1.05 |
| ***Heavy Metals*** | | | | | | | |
| Lead (mg/kg) | ≤2 | NT[a] | NT | NT | 0.047 | 0.091 | 0.047 |

dwb = dry weight basis; NT = not tested.

[a] Lead is tested once a year as part of the control plan. Frequency of testing was determined based on the usual levels for lead.

## 2.3.3 Additional Chemical Characterization

Analyses of the polyphenol content and nutritional composition were conducted on samples of quillaia extract type 2 liquid. The analytical results for the liquid product also are considered representative of the powder product, as quillaia extract type 2 powder is obtained by adding a final drying step to the liquid extract after purification, concentration, and pasteurization (see Part 2.2.2).

Polyphenols are a minor component of quillaia extract type 2. The polyphenol content of 6 lots of quillaia extract type 2 liquid was determined, and the levels ranged from 1.31 to 5.22% of the extract. A summary of the polyphenols content in 6 lots of quillaia extract type 2 is presented in Table 2.3.3-1.

**Table 2.3.3-1 Summary of Polyphenols Batch Analysis Results for 6 Lots of Quillaia Extract Type 2 Liquid**

| Specification Parameter | Manufacturing Lot | | | | | | Method |
|---|---|---|---|---|---|---|---|
| | Q059/003/ A17 | Q059/005/ D17 | Q059/007/ D17 | Q059/010/ D17 | Q060/001/ D17 | Q065/002/ D17 | |
| Polyphenols (%) | 1.37 | 1.52 | 1.31 | 4.68 | 5.22 | 4.91 | Folin Ciocalteu |

Three of the 6 lots of quillaia extract type 2 (liquid) were analyzed for nutritional content and are presented in Table 2.3.3-2.

**Table 2.3.3-2 Summary of Nutritional Analysis Results for 3 Lots of Quillaia Extract Type 2 Liquid**

| Parameter | Manufacturing Lot | | | Method |
|---|---|---|---|---|
| | Q059/003/D17 | Q059/005/D17 | Q059/007/D17 | |
| Starch (%) | <0.3 | <0.3 | <0.3 | Continuous flow |
| Fructose (%) | <0.1 | <0.1 | <0.1 | HPAEC-PAD |
| Glucose (%) | <0.1 | <0.1 | <0.1 | HPAEC-PAD |
| Lactose (%) | <0.1 | <0.1 | 0.13 | HPAEC-PAD |
| Maltose (%) | <0.1 | <0.1 | <0.1 | HPAEC-PAD |
| Sucrose (%) | <0.1 | <0.1 | <0.1 | HPAEC-PAD |

**Table 2.3.3-2 Summary of Nutritional Analysis Results for 3 Lots of Quillaia Extract Type 2 Liquid**

| Parameter | Manufacturing Lot | | | Method |
|---|---|---|---|---|
| | Q059/003/D17 | Q059/005/D17 | Q059/007/D17 | |
| ***Compositional Analysis*** | | | | |
| Total nitrogen (g/100 g) | <0.08 | <0.08 | <0.08 | Kjeldahl (titration) |
| Protein (g/100 g) | <0.5 | <0.5 | <0.5 | Kjeldahl (titration) |
| High molecular weight fibers + resistant starch (%) | ND | 0.9 | ND | AOAC 2009.01 |
| Dietary fiber with low molar masses (%) | ND | <0.3 | ND | AOAC 2009.01 |
| Total dietary fiber (%) | ND | 0.9 | ND | AOAC 2009.01 |
| Galactose (%) | <0.1 | <0.1 | <0.1 | HPAEC-PAD |
| ***Element Analysis*** | | | | |
| Calcium (mg/kg) | 810 ± (160) | 790 ± (160) | 770 ± (150) | ICP-OES |
| Magnesium (mg/kg) | 610 ± (120) | 580 ± (120) | 580 ± (120) | ICP-OES |

HPAEC-PAD = high-performance anion-exchange chromatography with pulsed amperometric detection; ICP-OES = inductively-coupled plasma optical emission spectroscopy; ND = not determined.

## 2.4 Stability Data

Quillaia extract type 2 (liquid and powder) should be stored between 5 and 25°C, sheltered from light, moisture, and oxygen. The shelf-life for quillaia extract type 2 is 24 months when stored under the recommended conditions and in its original packaging.

Three lots of quillaia extract type 2 liquid were stored under ambient conditions in Naturex's Chilean facility (20 to 25°C and 50 to 75% relative humidity) for 22 to almost 30 months and re-tested for appearance, flavor, Brix, saponin content, aerobic plate count, yeast, and mold. The results are summarized below in Table 2.4-1. The results demonstrate that the saponin content remained stable and the microbiological counts did not exceed the specified limits when quillaia extract type 2 liquid was stored approximately 30 months. Thus, quillaia extract type 2 remained stable for the duration of the shelf-life of 24 months. On the basis that the chemical and microbiological parameters are known to be more susceptible to instability in liquid form compared to powder form, the stability studies performed on a liquid product are considered as the worst case and thus representative for both preparations. Therefore, the powder products also are concluded to be stable for the duration of the shelf-life of 24 months.

**Table 2.4-1 Results of Stability Testing for Quillaia Extract Type 2 Liquid When Stored under Ambient Conditions 20 to 25°C and 50 to 75% Relative Humidity) for up to Approximately 30 Months**

| Specification Parameter | Limit | Manufacturing Lot | | | | | |
|---|---|---|---|---|---|---|---|
| | | Q033/001/A16 | | Q193/005/A16 | | Q272/001/A16 | |
| | | Batch Release | Retest (~30 Months) | Batch Release | Retest (24 Months) | Batch Release | Retest (22 Months) |
| **Testing Date** | | **02 Feb 2016** | **21 Jul 2018** | **11 Jul 2016** | **21 Jul 2018** | **28 Sept 16** | **21 Jul 2018** |
| ***Identity*** | | | | | | | |
| Appearance | Clear brown liquid | Conforms | Conforms | Conforms | Conforms | Conforms | Conforms |
| Flavor | Bittersweet | Conforms | Conforms | Conforms | Conforms | Conforms | Conforms |
| Brix | >20 | 22 | 22.8 | 22.4 | 22.5 | 21 | 20.9 |
| ***Chemical Specifications*** | | | | | | | |
| Saponins (% dwb) | 65 to 75 | 67.45 | 68.6 | 74.113 | 74.54 | 74.378 | 70.95 |
| ***Microbiological Specifications*** | | | | | | | |
| Aerobic Plate Count (cfu/g) | <100 | Conforms | Conforms | Conforms | Conforms | Conforms | Conforms |
| Yeast (cfu/g) | <10 | Conforms | Conforms | Conforms | Conforms | Conforms | Conforms |
| Mold (cfu/g) | <10 | Conforms | Conforms | Conforms | Conforms | Conforms | Conforms |

cfu = colony-forming units; dwb = dry weight basis.

# Part 3. §170.235 Dietary Exposure

## 3.1 Current Regulatory Status

In the U.S., quillaia extract, type not differentiated, is permitted for direct addition to food for human consumption under its common name, quillaia (soapbark) under 21 CFR §172.510 (U.S. FDA, 2019). Quillaia extract type 1 was concluded to be GRAS by the ABA for use as a foaming agent in semi-frozen carbonated and non-carbonated beverages at levels not to exceed 500 mg/kg (dried basis) in beverage concentrate prior to the incorporation of water and carbon dioxide or air in retail establishments. The aforementioned GRAS uses of quillaia were notified to the offices of the U.S. FDA by the ABA under the voluntary GRAS notification program and the notice was filed by the Agency under GRN 165 without objection (GRN No. 000165, U.S. FDA, 2005). Quillaia extract also has been concluded to be GRAS by the Expert Panel of the Flavor and Extract Manufacturers Association. In the most recent evaluation in 2015, the FEMA Expert Panel concluded the use of quillaia extract (assumed to be type 1 on the basis of the use-levels in comparison to previous FEMA GRAS conclusions and the year of the earlier conclusions) was GRAS (Cohen *et al*., 2015). Dietary supplements containing quillaia extract also are expected to be present in the U.S. marketplace and appear to have been in use prior to 15 October 1994, due to its inclusion the Utah Natural Products Alliance master 'Old Dietary Ingredient List', as provided by the American Herbal Products Association (UNPA, 1998).

A summary of the permitted food-uses and use-levels organized according to the food categories in 21 CFR §170.3 (U.S. FDA, 2019) is provided below in Table 3.1-1. All permitted food-uses of quillaia extract were assumed to be type 1 and converted to a saponin basis assuming a maximum saponin content of 26% w/w on the basis of the upper limit in the specifications for quillaia extract type 1 as established by JECFA (JECFA, 2006b).

**Table 3.1-1 Summary of the Current[a] Food-Uses and Use-Levels for Quillaia Extract in the U.S.**

| Food Category (21 CFR §170.3) (U.S. FDA, 2019) | Current Food-Uses | Quillaia Extract Use-Level, Ave./Max. (ppm)[b] | Quillaia Extract Use-Level, as Saponins, Ave./Max. (mg/100 g)[c] |
|---|---|---|---|
| Baked Goods and Baking Mixes | Baked Goods | 24/30 | 0.6/0.8 |
| Beverages, Alcoholic | Alcoholic Beverages | 90/100 | 2.3/2.6 |
| Beverages and Beverage Bases[d] | Semi-Frozen Carbonated and Non-Carbonated Beverages | 500 | 13.0 |
| | Other Non-Alcoholic Beverages[e] | 91.5/103 | 2.4/2.7 |
| Breakfast Cereals | Breakfast Cereals | 15/75 | 0.4/2.0 |
| Cheeses | Cheese | 15/75 | 0.4/2.0 |
| Chewing Gum | Chewing Gum | 7.5/30 | 0.2/0.8 |
| Coffee and Tea | Instant Coffee and Tea | 1.5/30 | <0.1/0.8 |
| Condiments and Relishes | Condiments and Relishes | 15/75 | 0.4/2.0 |
| Confections and Frostings | Confections and Frostings | 7.5/30 | 0.2/0.8 |
| Dairy Product Analogues | Imitation Dairy | 7.5/30 | 0.2/0.8 |
| Egg Products | Egg Products | 7.5/30 | 0.2/0.8 |
| Fish Products | Fish Products | 7.5/30 | 0.2/0.8 |
| Frozen Dairy Desserts | Frozen Dairy | 15/75 | 0.4/2.0 |
| Fruit and Water Ices | Fruit Ices | 7.5/30 | 0.2/0.8 |
| Gelatins, puddings, and fillings | Gelatins and Puddings | 7.5/30 | 0.2/0.8 |
| Gravies and Sauces | Gravies | 7.5/30 | 0.2/0.8 |
| Grain Products and Pastas | Other Grains | 7.5/30 | 0.2/0.8 |
| Hard Candy | Hard Candy | 18/30 | 0.5/0.8 |
| Herbs, Seeds, Spices, Seasonings, Blends, Extracts, and Flavorings | Seasonings and Flavors | 15/75 | 0.4/2.0 |
| Jams and Jellies | Jams and Jellies | 7.5/30 | 0.2/0.8 |
| Meat Products | Meat Products | 7.5/75 | 0.2/2.0 |
| Milk Products | Milk Products | 1.5/30 | <0.1/0.8 |
| Nuts and Nut Products | Nut Products | 30/120 | 0.8/3.1 |
| Poultry Products | Poultry | 15/75 | 0.4/2.0 |
| Processed Fruits and Fruit Juices | Processed Fruits | 1.5/30 | <0.1/0.8 |
| Processed Vegetables and Vegetable Juices | Processed Vegetables | 7.5/30 | 0.2/0.8 |
| | Reconstituted Vegetables | 7.5/30 | 0.2/0.8 |
| Snack Foods | Snack Foods | 15/75 | 0.4/2.0 |
| Soft Candy | Soft Candy | 16/30 | 0.4/0.8 |
| Soups and Soup Mixes | Soups | 15/75 | 0.4/2.0 |

**Table 3.1-1 Summary of the Current[a] Food-Uses and Use-Levels for Quillaia Extract in the U.S.**

| Food Category (21 CFR §170.3) (U.S. FDA, 2019) | Current Food-Uses | Quillaia Extract Use-Level, Ave./Max. (ppm)[b] | Quillaia Extract Use-Level, as Saponins, Ave./Max. (mg/100 g)[c] |
|---|---|---|---|
| Sugar Substitutes | Sugar Substitutes | 7.5/30 | 0.2/0.8 |
| Sweet Sauces, Toppings and Syrups | Sweet Sauces | 15/75 | 0.4/2.0 |

Ave./Max. = average usual and maximum use-level for flavoring substance; CFR = Code of Federal Regulations; U.S. = United States.

[a] Current uses incorporate the specific uses as included in FEMA GRAS 27 (Cohen *et al*., 2015) (FEMA No. 2973) and GRN No. 000165 (U.S. FDA, 2005).

[b] Use-levels are transcribed as expressed in FEMA GRAS 27 (Cohen *et al*., 2015) (FEMA No. 2973), which were assumed to be for quillaia extract type 1, and as in GRN No. 000165, which was for quillaia extract type 1, and on the basis of the total ingredient.

[c] Use-levels for 'current uses' have been converted to a saponin basis, assuming a maximum saponin content of 26% w/w for quillaia extract type 1 on the basis of the upper limit in the specifications for quillaia extract type 1, established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006b). These are the values used in the intake assessment described in Part 3.2.2. Average usual use-level for flavoring use have been used in the assessment.

[d] Different use-levels for 'beverages and beverage bases' included in GRN No. 000165 (Semi-Frozen Carbonated and Non-Carbonated Beverages and Brewed Sodas) and FEMA GRAS 27 (Other Beverages).

[e] Only brewed sodas were included, as identified in GRN No. 000165.

## 3.2 Estimated Intake of Quillaia Extract Type 2 and Saponins from Permitted and Proposed Uses

### 3.2.1 Methods

Assessments of the anticipated intake of saponins (reference component) from all permitted and proposed uses of quillaia extract type 1 and type 2 (see Tables 3.1-1 and 1.3-1, respectively), and intake of quillaia extract type 2 (ingredient) from all proposed uses (see Table 1.3-1) were conducted using data available in the 2013-2014 cycle of the U.S. National Center for Health Statistics' National Health and Nutrition Examination Survey (NHANES) (CDC, 2015, 2016; USDA, 2016). A description of the survey and methodology employed in the intake assessment along with the pertinent results is presented herein.

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2013-2014. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (CDC, 2016; USDA, 2016). The NHANES data were employed to assess the mean and 90$^{th}$ percentile intakes of saponins and quillaia extract type 2 for each of the following population groups:

- Infants and young children, ages 0 to 2 years;
- Children, ages 3 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (all age and gender groups combined).

The use-levels for all permitted and proposed uses of quillaia extract type 1 and type 2 also are included in Tables 3.1-1 and 1.3-1, respectively. As discussed above, all permitted food-uses of quillaia extract were assumed to be type 1 and converted to a saponin basis assuming a maximum saponin content of 26% w/w on the basis of the upper limit in the specifications for quillaia extract type 1 as established by JECFA (JECFA, 2006b). For the FEMA GRAS uses, the *average* use-levels were utilized in the dietary intake assessment. The use-levels for the proposed food-uses are expressed on the basis of the saponin content (reference component) and were converted to the equivalent level of the ingredient itself based on the minimum saponin content of 65%, on the basis of the lower limit in the specification for quillaia extract type 2 as per Naturex's specification (see Table 2.3.1-1). Where there was overlap in the permitted and proposed food-uses, the proposed use-levels were utilized in the dietary exposure assessment, as they were higher than the permitted use-levels. In cases where a single food-use within a food category was proposed at a higher use-level than current uses, the proposed use-level was used for the identified food-use and the current use-level was used for all remaining food-uses within the food category [*e.g.*, the proposed use-level for 'mustard' (120 mg/100 g, as saponins) was higher than the current permitted use as a flavoring (0.4 to 2.0 mg/100 g, as saponins)].

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intakes of saponins and quillaia extract type 2 by the U.S. population[1]. Estimates for the daily intake of saponins and quillaia extract type 2 represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2013-2014; these average amounts comprised the distribution from which the mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of saponins or quillaia extract type 2 averaged over all individuals surveyed, regardless of whether they consumed food products in which quillaia extract is permitted or proposed for use, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing quillaia extract during the 2 survey days). "Consumer-only" intake refers to the estimated intake of saponins or quillaia extract type 2 by those individuals who reported consuming food products in which the use of quillaia extract is currently permitted or under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which quillaia extract is permitted or proposed for use on either Day 1 or Day 2 of the survey.

### 3.2.2 Estimated Intakes of Saponins from Permitted Uses of Quillaia Extract Type 1 and Proposed Uses of Quillaia Extract Type 2

Summaries of the estimated daily intake of saponins from all permitted and proposed uses of quillaia extract, types 1 and 2, are provided in Table 3.2.2-1 on an absolute basis (mg/person/day) and in Table 3.2.2-2 on a body weight basis (mg/kg body weight/day).

Based on the range of food-uses included in both the current and proposed conditions of use, almost all individuals included in the NHANES 2013-2014 were consumers of products in which quillaia extract is permitted or proposed for use (*i.e.*, 100% of consumers above 3 years, and 78% of consumers up to 3 years; see Table 3.2.2-1). A high proportion of consumers results in *per capita* and consumer only intakes that are very similar. As such, the consumer-only estimates are discussed in detail herein.

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use-level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of saponins from quillaia extract were determined to be 31 and 75 mg/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of saponins on an absolute basis, at 38 and 91 mg/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 10 and 17 mg/person/day, respectively (Table 3.2.2-1).

**Table 3.2.2-1 Summary of the Estimated Daily Intake of Saponins from Quillaia Extract Based on Current and Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)**

| Population Group | Age Group (Years) | *Per Capita* Intake (mg/day) | | Consumer-Only Intake (mg/day) | | | |
|---|---|---|---|---|---|---|---|
| | | Mean | 90th Percentile | % | n | Mean | 90th Percentile |
| Infants and Young Children | 0 to <3 | 8 | 12 | 78.2 | 491 | 10 | 17 |
| Children | 3 to 11 | 17 | 40 | 100 | 1,284 | 17 | 40 |
| Female Teenagers | 12 to 19 | 22 | 62 | 100 | 577 | 22 | 62 |
| Male Teenagers | 12 to 19 | 20 | 55 | 100 | 559 | 20 | 55 |
| Female Adults | 20 and up | 32 | 73 | 100 | 2,387 | 32 | 73 |
| Male Adults | 20 and up | 38 | 91 | 100 | 2,089 | 38 | 91 |
| Total Population | All ages | 30 | 75 | 99.1 | 7,387 | 31 | 75 |

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of saponins were determined to be 0.5 and 1.0 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean intakes of 0.8 mg/kg body weight/day, while children were identified as having the highest 90th percentile consumer-only intakes, at 1.5 mg/kg body weight/day. Female and male teenagers had the lowest mean, and male teenagers had the lowest 90th percentile, consumer-only intakes of 0.3 and 0.8 mg/kg body weight/day, respectively (Table 3.2.2-2).

**Table 3.2.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Saponins from Quillaia Extract from Current and Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)**

| Population Group | Age Group (Years) | *Per Capita* Intake (mg/kg bw/day) | | Consumer-Only Intake (mg/kg bw/day) | | | |
|---|---|---|---|---|---|---|---|
| | | Mean | 90th Percentile | % | n | Mean | 90th Percentile |
| Infants and Young Children | 0 to <3 | 0.7 | 0.9 | 78.1 | 489 | 0.8 | 1.2 |
| Children | 3 to 11 | 0.6 | 1.5 | 100 | 1,275 | 0.6 | 1.5 |
| Female Teenagers | 12 to 19 | 0.3 | 0.9 | 100 | 568 | 0.3 | 0.9 |
| Male Teenagers | 12 to 19 | 0.3 | 0.8 | 100 | 557 | 0.3 | 0.8 |
| Female Adults | 20 and up | 0.4 | 1.0 | 100 | 2,373 | 0.4 | 1.0 |
| Male Adults | 20 and up | 0.4 | 1.0 | 100 | 2,080 | 0.4 | 1.0 |
| Total Population | All ages | 0.5 | 1.0 | 99.1 | 7,342 | 0.5 | 1.0 |

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

### 3.2.3 Estimated Intakes of Quillaia Extract Type 2 from Proposed Uses

In order to determine the estimated daily intakes of quillaia extract type 2, the maximum potential use-level of quillaia extract type 2 for each proposed food-use was calculated by assuming a minimum saponin content of 65%, as per Naturex's specification (see Table 2.3.1-1). The resultant intakes of quillaia extract type 2 from proposed food-uses is provided in Table 3.2.3-1 on an absolute basis (mg/person/day), and in Table 3.2.3-2 on a body weight basis (mg/kg body weight/day).

There was a high percent of consumers of products in which quillaia extract type 2 is proposed for use, whereby 75.9% or greater of NHANES subjects above 3 years, and 54.3% of individuals up to 3 years, were identified as consumers (Table 3.2.3-1). As per Part 3.2.2, the consumer-only estimates are discussed in detail herein.

Among the total population (all ages), the mean and 90$^{th}$ percentile consumer-only intakes of quillaia extract type 2 were determined to be 45 and 109 mg/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90$^{th}$ percentile consumer-only intakes of quillaia extract type 2 on an absolute basis, at 53 and 127 mg/person/day, respectively, while infants and young children had the lowest mean and 90$^{th}$ percentile consumer-only intakes of 21 and 33 mg/person/day, respectively (Table 3.2.3-1).

**Table 3.2.3-1 Summary of the Estimated Daily Intake of Quillaia Extract Type 2 Based on Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)**

| Population Group | Age Group (Years) | *Per Capita* Intake (mg/day) | | Consumer-Only Intake (mg/day) | | | |
|---|---|---|---|---|---|---|---|
| | | Mean | 90$^{th}$ Percentile | % | n | Mean | 90$^{th}$ Percentile |
| Infants and Young Children | 0 to <3 | 11 | 19 | 54.3 | 320 | 21 | 33 |
| Children | 3 to 11 | 23 | 56 | 89.5 | 1,098 | 26 | 59 |
| Female Teenagers | 12 to 19 | 31 | 89 | 81.8 | 463 | 37 | 103 |
| Male Teenagers | 12 to 19 | 27 | 74 | 75.9 | 434 | 36 | 82 |
| Female Adults | 20 and up | 44 | 106 | 94.1 | 2,199 | 46 | 109 |
| Male Adults | 20 and up | 48 | 120 | 91.1 | 1,869 | 53 | 127 |
| Total Population | All ages | 40 | 102 | 89.2 | 6,383 | 45 | 109 |

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (all ages) mean and 90$^{th}$ percentile consumer-only intakes of quillaia extract type 2 were determined to be 0.7 and 1.5 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90$^{th}$ percentile consumer-only intakes of 1.7 and 2.5 mg/kg body weight/day, respectively. Male teenagers had the lowest mean and 90$^{th}$ percentile consumer-only intakes of 0.5 and 1.3 mg/kg body weight/day, respectively (Table 3.2.3-2).

**Table 3.2.3-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Quillaia Extract Type 2 from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)**

| Population Group | Age Group (Years) | *Per Capita* Intake (mg/kg bw/day) | | Consumer-Only Intake (mg/kg bw/day) | | | |
|---|---|---|---|---|---|---|---|
| | | Mean | 90th Percentile | % | n | Mean | 90th Percentile |
| Infants and Young Children | 0 to <3 | 0.9 | 1.4 | 54.0 | 318 | 1.7 | 2.5 |
| Children | 3 to 11 | 0.8 | 2.3 | 89.6 | 1,092 | 0.9 | 2.4 |
| Female Teenagers | 12 to 19 | 0.5 | 1.4 | 81.7 | 455 | 0.6 | 1.4 |
| Male Teenagers | 12 to 19 | 0.4 | 1.1 | 75.8 | 432 | 0.5 | 1.3 |
| Female Adults | 20 and up | 0.6 | 1.4 | 94.1 | 2,187 | 0.6 | 1.5 |
| Male Adults | 20 and up | 0.6 | 1.4 | 91.1 | 1,861 | 0.6 | 1.4 |
| Total Population | All ages | 0.6 | 1.5 | 89.2 | 6,345 | 0.7 | 1.5 |

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

## 3.2.4 Summary and Conclusions

Consumption data and information pertaining to the permitted and proposed food-uses of quillaia extracts type 1 and 2 were used to estimate the *per capita* and consumer-only intakes of the reference component (*i.e.*, saponins) and of quillaia extract type 2 for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates suitably conservative. For example, it was assumed in this exposure assessment that all food products within a food category contained quillaia extract type 2 at the maximum specified level of use for the proposed food-uses. In reality, the levels added to specific foods will vary depending on the nature of the food product, and it is unlikely that quillaia extract type 2 will have 100% market penetration in all identified food categories. In addition, all permitted conditions of use of quillaia extract type 1, considering both flavoring use and uses previously concluded to be GRAS, were included in the exposure assessment to achieve a worst-case exposure to saponins, which are the reference component of quillaia extract type 2. The likelihood of an individual consuming all of the permitted and proposed food-uses containing quillaia extracts type 1 and 2 within the diet over a lifetime is low, and as such, the values presented herein represent worst-case estimates of exposure.

In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of saponins from all permitted and proposed food-uses in the U.S. of quillaia extract types 1 and 2 by the total U.S. population were estimated to be 31 and 75 mg/person/day, respectively, equivalent to 0.5 and 1.0 mg/kg body weight/day. Among the individual population groups, the highest mean and 90th percentile consumer-only intakes of saponins on an absolute basis were determined to be 38 and 91 mg/person/day, respectively (*i.e.*, 0.4 and 1.0 mg/kg body weight/day), as identified among male adults. When expressed on a body weight basis, infants and young children had the highest mean intakes, at 0.8 mg/kg body weight/day, while children aged 3 to 11 years were determined to have the highest 90th percentile consumer-only intakes of 1.5 mg/kg body weight/day.

When considering the intakes of quillaia extract type 2 on a consumer-only basis, the resulting mean and 90$^{th}$ percentile intakes of quillaia extract type 2 by the total U.S. population from proposed food-uses in the U.S., were estimated to be 45 and 109 mg/person/day, respectively, equivalent to 0.7 and 1.5 mg/kg body weight/day. Among the individual population groups, the highest mean and 90$^{th}$ percentile consumer-only intakes of quillaia extract type 2 on an absolute basis were determined to be 53 and 127 mg/person/day, respectively (*i.e.*, 0.6 and 1.4 mg/kg body weight/day), as identified among male adults. When expressed on a body weight basis, infants and young children had the highest mean and 90$^{th}$ percentile consumer-only intakes of 1.7 and 2.5 mg/kg body weight/day, respectively.

# Part 4. §170.240 Self-Limiting Levels of Use

The use of quillaia extract type 2 will be self-limiting due to its technological function as an emulsifier. Levels of quillaia extract type 2 greater than those proposed for use (see Table 1.3-1) will not achieve a greater emulsification effect.

# Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

# Part 6. §170.250 Narrative and Safety Information

## 6.1 Introduction

Naturex has conducted a scientific procedures GRAS evaluation of the use of quillaia extract type 2 as an emulsifier in various food categories, as defined in Part 1.3. Naturex's quillaia extract type 2 meets all limits established by JECFA for quillaia extract type 2 (JECFA, 2014). The safety of quillaia extracts was evaluated by JECFA, the Scientific Committee on Food (SCF), and the European Food Safety Authority (EFSA) (SCF, 1978; JECFA, 1982, 1986, 2002, 2004, 2006a; EFSA, 2019). While the majority of the toxicological studies summarized in these reviews were conducted using quillaia extracts that were representative of quillaia extract type 1, JECFA concluded that no further studies on quillaia type 2 extracts were required due to the similarity between the saponin profiles of the types 1 and 2 extracts. Saponins were considered to be the reference component responsible for toxicity, and there was no difference in toxicity between the type 1 and type 2 extracts when assessed based on their saponin content. Thus, the toxicological data on quillaia extract type 1 are relevant to the safety assessment of quillaia extract type 2.

The safety of quillaia extract type 2 is based on the toxicological data that were summarized in the JECFA, SCF, and EFSA reviews and supported by data on the metabolic fate of related saponins, unpublished genotoxicity studies on quillaia extract type 1, and published human studies. The toxicological studies reviewed by the SCF, JECFA, and/or EFSA included acute toxicity data for type 1 and type 2 extracts, a 13-week toxicity study in the rat (Gaunt *et al.*, 1974), an 84-week study in mice (Phillips *et al.*, 1979), and a 108-week study in rats (Drake *et al.*, 1982). Acute toxicity studies are summarized in Part 6.4.1, and sub-chronic and chronic repeat-dose toxicity studies are summarized in Part 6.4.2. The pivotal study on the safety of quillaia extract type 2 is the 108-week study in rats (Drake *et al.*, 1982).

Data on the metabolic fate, genotoxicity, and reproductive effects of quillaia extracts were not included in the reviews conducted by the SCF or JECFA, as no data on these endpoints were available at the time of the reviews. A search of the literature using the PubMed database for studies related to the safety of quillaia extracts did not reveal any studies on the metabolic fate of quillaia extracts or any new traditional toxicity studies. In the absence of data on quillaia extract or quillaia saponins, the absorption and metabolic fate of quillaia extracts were assessed using information from the Committee for Veterinary Medicinal Products report (EMEA, 1996) and on related saponins, and these data are discussed in Part 6.3. Summaries of recently conducted, unpublished genotoxicity studies that were included in the evaluation conducted by EFSA are presented in Part 6.4.3; these studies were commissioned due to the EFSA re-evaluation of quillaia extracts, following the *Guidance for submission for food additive evaluations* and which considers that "*The genotoxic potential of any new additive has to be assessed as part of the evaluation process*" (EFSA, 2012). Other, non-traditional preclinical studies identified in the literature and relevant to the safety of quillaia extract type 2 are summarized in Part 6.4.4, while human studies relevant to safety are summarized in Part 6.5. The similarity in structure between quillaia saponins and bile salts, the potential for interactions between quillaia saponins and bile acids, and the potential for such an interaction to affect liver weight is discussed in Part 6.7.

Naturex hereby certifies that all data and information presented in this GRAS Notice represent a complete, representative, and balanced submission and considered all unfavorable as well as favorable information known to Naturex and pertinent to the evaluation of the safety and GRAS status of the use of Naturex's quillaia extract type 2 as an ingredient for addition to food.

## 6.2 Safety Assessments by Regulatory Bodies and Expert Panels

### 6.2.1 Joint FAO/WHO Expert Committee on Food Additives (JECFA)

Quillaia extracts were evaluated by JECFA during their 26$^{th}$, 29$^{th}$, 57$^{th}$, 61$^{st}$, and 65$^{th}$ meetings. Quillaia was initially evaluated at JECFA's 26$^{th}$ meeting, where a 13-week toxicity study in the rat and 2 long-term studies, an 84-week study in mice and a 108-week study in rats, were reviewed (JECFA, 1982). In the 84-week study, slightly decreased body weight gain and a few organ weight changes were reported in mice at the highest dietary level, which led JECFA to select the mid-dose as the no-observed-adverse-effect level (NOAEL), 0.5% in the diet (0.7 g/kg body weight/day). From the 108-week study in rats, JECFA also noted that there were minor changes in body weight gain and some differences in relative organ weights. JECFA (1982) reported the mid-dose level, 1.0% in the diet (0.5 g/kg body weight/day), to be the NOAEL. However, the body weights were reduced only in high-dose male rats and were concluded to be a result of reduced food consumption. The significant differences in relative organ weights occurred in 1 sex only and there were no corresponding histological effects. Contrary to JECFA's chosen NOAEL, the authors of the study reported a NOAEL of 3.0% in the diet (1.5 g/kg body weight/day), the highest dose tested (Drake *et al*., 1982). Despite the available studies, an acceptable daily intake (ADI) was not established during JECFA's 26$^{th}$ meeting due to the lack of specifications for quillaia. Tentative specifications were prepared by JECFA at the 29$^{th}$ meeting, and an ADI of 0 to 5 mg/kg body weight/day was established based on the NOAEL of 1.0% in the diet (0.5 g/kg body weight/day) from the long-term rat study (JECFA, 1986).

A review of all relevant information on the toxicity and dietary exposure of quillaia was requested by the Codex Committee on Food Additives and Contaminants and reviewed at JECFA's 57$^{th}$ meeting (JECFA, 2002). Revisions were made to the specifications previously determined at the 29$^{th}$ meeting to clarify the differences between unpurified and semi-purified extracts. In addition, the ADI previously determined by JECFA was changed to a temporary ADI of 0 to 5 mg/kg body weight for unpurified extract, pending

clarification of the specifications. The long-term rat study previously described was used to establish the temporary ADI.

At its 61st meeting, new information related to the chemical characterization of quillaia extracts and additional specifications were reviewed by the Committee (JECFA, 2004). The Committee required separate specifications for the 2 types of quillaia: type 1 ('unpurified' – saponin content between 20% and 26%) and type 2 ('semi-purified' – saponin content between 65% and 90%). Four major saponins (*i.e.,* QS-7, QS-17, QS-18, and QS-21) were identified in the saponin fraction of type 1 quillaia extracts and were determined to be representative of the total saponin content. The assay conducted for the quantification of saponin content was based on the 4 above-mentioned saponins. The Committee reviewed a study demonstrating that extracts from quillaia trees have 2 different saponin profiles. The authors reported that 1 profile was a subset of the other and concluded that genetic factors were responsible for the variation in saponin profiles. A commercial product was reported to have a mixture of the 2 profiles, which was determined to be the result of mixing bark from trees that possess the different profiles. In 1 of the profiles, there were 2 major peaks that were reported to be identical to QS-18 and QS-21, which were identified 10 years prior. Due to the fact that the saponin profiles of trees were considered to be genetically determined and unlikely to change over a 20-year period, the data submitted for toxicological and dietary exposure assessment were concluded to be specific to the material described as a type 1 extract by the Committee. Thus, the "temporary" designation on the ADI of 0 to 5 mg/kg body weight for quillaia extract type 1 was removed. The saponin profile of type 2 extracts obtained using ultrafiltration were determined by the Committee to be similar to the saponin profile of type 1 extracts (*i.e.,* QS-7, QS-17, QS-18, and QS-21). However, there were limited data to characterize the saponin fraction of type 2 extracts obtained by affinity chromatography and the non-saponin fraction, and thus, the Committee could not establish an ADI for type 2 extracts.

At its 65th meeting, the Committee reviewed information regarding the production and composition of type 2 extracts prepared by membrane ultrafiltration and chromatography and acute toxicity data for type 1 and 2 extracts (JECFA, 2006a). Due to the similarity reported between the saponin profile of type 2 extract (prepared by membrane ultrafiltration or chromatography) and type 1 extract by chromatographic analysis, the Committee concluded that no further toxicity studies on quillaia type 2 extracts were required. In the acute studies, there were no differences in toxicity between the type 1 and type 2 extracts when assessed based on their saponin content. The Committee considered that the toxicity of the extracts was a result of the saponin content and determined a group ADI of 0 to 1 mg/kg body weight (expressed as quillaia saponins) based on the lower end of the specified range of saponins in the type 1 extract (*i.e.,* 20%). The previously determined ADI of 0 to 5 mg/kg body weight (expressed as quillaia extract) was withdrawn.

### 6.2.2 European Union

#### *6.2.2.1 Scientific Committee for Food (SCF) Evaluation*

Quillaia's current approvals in the European Union resulted from the initial safety evaluation of the SCF that was published in the *Report of the Scientific Committee for Food – Seventh Series (December 1978) (Cat. N°CB-NW-78-007-EN-C -DA-DE-EN-FR-IT-NL) – page 42. Emulsifiers, stabilizers, thickeners and gelling agents (Opinion expressed on 30 November 1978)*[2]. The evaluation was based on the natural extract of quillaia bark as specified in the British Pharmacopoeia (BP, 1973), wherein quillaia extract was described by the SCF as "*an aqueous extract of the dried inner part of the bark of Quillaia Saponaria Molina and of the other species of quillaia (Rosaceae) and contains 3 or possibly 4 saponins (2 major, 1 minor, 1 trace) constituting about 10% of the extract. The sugars glucose, galactose, arabinose, xylose, rhamnose, and 2 further unidentified sugars are also present. The 2 major saponins are quillaia sapogenin which has a triterpenoid structure and quillaic acid*" (SCF, 1978). The evaluation included assessments of the composition, pharmacological properties, and source material of the extract, and the results of 2 long-term studies in the mouse and rat. Subsequently, the SCF established an ADI of 5 mg/kg body weight for the spray-dried extract. No details were provided in the SCF report on how the ADI was derived.

#### *6.2.2.2 European Food Safety Authority (EFSA)*

EFSA published a scientific opinion on quillaia extract based on their re-evaluation of the safety of quillaia extract as a food additive for use as an emulsifier and in flavorings (EFSA, 2019).

As part of the assessment, EFSA reviewed a number of repeated-dose studies on quillaia extract including a 90-day study in rats (Gaunt *et al.*, 1974), an 84-week study in mice (Phillips *et al.*, 1979), and a 2-year study in rats (Drake *et al.*, 1982). In the 90-day study, the NOAEL was determined to be 400 mg/kg body weight/day on the basis of changes in organ weight at higher doses; however, it was noted that there were no histopathological changes. The NOAEL in the 84-week study in mice was determined to be 750 mg/kg body weight/day on the basis of lower body weight gain and organ weight changes at higher dose levels. Again, there were no accompanying histopathological changes.

In the 2-year study in rats, the NOAEL was determined to be 1,500 mg/kg body weight/day (highest dose tested), with no evidence of carcinogenicity. The Panel did, however, note that the gut epithelium may be a target for quillaia extract, given that changes in the weight of gastrointestinal organs were observed in several studies (Gaunt *et al.*, 1974; Phillips *et al.*, 1979; Drake *et al.*, 1982; Kawaguchi *et al.*, 1994); however, these effects did not coincide with any histopathological changes attributed to the test-substance.

Furthermore, no evidence of genotoxicity was reported in various *in vitro* and *in vivo* tests. On the basis of the available data, EFSA concluded that the 2-year study in rats was the most comprehensive and robust and established an ADI for quillaia extract of 3 mg saponins/kg body weight/day on the basis of a NOAEL of 1,500 mg quillaia extract/kg body weight/day, factoring in that quillaia extract type 1 contains *ca.* 20% saponins which are the bioactive substances considered responsible for toxicity on the basis that the $LD_{50}$ values for the Type 1 and 2 extracts were about the same when expressed on a saponin basis.

[2] http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_07.pdf (SCF, 1978).

## 6.3 Absorption, Distribution, Metabolism and Excretion

No data on the metabolic fate of quillaia extracts or quillaia saponins were identified. *In lieu* of product-specific data, the absorption and metabolic fate of quillaia extracts were assessed using information from the Committee for Veterinary Medicinal Products report on related saponins (EMEA, 1996) and using information on related saponins, such as glycyrrhizinic acid (*i.e.*, glycyrrhizin), β-aescin (β-escin) from horse chestnut (*Aesculus hippocastanum*), *Pulsatilla* saponin D, DS-1 from *Dianthus superbus,* anhuienoside C a triterpenoid saponin from the rhizome of *Anemone flaccida* Fr. Schmidt (known as "Di Wu" in Chinese), asperosaponin VI (also named akebia saponin D) from *Dipsacus asper* Wal, hederacolchiside E from *Pulsatilla koreana*, and BTS-1 from *Gypsophila oldhamiana*.

All of these saponins are glycosides of the pentacyclic triterpenoid, oleanolic acid. The difference between the oleanolic acid and quillaic acid (the aglycone of quillaia saponins) is the absence of a hydroxyl and a ketone functional group at C-16 and C-23 positions of oleanolic acid, respectively. The main distinguishing features among these saponins is the structural diversity in the sugar chains attached at C-28 and/or C-3 positions on the aglycone that forms the backbone of each saponin.

Additional information on potential metabolic pathways was also obtained from studies conducted with ginsenoside saponins. Although the core aglycone structure of the ginsenosides is a 4-ring structure as opposed to a 5-ring structure, the complexity of ginsenoside chain attachments is similar to those of quillaia saponins, and thus provide information on the influence of the side chains on saponin metabolism.

As discussed above in Part 2.1.6, quillaia saponins have 2- to 5-unit sugar chains attached at C-3 and C-28 of the aglycone and an 18-carbon acyl chain attached to the fucose first sugar unit at C-28 position in the majority of the saponins (see Figure 2.1.6-1 above).

### 6.3.1 Gastrointestinal Metabolism of Related Saponins

#### *6.3.1.1 Committee for Veterinary Medicinal Products Review*

The European Committee for Veterinary Medicinal Products (EMEA, 1996) noted that the hydrolysis of saponins to their aglycone sapogenins and sugars by gastrointestinal microflora has been demonstrated *in vitro*. Although no study details were provided, Gutierrez and Davis (1962) and Gestetner *et al.* (1968) were cited in the Maximum Residue Limit Expert Report submitted to the European Agency for the Evaluation of Medicinal Products (EMEA) for quillaia saponins [Intervet International B.V., 1994 (unpublished)]. Colonies of saponin-digesting bacteria were isolated from the rumen fluid obtained from yearling steers that were permitted to graze on lush, pre-bloom Ladino clover (*Trifolium repens*) (Gutierrez and Davis, 1962). Inoculations of the harvested bacteria with alfalfa saponins yielded a "slime" that consisted primarily of residual sapogenins. Micro-organisms from the cecum and colon of mice, rats, and chicks have also been demonstrated to metabolize soybean saponins to sapogenins (Gestetner *et al.*, 1968). Due to the liberation of several sugars from soybean and alfalfa saponins, a glycosidase enzyme(s) with low specificity was suggested by Gestetner *et al.* (1968) to be responsible for the gastrointestinal metabolism.

#### *6.3.1.2 In Vitro and Pre-Clinical Data*

The results of i*n vitro* studies with glycyrrhizin, escin Ia, and anhuienoside C demonstrate that these saponins are extensively metabolized by the gastrointestinal flora (Okamura *et al.*, 2003; Yang *et al.,* 2004; Zhao *et al.*, 2015). Anhuienoside C was stable in fasted state simulating gastric fluid for at least 3 hours and was sequentially deglycosylated into 4 metabolites by the intestinal microflora from male Sprague-Dawley

rats (Zhao *et al.*, 2015). The final metabolite of this saponin was the aglycone oleanolic acid. Zhao *et al.* (2015) suggested that the oral bioavailability of anhuienoside C was primarily limited by its bacterial metabolism.

The results of preclinical studies further demonstrate the gastrointestinal metabolism of saponins by the microflora. In male Wistar and Sprague-Dawley rats, glycyrrhizin (*i.e.*, glycyrrhizinic acid) was metabolized to glycyrrhetic acid by the gastrointestinal microflora *via* the removal of 2 glucuronic acid molecules attached at carbon 3 following an oral administration of 10 mg/kg body weight glycyrrhizin (Takeda *et al.*, 1996).

Ginsenoside saponins are also metabolized extensively in the gastrointestinal tract of animals and humans by the intestinal microflora following oral administration (reviewed in Hasegawa, 2004; Qi *et al.*, 2011; Wang *et al.*, 2011; Yu *et al.*, 2012; Wan *et al.*, 2013). Deglycosylation and oxygenation were considered the primary and secondary metabolic pathways for these compounds, respectively (reviewed in Hasegawa, 2004; Qian and Cai, 2010; and 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 stepwise manner from the terminal sugar. Using an ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF-MS) technique, notoginsenoside Fc, a protopanaxadiol (PPD)-type saponin, was proposed to go through a series of deglycosylation reactions, forming 8 metabolites, which were identified in the feces of male Sprague-Dawley rats following a single oral dose of 100 mg/kg (He *et al.*, 2015).

#### *6.3.1.3 Human Data*

Similar findings to the animal models were reported in studies of saponin metabolism utilizing human microflora. A “fair number” of bacteria from the human gastrointestinal flora were reported to have β-glucuronidase activity, and thus, are capable of metabolizing glycyrrhizinic acid to glycyrrhetic acid (Hattori *et al.*, 1985). Some microbial glucuronidases remove both glucuronide moieties from glycyrrhizin, while others will only remove 1 (Kim *et al.,* 1999, 2000); however, a fewer number of bacteria could reduce 3-dehydro-glycyrrhizinic acid to glycyrrhizinic acid or 3-epi-glycyrrhizinic acid (Hattori *et al.*, 1985).

The results of *in vitro* studies using human gastrointestinal microflora isolated from feces indicate that the sugar side chains from ginsenosides were removed following anaerobic incubation (reviewed in Yu *et al.*, 2012). Incubation of American ginseng (*Panax quinquefolius* L.) root extract with human intestinal microflora obtained from the fresh feces 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.

The results of *in vitro* studies with microflora from rats and humans, and *in vivo* preclinical metabolism studies on other saponins, including glycyrrhizin, escin Ia, anhuienoside C, soybean saponins, and ginsenosides, consistently demonstrate that saponins are metabolized *via* sequential deglycosylation by gastrointestinal microflora. Based on the structural similarity of quillaia saponins to the saponins investigated, quillaia saponins are expected to also undergo sequential deglycosylation by the gastrointestinal microflora following oral administration.

### 6.3.2 Absorption of Related Saponins

#### *6.3.2.1 Committee for Veterinary Medicinal Products Review*

The absorption of quillaia saponins was assessed by the Committee for Veterinary Medicinal Products, who concluded that "*saponins are not significantly absorbed after oral administration*" (EMEA, 1996). Groups of 10 male albino mice, 3 male rats, and 3 male Leghorn chicks were fed diets containing 20% heated soybean flour for 10 days in a study by Gestetner *et al*. (1968), as summarized by EMEA (1996). Soybean saponins were detected (detection limit 40 µg) in the small intestine, but not in the cecum, colon, or blood of the mice, rats, and chicks that had consumed the soybean flour diet; however, the hydrolysis product sapogenins (detection limit 4 µg) were detected in the cecum 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 soybean saponins nor their hydrolysis products were absorbed from the digestive tract. 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 saponin extract at the corresponding pH levels for 3 hours 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, indicating that the micro-organisms from the cecum and colon were likely responsible for the metabolism of saponins to sapogenins.

EMEA (1996) further noted that the inability of the hemolytic 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 suggested that the large surface area of the gastrointestinal tract in comparison to the concentration of ingested saponins could explain their low oral toxicity.

#### *6.3.2.2 In Vitro Data*

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

#### *6.3.2.3 Pre-Clinical Data*

The previously described study by Gestetner *et al*. (1968) demonstrated that neither soybean saponins nor their hydrolysis products (sapogenins) were absorbed from the digestive tract of mice, rats, or chicks, within the limits of detection, following consumption of diets containing 20% heated soybean flour for 10 days. 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 feces using thin layer chromatography. Yoshikoshi *et al.* (1995) suggested that these results indicated that the majority of soybean saponins were hydrolyzed to their aglycones in the gastrointestinal tract.

More recent data of other related saponins supports these earlier conclusions, with the oral bioavailability of escin saponins, glycyrrhizin, pulsatilla saponin D, and DS-1 from *Dianthus superbus* in rats ranging from 0.16% for escin Ib to 4.0% for purified glycyrrhizin saponin (Wang *et al.*, 1995; Wu *et al.*, 2012, 2014; Ouyang *et al.*, 2015; Ren *et al.*, 2017). The oral bioavailability of glycyrrhizin following oral administration of a glycyrrhiza extract was 1.7% (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. Ren *et al.* (2017) suggested that the large molecular mass, high hydrogen-bonding capacity, and poor water solubility of saponins may be possible reasons for their low oral bioavailability. Pharmacokinetic parameters were also investigated for the related saponins asperosaponin VI, BTS-1, and hederacolchiside E in rats (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).

#### *6.3.2.4 Human Data*

The pharmacokinetics of the escin saponins was investigated in 10 healthy male humans following a single oral dose of saponin (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. When measured in the plasma of the participants, the maximum serum concentrations for these saponins ranged from 0.38 to 1.82 ng/mL. Similar to what was reported in rats, multiple peaks were reported in the plasma concentration-time curves for the escin saponins in some subjects, suggesting that either tissue redistribution or enterohepatic recycling was occurring (Wu *et al.*, 2010).

Considering the similarities among the backbone structure (*i.e.,* the aglycone) and the corresponding glycosides of the related saponins compared to quillaia, it is expected that quillaia saponins will also have low oral bioavailability.

## 6.4 Toxicological Studies

### 6.4.1 Acute Toxicity

Acute oral toxicity data are available for quillaia saponins, and quillaia extracts type 1 and 2. An oral $LD_{50}$ of 1,600 mg/kg body weight in mice was reported for quillaia saponins (no further study details available) (Efimvoa *et al.,* 1966).

JECFA summarized an additional study in its 65$^{th}$ report (JECFA, 2006a), which addressed the acute oral toxicity of *Q. saponaria* extract type 1 and type 2 (reference not provided). In this study, single oral doses of 3,000 to 20,000 mg of type 1 or type 2 *Q. saponaria* extract/kg body weight (extracts unpurified or purified by ultrafiltration, respectively) were administered to Sprague-Dawley rats (5/sex/group) followed by a 14-day observation period. Median lethal doses were determined to be 11,400 and 6,600 mg/kg body weight for type 1 and type 2 extracts (as administered), respectively. However, on the basis of saponin content (*i.e.,* 8.8% and 14% saponin on an as is basis, equivalent to 20% and 72% on a dry matter basis, for the standardized type 1 and 2 extracts, respectively), the $LD_{50}$ values for type 1 and type 2 extracts were calculated to be 1,000 and 900 mg/kg body weight, respectively. JECFA concluded that on the basis of the saponin contents of the extracts, "*the LD50s for the two extracts were the same: about 900 mg/kg body weight*".

### 6.4.2 Repeat-Dose Toxicity

#### *6.4.2.1 Sub-chronic Toxicity*

The safety of *Q. saponaria* extracts has been investigated in 3 sub-chronic toxicity studies with rats (Oser, 1966; Gaunt *et al*., 1974; Kawaguchi *et al*., 1994). The JECFA (1982, 2002) reviewed the studies by Gaunt *et al*. (1974) and Kawaguchi *et al.* (1994), while Oser (1966) was referenced in the introduction of Gaunt *et al*. (1974). Detailed descriptions of the above-mentioned studies are provided below.

In the study conducted by Gaunt *et al*. (1974), weanling CFE strain specific pathogen-free (SPF) male (130 to 175 g) and female (105 to 135 g) rats (15/sex/group) were provided with diets containing 0%, 0.6%, 2.0%, or 4.0% *Q. saponaria* extract[3] for 13 weeks. An additional component of this study provided diets containing 0%, 2.0%, or 4.0% *Q. saponaria* extract to rats (5/sex/group) for 2 or 6 weeks. Throughout the study, rats were observed for general condition and behavior. Body weight and food intake were measured at baseline and every week throughout the study period. At Week 6, blood samples were collected from 5 males and 5 females in the control and 4.0% dose groups for *in vitro* hemolysis analysis. At Weeks 6 and 13, urine samples were collected to measure the amount of urine produced after a water load of 25 mL/kg body weight in a 2-hour period and 16 to 20 hours later. Following the feeding period, rats were euthanized by exsanguination under barbiturate anesthesia. An autopsy was conducted, and the brain, pituitary, thyroid, heart, liver, spleen, stomach, small intestine, cecum, kidneys, adrenals, and gonads were weighed. Macroscopic examinations were conducted on these organs and on the esophagus, colon, rectum, lung, lymph nodes, skeletal muscle, trachea, uterus, urinary bladder, and pancreas in control and high-dose (4.0%) rats. Hematological analysis was performed on the terminal blood that was obtained at autopsy, and hemoglobin content, packed cell volume, and counts of erythrocytes, reticulocytes, and leucocytes were assessed. Serum obtained at autopsy was used to examine concentrations of urea, glucose, total protein, and albumin, and for activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH). One week prior to the end of the study, urine was collected from all rats to examine the microscopic constituents and content of blood, bile, and ketones. In addition, specific gravity and volume of urine produced following a 6-hour period without water were measured. The mean daily intakes of *Q. saponaria* extract over the 13-week study period were determined to be 360, 1,180, and 2,470 mg/kg body weight/day (providing intakes of approximately 72, 236, and 494 mg *Q. saponaria* saponins/kg body weight/day) in males and 440, 1,370, and 3,030 mg/kg body weight/day (providing intakes of 88, 274, and 606 mg *Q. saponaria* saponins/kg body weight/day) in females (for dietary concentrations of 0.6%, 2.0%, and 4.0%, respectively).

No adverse effects were reported with respect to the behavior and general condition, hematological, serum biochemical, urinary analyses, and gross or histopathology of rats from either of the two study components.

No quantitative results were presented for the rats that were provided with 0%, 2%, or 4% *Q. saponaria* extract in the diet for 2 or 6 weeks, and results were limited to organ weights. The authors reported that "*significant differences in organ weights were confined almost entirely to males and consisted of decreases in the* [absolute] *weights of the liver, spleen, kidneys, adrenals, and pituitary, in some cases only at week 2 or 6*". The changes were reported in groups that had lower average body weights. Significant decreases were reported in the relative liver weights of males fed 2% and 4% *Q. saponaria* extract (duration of exposure not reported), relative spleen weight of high-dose females in the 2-week group, and relative kidney weights of high-dose males in the 6-week group. A significant increase in the relative testis weight in

[3] Saponin content not reported; however, it was assumed to be 20% based on JECFA's conclusion that the test material in the toxicology studies was representative of a type 1 extract and the lower limit of 20% saponins in the type 1 extract.

high-dose males in the 2-week group receiving *Q. saponaria* was reported compared to sex-specific controls.

Detailed results were reported for animals that were fed diets containing 0%, 0.6%, 2.0%, or 4.0% *Q. saponaria* extract for 13-weeks. Body weights of high-dose male and female rats were significantly reduced compared to their respective control groups up to study Days 78 and 14, respectively. It was reported that the greatest difference in weight gain occurred during the initial days of the study (high-dose animals given 4% *Q. saponaria* in the diet lost weight during the first 24 hours), and the mean weight gain over the entire study period in both males and females was not significantly different from controls, which suggested that the reduction in weight gain was likely due to unpalatability of the diet.

Mean intakes of feed over the entire study period were reduced in all groups fed *Q. saponaria* extract compared to sex-specific controls, and the differences achieved statistical significance in females provided diets containing 2% or 4% *Q. saponaria* ($P<0.01$; compared to female controls). The authors reported that the largest difference in feed intake occurred during the beginning of the study. A significant reduction in mean water intake over the entire study period was reported in males given diets containing 2% or 4% *Q. saponaria* ($P<0.05$ and $P<0.01$, respectively, compared to male controls). The study authors reported that the reductions in water intake were likely related to the reduced feed consumption.

Significant differences in terminal organ weights were considered "difficult to interpret" by the study authors due to their occurrences in only 1 sex and lack of corresponding histopathological effects. Significant decreases in males relative to sex-specific controls were reported for absolute liver weight (0.6%, 2.0%, 4.0% diet groups; $P<0.05$, 0.01, and 0.001, respectively), kidney weight (2 and 4% diet groups; $P<0.05$), and adrenal gland weight (in 4% diet group; $P<0.05$). Significant decreases were reported in organ weights relative to body weight for the brain (males in 4% diet group; $P<0.05$), liver (males in 2% and 4% diet groups; $P<0.001$), and kidney (males in 2% and females in 4% diet groups; $P<0.05$), while significant increases were reported relative to body weight for the stomach (males in 2% and 4%, and females in 4% diet groups; $P<0.01$), cecum (females in 4% diet group; $P<0.05$), and thyroid (males in 0.6% and females in 2% diet groups; $P<0.05$). Quillaia saponins were previously reported to cause irritation to the gastrointestinal tract of hens and dogs (Bäck, 1917), and it was suggested that the increase in relative stomach weights in the current study was a result of local irritant effects. However, there were no effects suggestive of gastrointestinal irritation (including diarrhea) reported in the present study in any of the rats consuming *Q. saponaria* extract. The authors reported that the organ weight changes could not be attributed to changes in body weight, leading them to conclude that "*until evidence to the contrary is produced, the dietary levels producing changes in both absolute and relative organ weights must be regarded as having a toxic effect*". A NOAEL was determined by the authors to be 0.6% in the diet (*i.e.,* approximately 400 mg/kg body weight/day) and a lowest-observed-adverse-effect level (LOAEL) was determined to be 2% in the diet (*i.e.,* approximately 1,200 mg/kg body weight/day).

The selection of the lowest dose-level of 0.6% as the NOAEL was conservative since the organ weight changes were not consistent among male and female rats (in males, decreased absolute and relative liver weights, as well as absolute kidney weights were reported in the mid- and high-dose groups and decreased relative kidney weights were reported in the mid-dose group, while in females, relative kidney weights were reported to be decreased in the high-dose group). The reported changes in organ weights did not result in any adverse effects on organ structure or function based on the lack of hematological, serum biochemical, urinary, or gross or histological abnormalities. Given the lack of consistent definitive compound-related adverse effects, a NOAEL of 4% *Q. saponaria* extract in the diet (*i.e.,* approximately 2,500 mg/kg body weight/day) is more appropriate.

Kawaguchi *et al*. (1994) investigated the toxicity of saponins from *Thea sinensis* L. and included a group that were administered *Q. saponaria* saponins for comparative purposes. Jcl:Wistar rats (12/sex/group) were administered 1,200 mg *Q. saponaria* extract/kg body weight/day (providing 240 mg *Q. saponaria* saponins/kg body weight/day) by gastric intubation for 90 days. Food and water consumption were reported prior to the initial administered dose, on Day 2 of the study, and on a weekly basis for the rest of the study period, and general clinical observations were reported daily. During the final week of the study period, 3- and 21-hour urine samples were collected, and ocular fundus and slit-lamp examinations were conducted (6/sex/group). Urinalysis, hematological parameters, and blood biochemical parameters were measured post-exposure. After biological samples were obtained, the animals were euthanized and subjected to gross organ and histological examinations. Heparinized blood samples were collected at Day 47 and at sacrifice from an additional 10 rats/sex/group for hemolytic assessment.

No differences were reported in urinalysis, ophthalmological, or hemolytic parameters in rats receiving *Q. saponaria* extract compared to the control rats. In the *Q. saponaria* group, 1 rat of each sex died, and 5 males and 1 female were euthanized due to their deteriorating condition. Rats from the *Q. Saponaria* group demonstrated numerous clinical symptoms of toxicity (*e.g.,* salivation, regurgitation of liquid administered, abnormal respiratory sounds, diarrhea), whereas control rats showed no abnormalities in their general condition and none were euthanized prior to the end of the study. Some significant differences in body weight gain, food consumption, organ weight differences, and blood biochemistry were also reported; however, with the exception of decreased triglycerides and increased absolute weight and weight relative to body weight of the stomach, the above-mentioned differences were reported only in 1 sex and/or were not seen or were changed in the opposite direction in the rats that were prematurely killed. With respect to histopathological examinations of the surviving animals in the *Q. saponaria* group, inflammatory changes were reported in the forestomach of both sexes, and the larynx, trachea, and lung of males, and atrophy was reported in the thymus of 1 male. Similar histopathological findings were reported for the animals that were euthanized prior to study completion. The authors did not comment on the histopathological changes in the *Q. saponaria* group; however, they reported that any observed inflammatory changes in the groups receiving saponins from *Thea sinensis* L could likely be attributed to the mucosal irritant properties of the test article. Although not discussed by the authors, it is possible that the changes in the *Q. saponaria* group resulted from irritation to *Q. saponaria* extract due to the gavage method of administration, gastro-esophageal reflux, and any subsequent aspiration of the test substance (Eichenbaum *et al*., 2011). Overall, interpretation of the results reported by Kawaguchi *et al*. (1994) proves to be difficult, as only a single dose of *Q. saponaria* extract was used in the study. JECFA (2002) reviewed the study conducted by Kawaguchi *et al*. (1994) and concluded that the reported data were "*irrelevant to the toxicological assessment of quillaia extracts as sufficient data were not available on the specifications of the test material and because the animals were given the compound by gavage*".

Oser (1966) reported on the toxicity of *Q. saponaria* saponin and *Yucca mohavensis* extract. *Q. saponaria* saponin was provided in a basal diet at concentrations of 0% or 0.05% [equivalent to approximately 45 mg/kg body weight/day (EFSA, 2012)] to young FDRL albino rats (5/sex/group) over a 12-week period. No details were provided on the composition of *Q. saponaria* saponin (including saponin content). Body weights, feed consumption and behavior were recorded, however, the timing of these assessments was not reported. Blood samples were collected at Weeks 4, 8, and 12 for determination of erythrocyte counts, hemoglobin, and erythrocyte fragility. At Week 12, leucocyte counts, blood glucose, and non-protein nitrogen were assessed in the same blood samples. At study termination, autopsies were conducted, and organ weights were determined for the liver, kidneys, spleen, heart, and adrenals. Liver, kidney, adrenals, gonads, lymph nodes, and bone marrow were examined histologically, and 15 additional organs or tissues (further details not reported) were examined in 3 rats per sex group. In the rats that received *Q. saponaria* saponin, there were no adverse effects in any of the parameters reported compared to the control rats and

no pre-terminal deaths were reported. It was reported that "Quillaia (*Q. saponaria* Mol.)" was proposed as an addition to the 'safe' "Natural Flavoring Substances and Natural Substances Used in Conjunction with Flavors" in the 30 March 1966 issue of the Federal Register (Oser, 1966).

#### *6.4.2.2 Chronic Toxicity*

The potential chronic toxicity of *Q. saponaria* extracts was investigated in an 84-week study in mice (Phillips *et al*., 1979) and a 2-year study in rats (Drake *et al*., 1982). These studies were reviewed by JECFA and are summarized and discussed below.

Phillips *et al*. (1979) reported on the long-term toxicity of *Q. saponaria* extract in TO strain, SPF mice. *Q. saponaria* extract[4] was administered as a dietary admixture at concentrations of 0%, 0.1%, 0.5%, or 1.5%; diets were supplemented and provided to groups of mice (48/sex/group) over an 84-week period. The general condition and behavior of mice was monitored frequently (no further details provided), and body weights of 16 males per group were recorded "at regular intervals". At Weeks 26 and 52, blood samples were collected from 10 male and 10 female mice from the control, mid- and high-dose groups, and all surviving mice at Week 84, for the determination of hemoglobin concentration, packed cell volume, and counts of reticulocytes, total erythrocytes, and total leucocytes. All animals that survived until the end of the study period were euthanized by exsanguination under barbiturate anesthesia and subjected to gross examination. The brain, heart, liver, kidneys, spleen, stomach, small intestine, cecum, and testes were weighed and examined microscopically. 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 any other tissues that showed abnormalities were examined histopathologically.

The mice that received diets containing 0%, 0.1%, 0.5%, or 1.5% *Q. saponaria* extract (equivalent to approximately 0, 140, 700, and 2,200 mg *Q. saponaria* extract/kg body weight/day, respectively) consumed approximately 0, 28, 140, and 440 mg *Q. saponaria* saponins/kg body weight/day, respectively. Behavior and general condition of the animals were not adversely affected by consumption of *Q. saponaria* extract. Mortality, histopathological abnormalities, and the incidence of tumors were not significantly different between animals consuming *Q. saponaria* extract and control animals. A slightly lower weight gain was reported in high dose males and significantly reduced terminal body weights were reported in high dose males ($P<0.05$); however, body weights were similar to controls at each individual timepoint assessed during the administration period. Although feed intake was not measured during the study, the reported decrease in terminal body weights was suggested by the study authors to have been due to the unpalatability of the test compound and local irritation of the gastrointestinal tract, since transient decreases in body weight had previously been attributed to unpalatability of *Q. saponaria* extract as a result of decreased feed intake in a 90-day study in rats by Gaunt *et al.* (1974). There was no evidence of gastrointestinal irritation in either the current study or the study by Gaunt *et al.* (1974).

In high-dose males, significant increases were reported in relative brain and stomach weights ($P<0.05$; compared to male controls). The absolute weight of the small intestine was significantly increased in high-dose females; and significant decreases in the absolute weights of the liver and kidney in high-dose males and testes in mid- and high-dose males ($P<0.05$; compared to sex-specific controls) were reported. The authors reported that decreased liver and increased gastrointestinal weights in rats following consumption of *Q. saponaria* extract were previously reported in the study by Gaunt *et al*. (1974) and thus, may

[4] Saponin content not reported; however, it was assumed to be 20% based on JECFA's conclusion that the test material in the toxicology studies was representative of a type 1 extract and the lower limit of 20% saponins in the type 1 extract.

represent a toxic effect. No evidence of any pathological effect on the gastrointestinal tract was reported in the present study. No histopathological abnormalities were reported in any of the organs that were significantly different from controls in weight, and the weight changes were only observed in 1 sex. The study authors considered the observed alterations in organ weights as "*unlikely to be of toxicological significance*".

A significant reduction in red blood cell counts was reported in the mid- and high-dose males ($P<0.05$ and 0.01, respectively), as well as high-dose females ($P<0.01$) at Week 26. At Week 84, the reduction in red blood cell counts remained significant only in mid-dose males ($P<0.01$). In addition, a significant reduction in mid-dose females ($P<0.05$) and a significant increase in high-dose males ($P<0.05$) were reported for packed cell volume. The study authors did not address the toxicological relevance of the hematological variations. These changes do not represent a safety concern, since the reductions in red blood cell counts were transient and did not demonstrate a dose-response relationship at Week 84 and the changes in packed cell volume were in opposing directions in the male and female rats at Week 84. Based on the results, a NOAEL of 0.5% in the diet (*i.e.,* approximately 700 mg/kg body weight/day, and representing the mid-dose level) for *Q. saponaria* extract in mice was determined by the authors. This NOAEL was established due to the slight decrease in body weight gain and organ weight changes (of "doubtful significance") in the high-dose animals. The authors acknowledged that the organ weight changes [*i.e.,* decreased absolute weights of the liver and kidney in high-dose males, increased relative (to body weight) weight of the brain and stomach in high-dose males, and increased absolute weight of the small intestine in high-dose females], on which the NOAEL was in part based, were of "doubtful significance". Since organ weight changes were in 1 sex, changes in absolute organ weights were not paralleled by changes in the relative (to body weight) organ weights, and no histopathological abnormalities were reported, it is unlikely that the above-mentioned changes in organ weights were a result of the consumption of *Q. saponaria* extract.

The reduction in terminal body weights of high-dose male mice was the second endpoint on which the NOAEL was based. There were no significant differences in the terminal body weights of female high-dose mice compared to their respective controls. Although feed intake and palatability of the diet was not assessed in the current study, unpalatability of the test compound was suggested by the study authors to be the cause of the decreased body weight on the basis that transient decreases in body weight had previously been attributed to unpalatability of *Q. saponaria* extract as a result of decreased feed intake in the study by Gaunt *et al.* (1974). As feed intake was not measured in the present study, it is not possible to determine whether the reduced body weights could be attributed to decreased feed intake. However, in a palatability study carried out by Drake *et al*. (1982), a greater preference for the control diet as opposed to a diet supplemented with *Q. saponaria* extract was demonstrated in rats. Based on the preference of rats, it can be assumed that the mice in the Phillips *et al*. (1979) study also showed greater preference to the control diet over the *Q. saponaria* extract diet, which resulted in decreased feed consumption due to unpalatability, thus leading to the observed decrease in terminal body weight in high-dose males. Based on the lack of definitive compound-related adverse effects, a proposed NOAEL of the highest concentration tested, 1.5% *Q. saponaria* extract in the diet (*i.e.,* approximately 2,200 mg/kg body weight/day, providing 440 mg *Q. saponaria* saponins/kg body weight/day) is more appropriate.

Drake *et al.* (1982), reported on the long-term toxicity of *Q. saponaria* extract in Wistar rats in a 2-year study. The saponin content of *Q. saponaria* extract was not reported; however, the specifications were reported to conform to the British Pharmacopoeia (BP, 1973), and those in the United Kingdom *Emulsifiers and Stabilisers in Food Regulations 1975* (Statutory Instrument, no. 1486 – MAFF, 1975), and JECFA concluded that the test material was representative of a type 1 extract (JECFA, 2004). On this basis, the lower limit for saponin content in the quillaia extract type 1 monograph (*i.e.*, 20%) was used to calculate the

intakes of saponins. Prior to the main study, the acceptability of diets containing *Q. saponaria* extract was investigated in 2 short studies in male rats. In 1 of the studies, the test animals were permitted to choose between a control diet, or a diet supplemented with 0.3%, 1.0%, or 3.0% *Q. saponaria* extract for a 21-day period. The mean consumption of the control diet was calculated to be 23, 23.6, and 27.4 g and the diet containing *Q. saponaria* extract was calculated to be 1.3, 2.0, and 0.8 g over the 21-day study period, respectively for the 3 dose groups. Based these data the control diet was preferred compared to the diet supplemented with *Q. saponaria* extract. In the second study, the male rats were not given a choice of diet. The animals were provided with either a control diet or diet containing 0.3%, 1.0%, or 3.0% *Q. saponaria* extract over a 7-day period. Mean feed intakes were calculated to be 25, 29, 26, and 21 g/day for dose groups consuming 0%, 0.3%, 1.0%, or 3.0% *Q. saponaria* extract, respectively. The mean body weight gain of the animals was reported to be 26, 29, 30, and 11 g/day, respectively. The authors did not report the statistical significance of these results; however, the reported feed intake and body weight gain of animals suggests that rats are more likely to consume smaller amounts of diets high in *Q. saponaria* extract.

In the 2-year study, Wistar rats (48/sex/group) were given diets containing 0%, 0.3%, 1.0%, or 3.0% *Q. saponaria* extract. Measurements of body weight and feed and water consumption were conducted every 2 months; urine was collected from control and high-dose groups (10 animals/group) at Weeks 13, 24, and 78. Urinalysis consisted of appearance, microscopic constituents, protein content, glucose, ketones, bile salts, and blood. At these timepoints, additional samples were collected for concentration and dilution tests (*i.e.,* over 6 hours without water, over 4 hours after 16 hours of water deprivation, over 2 hours following a water load) to determine volume and specific gravity, and the number of cells in the 2-hour sample. At Weeks 15, 25, and 52, blood was collected from 10 animals/sex/group, and from all animals at sacrifice (Week 108) for hematological analysis including hemoglobin, packed cell volume, total erythrocyte and leucocyte counts, and differential leucocyte counts. Blood samples collected at sacrifice were analyzed for urea, glucose, total protein, albumin, AST, ALT, and LDH. Macroscopic examination was conducted at sacrifice, and the weights of the brain, heart, liver, spleen, kidney, stomach, small intestine, cecum (empty and full), adrenal glands, gonads, pituitary and thyroid were measured. The above-mentioned tissues and the salivary glands, thymus, lymph nodes, pancreas, aorta, nasal bones, lungs, trachea, esophagus, colon, rectum, skeletal muscle, spinal cord, sciatic nerve, uterus or prostate and seminal vesicles, urinary bladder, mammary tissue, eye and Harderian gland, and any other abnormal tissues underwent histological examination.

The mean dietary intake of *Q. saponaria* extract was calculated to be 120, 390, and 1,175 mg/kg body weight/day and 150, 500, and 1,500 mg/kg body weight/day in males and females, respectively, for groups consuming 0.3%, 1.0%, and 3.0% *Q. saponaria* extract, respectively. The intakes reported provided male and female rats with approximately 24, 78, and 235, and 30, 100, and 300 mg *Q. saponaria* saponins/kg body weight, respectively. During Weeks 87 and 91, significant increases in the total number of deaths (including animals killed *in extremis*) were reported in males consuming diets containing 1 % *Q. saponaria* extract ($P<0.01$ and $P<0.05$, respectively). There were no treatment-related deaths.

Significant reductions in body weight were reported in males consuming 3% *Q. saponaria* extract at Weeks 4, 42, 48, 54, and 89 ($P<0.05$), and at Weeks 63, 71, and 80 ($P<0.01$), compared to controls. Female rats consuming 0.3% *Q. saponaria* extract in the diet showed significant increases in body weight at Weeks 4, 8, 16, and 25 ($P<0.05$) compared to controls. However, despite the significant decreases in body weight in the high-dose males at various individual timepoints during the study, no significant differences in body weight were noted between groups at Week 106. Feed intake was lower in the high-dose male and female groups throughout the study compared to sex-specific controls, although these differences did not reach statistical significance, and there were no significant differences in water intake between groups.

At Week 78, a significant increase in specific gravity of the urine samples collected 4 hours after a 16-hour period of no water consumption was reported in males receiving diets containing 3% *Q. saponaria* extract compared to male controls (P<0.01). No other significant effects were reported in the urinalysis. There were no significant treatment-related biochemical/clinical chemical effects.

Significant differences from sex-specific controls were reported in hematological parameters, consisting of increased total white blood cell counts in Week 15 (in males given diet containing 1% or 3% *Q. saponaria* extract; P<0.05 and P<0.01, respectively) and in Week 25 (in males given diet containing 3% *Q. saponaria* extract; P<0.01), increased neutrophil and decreased leukocyte counts at Week 15 (in males given diet containing 3% *Q. saponaria* extract; P<0.01), increased hemoglobin concentration at Week 15 (in males given diet containing 1% *Q. saponaria* extract; P<0.05), and increased red blood cell counts at sacrifice (in females given diet containing 1% *Q. saponaria* extract; P<0.05). These effects were, however, transient and/or in 1 sex only. Total white blood cell counts were significantly decreased at sacrifice in high-dose males and females (P<0.05). Although not statistically analyzed, total white blood cell counts at sacrifice were decreased in all *Q. saponaria* extract groups and the controls compared to the counts recorded at Weeks 15, 25, and 52 for each group. The authors suggested that this reduction could be a result of blood samples being taken from the tail vein during mid-study collection and from the aorta at termination of the study. Non-significant decreases in body weights in the second half of the study and food consumption throughout were reported in the high-dose animals compared to the controls, which led the authors to suggest that the reduction in terminal white blood cell counts in the high-dose animals was likely "*a reflection of the decreased growth rate*", as decreased food intake and body weights were previously reported to be associated with a decrease in white blood cell counts (Oishi *et al*., 1979). Drake *et al.* (1982) also noted that total white blood cell counts were significantly increased at Week 15 in males but not females in the current study, and not affected by consumption of *Q. saponaria* extract in the 90-day study in rats by Gaunt *et al.* (1974). Drake *et al.* (1982) concluded that the observed differences from controls in white blood cell counts were not compound-related (Drake *et al*., 1982).

Sporadic significant differences in absolute and relative organ weights compared to sex-specific controls were reported in 1 sex only and/or were not associated with a dose-response relationship. Significant differences in absolute organ weights consisted of decreases in the weight of the heart (high-dose males, P<0.05), kidneys (high-dose males, P<0.05), and thyroid (mid- and high-dose males, P<0.05), and significant increases in the weight of the stomach (low-dose females, P<0.05), small intestine (low- and high-dose females, P<0.05, 0.01, respectively), and full and empty cecum [low- (P<0.05, 0.0001, respectively) and high-dose (P<0.0001 for both) females]. Significant differences in relative (to body weight) organ weights consisted of a significant decrease in liver weight of mid-dose males (P<0.05), and significant increases in the weight of the liver (high-dose females, P<0.05), stomach (high-dose females, P<0.05), small intestine (high-dose females, P<0.01), and full and empty cecum [low- (P<0.05, 0.0001, respectively) and high-dose (P<0.0001 for both) females].

In the histopathological examinations, significantly increased incidences of cardiac fibrosis (P<0.05) and dilation of gastric mucosal glands (P<0.05) were reported in females receiving the low dose of *Q. saponaria* extract compared to controls. Since this was not dose-related, it was concluded to be unrelated to the consumption of *Q. saponaria* extract. No other adverse histopathological effects were reported. Various benign and malignant tumors were identified in both the control and *Q. saponaria* extract groups. Only the incidence of thyroid adenomas in females receiving the mid-dose of *Q. saponaria* extract was significantly increased compared to controls (P<0.05); the incidence in mid-dose females was lower than the incidence in male controls. The remaining incidences of tumors were reported by the study authors to be low and not dose related. Based on the results of the study, the highest concentration tested, 3% *Q. saponaria* extract in the diet (*i.e.,* approximately 1,500 mg *Q. saponaria* extract/kg body weight/day, providing 300 mg

*Q. saponaria* saponins/kg body weight/day), was selected as the NOAEL by the study authors. This NOAEL was selected due to the lack of significant differences between-groups in cumulative deaths, as well as a lack of compound-related, dose-dependent adverse effects on body and organ weights, hematology, serum biochemistry, urinalysis, and gross/histopathology. It is noteworthy that the lack of consistent compound-related, dose-dependent adverse effects is consistent with the low oral bioavailability of saponins and the expected low oral bioavailability of quillaia saponins (see Part 6.3.2). Absorption of a compound is required in order for it to exert systemic toxicity. Thus, the lack of absorption of quillaia saponins mitigates against systemic toxicity of *Q. saponaria* extracts. Furthermore, evidence of sequential deglycosylation of saponins in the gastrointestinal tract (see Part 6.3.1) and the lack of histopathological effects in the gastrointestinal tract and the absence of occurrences of diarrhea or evidence of any other signs of gastrointestinal irritation in the repeat dose toxicity studies with *Q. saponaria* extracts (Gaunt *et al.*, 1974; Phillips *et al.*, 1979; Drake *et al.*, 1982) supports that quillaia extracts also do not exert local toxicity following consumption.

The study by Drake *et al.* (1982) is considered the pivotal study on the safety of quillaia extract type 2. Although in their evaluation, JECFA (1982) reported the mid-dose level, 1.0% in the diet (0.5 g/kg body weight/day), to be the NOAEL on the basis of minor changes in body weight gain and some differences in relative organ weights, there were no consistent, statistically significant, dose-dependent adverse effects in this study to justify the selection of the mid-dose as the NOAEL. Body weights were reduced only in high-dose male rats and were concluded to be a result of reduced food consumption, which is considered to be a result of the unpalatability of the *Q. saponaria* extracts diets on the basis of the results of the diet acceptability studies conducted prior to the main 90-day study (Drake *et al.*, 1982). The significant differences in relative organ weights occurred in 1 sex only and there were no corresponding histological effects. Furthermore, in the recent re-evaluation of the safety of quillaia extracts by the EFSA, it was acknowledged that Drake *et al.* (1982) was the most comprehensive and robust study on which to establish an ADI, and EFSA concluded that the author's NOAEL of 3% *Q. saponaria* extract in the diet could be used to establish an ADI. Using this NOAEL and assuming 20% saponins in a type 1 extract, EFSA derived an ADI of 3 mg saponins/kg body weight/day for quillaia extracts. Therefore, the NOAEL that was determined by Drake *et al.* (1982) to be 3% *Q. saponaria* extract in the diet (*i.e.,* approximately 1,500 mg *Q. saponaria* extract/kg body weight/day, providing 300 mg *Q. saponaria* saponins/kg body weight/day) is the most appropriate NOAEL on which to base the safety of the intake of quillaia extract type 2. The resultant intake of 300 mg *Q. saponaria* saponins/kg body weight/day at the NOAEL from Drake *et al.* (1982) provides a 200-fold margin of exposure in comparison to the highest 90$^{th}$ percentile consumer-only intakes of 1.5 mg saponins/kg body weight/day that occurred in children aged 3 to 11 years resulting from the combined current uses of quillaia extract type 1 and the proposed uses of quillaia extract type 2 (see Part 3.2.2). In addition, the estimated intakes of saponins are conservative in nature. Thus, it can be concluded that the safety of the estimated intakes of saponins resulting from the current uses of quillaia extract type 1 and the proposed uses of quillaia extract type 2 is supported.

### 6.4.3 Mutagenicity and Genotoxicity

No published studies on the mutagenicity or genotoxicity of quillaia extracts or quillaia saponins were identified. Therefore, due to the lack of these studies in the literature, and the consideration that "*the genotoxic potential of any new additive has to be assessed as part of the evaluation process*" (EFSA, 2012), the EFSA Working Group on Applications of the Scientific Panel on Food Additives and Nutrient Sources Added to Food requested genotoxicity data on the food additive quillaia extract (E 999) as part of the re-evaluation process. In response, Naturex, in conjunction with other interested parties, commissioned the conduct of a bacterial reverse mutation assay, an *in vitro* mammalian cell micronucleus test, and an *in vivo* mammalian erythrocyte micronucleus test. Brief summaries of these studies are provided below.

As there are 2 types of quillaia extracts of varying purity, the quillaia extract type that contained the highest level of impurities (*i.e.*, type 1) was selected for the tests; doses were based on the saponin content to ensure that the components of an untested preparation would be represented by the preparation that was tested. All tests were conducted in accordance with the principles of Good Laboratory Practice (GLP) using a representative batch of quillaia extract type 1 that complies with JECFA specifications (JECFA, 2006b).

#### *6.4.3.1 Bacterial Reverse Mutation Assay (OECD TG 471)*

A bacterial reverse mutation assay was conducted with quillaia extract type 1 in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 471 and in compliance with GLP [OECD, 1997, 1998; Sequani Limited, 2018a (unpublished)].

Quillaia extract type 1 was tested using the plate incorporation method and the pre-incubation method in *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* strain WP2 *uvrA* both in the presence and absence of S9 mix. The concentrations of quillaia extract tested were based on the saponin content, to permit the highest exposure to both saponins and impurities (*via* selection of a type 1 extract). The tested concentrations of quillaia extract provided 0.05, 0.15, 0.5, 1.5, and 5 µL saponins/plate, equivalent to 0.2155, 0.6465, 2.155, 6.465, and 21.55 µL quillaia extract/plate.

There was no precipitation or signs of toxicity. There were no dose-related or statistically significant increases in revertant numbers reported in any strain at any level of quillaia extract [at concentrations up to the maximum recommended dose of 5 µL saponins/plate (21.55 µL quillaia extract/plate)] 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. See Appendix A for a tabular summary of the results.

In conclusion, 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.

#### *6.4.3.2 In Vitro Mammalian Cell Micronucleus Test (OECD TG 487)*

An *in vitro* mammalian cell micronucleus test was conducted with quillaia extract type 1 in accordance with the OECD TG 487 and in compliance with GLP [OECD, 1998, 2016a; Sequani Limited, 2018b (unpublished)].

TK6 cells were treated with quillaia extract type 1, negative control (sterile water), or positive controls (cyclophosphamide in the presence of S9 mix and mitomycin C in the absence of S9 mix) in both the presence and absence of S9 mix. The treatment period was 3 hours in the presence and absence of S9 mix and harvesting was 44 hours after initiation of treatment; continuous treatment (27 hours) was not performed due to the positive result seen after the 3-hour treatment in the presence of S9 mix.

Concentrations of quillaia extract type 1 were corrected for saponin content and the treatment levels selected for analysis based on the cytotoxicity limit 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.

In the presence of S9 mix, there was a statistically significant increase in the percentage of micronucleated (% MN) cells at 0.012 µL saponins/mL, compared with 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 aberrant cells at any other dose of quillaia extract in the presence of S9 mix.

There were statistically significant increases in % MN cells at all dose levels treated with quillaia extract 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. See Appendix A for a tabular summary of the results.

In conclusion, quillaia extract was considered to be either clastogenic or aneugenic in the presence of S9 mix under the conditions of the test, at concentrations up to 0.05172 µL quillaia extract/mL (0.012 µL saponins/mL). The results in the absence of S9 mix were equivocal, at concentrations up to 0.06034 µL quillaia extract/mL (0.014 µL saponins/mL).

#### *6.4.3.3 In Vivo Mammalian Erythrocyte Micronucleus Test (OECD TG 474)*

An *in vivo* mammalian erythrocyte micronucleus test was conducted with quillaia extract type 1 in accordance with the OECD TG 474 and in compliance with GLP [OECD, 1998, 2016b; Sequani Limited, 2018c (unpublished)].

Groups of 6 male Crl:WI(Han) strain rats were dosed by gavage with water (negative control) or quillaia extract type 1 at the regulatory maximum dose of 2,000 mg/kg body weight/day saponins (equivalent to 8,620 mg/kg body weight/day quillaia extract type 1) on 2 successive days, approximately 24 hours apart. A positive control group, also of 6 males, was given a single 15 mg/kg body weight oral (gavage) dose of cyclophosphamide.

Blood samples for micronucleus evaluation were taken from main study animals approximately 48 hours after the final dose. Blood samples were also collected from satellite animals at 4 hours after the second dose to demonstrate bone marrow exposure in peripheral blood.

The mean frequency of micronucleated reticulocytes for males given quillaia extract was similar to that of the negative control group. A statistically significant decrease in the percentage of reticulocytes for quillaia extract treated animals confirmed test item exposure and toxicity to the target tissue (bone marrow). Exposure of the bone marrow to the test item was further evidenced by quillaic acid (the backbone of quillaia saponins) being detected in all plasma samples taken from satellite animals 4 hours after the second dose of quillaia extract. See Appendix A for a tabular summary of the results.

In conclusion, there was no evidence of clastogenicity or aneugenicity following gavage administration of quillaia extract up to the regulatory maximum dose level of 2,000 mg/kg body weight/day saponins (equivalent to 8,620 mg/kg body weight/day quillaia extract) in male rats. Quillaia extract was therefore considered to be neither clastogenic nor aneugenic *in vivo* under the conditions of the study.

### 6.4.4 Other Studies of Toxicity

In a study conducted by Ilsley *et al*. (2005), male and female piglets (n=192; mean age: 29 ± 0.1 days) received a diet containing 0 or 750 mg *Q. saponaria* saponins[5]/kg with or without 0 or 200 mg curcumin/kg over a 20-day period to investigate the effects of dietary supplementation with *Q. saponaria* saponins on immune function, general health, growth, and performance in weanling piglets. From Day 8 to 20 of the study, the dose of *Q. saponaria* saponins was reduced to 300 mg/kg feed because of the increased feed intake of the piglets. Throughout the study, animals were provided with diets and water *ad libitum*. The animals were separated into 6 groups of 8 piglets/pen. Daily records were kept for pen health scores, which were evaluated based on a subjective scale that ranged from 1 to 5, with 1 being "excellent, with no obvious illness, good vigor, and no lameness", and 5 being "very poor, with the majority of pen in a very poor state of health". At Days 6 and 20, 8 piglets from each dietary group were killed for blood and tissue analysis. Blood samples were analyzed for serum immunoglobulins G and A (IgG and IgA), C-reactive protein (cRP) and interferon-γ (IFN-γ) to examine immune function. Body weights, average daily weight gain, average daily feed intake, and growth:feed intake ratio were recorded to assess piglet growth and performance. Small intestinal villus height and crypt depth were measured to examine intestinal epithelium effects of *Q. saponaria* supplementation.

An average daily intake of 70 mg/kg body weight/day was calculated for *Q. saponaria* saponins (NRC, 1998). There were no reported adverse effects on piglet health, intestinal morphology, or immune function. Consumption of *Q. saponaria* saponins did not affect piglet growth. On Days 15 to 20, piglets receiving the diet supplemented with *Q. saponaria* saponins only (*i.e.*, without curcumin) displayed a significantly higher feed intake compared to the controls (P=0.044), resulting in a decrease in the growth:feed intake ratio during this time period. The study authors suggested that the piglets in the *Q. Saponaria* saponins group may have had increased vigor during this time period that resulted in increased appetite and feed intake; however, they further suggested that it was more likely that the additional energy consumed was used for the observed physiologically beneficial immune system effects, rather than growth.

Fidan and Dündar (2008) reported on the effects of *Yucca schidigera, Q. saponaria,* and a mixture of both compounds over a 3-week period in streptozotocin-induced diabetic male albino Wistar rats (180 to 250 g body weight). Normal and diabetic rats were randomly assigned into 1 of the following 5 groups (10 rats/group): non-diabetic control (C), diabetic control (D), diabetes with *Y. schidigera* (DY), diabetes with *Q. saponaria* (DQ), and diabetes with *Y. schidigera* and *Q. saponaria* (DQY). The control groups (C and D) received a standard rat feed (SRF). The diabetic groups (DY, DQ, and DQY) were provided with SRF containing 100 ppm *Y. schidigera* powder, 100 ppm *Q. saponaria* powder[6] (equivalent to 9 mg *Q. saponaria* powder/kg body weight/day and providing 0.225 to 0.315 mg *Q. saponaria* saponins/kg body weight/day), or 100 ppm *Y. schidigera* and *Q. saponaria* powder[7] (equivalent to 9 mg *Y. schidigera* and *Q. saponaria* powder/kg body weight/day providing 0.225 mg *Q. saponaria* saponins/kg body weight/day), respectively. Rats were anaesthetized at the end of the study. Cardiac blood samples were collected and malondialdehyde concentration and DNA damage were examined. In addition, plasma samples were evaluated for protein carbonyls, total antioxidant capacity, nitric oxide, insulin, triglycerides, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and fasting glucose. Beneficial effects were reported in the DQ group compared to the D control group on plasma lipids (significantly decreased plasma cholesterol and triglyceride levels), antioxidant capacity (significantly decreased mononuclear leukocyte DNA damage, plasma malondialdehyde and protein carbonyl levels), and glucose

[5] Composition of *Q. saponaria* saponins not reported.
[6] *Q. saponaria* powder (Nutrafito; 100% plant powder) contained 2.5 to 3.5% triterpenoidal saponins.
[7] *Q. saponaria* and *Y.schidigera* powder (Nutrafito Plus; 100% plant powder) contained 2.5% triterpenoid and 0.5% steroidal saponins.

(significantly decreased fasting blood glucose levels). There were no adverse effects on any of the parameters assessed.

Turner *et al.* (2002) reported on a study in which 96 male and female weanling piglets (approximately 24 days of age and weighing 8.9 kg at baseline) were provided with diets containing 0, 125, 250, or 500 mg *Q. saponaria* extract/kg diet over a 28-day period and housed 2 animals/pen. The effects of dietary *Q. saponaria* extract[8] on growth performance and immune function, alone or in combination with an immune challenge (*i.e.,* orally administered *Salmonella typhimurium*) were reported. Body weight and feed consumption were measured once a week on Days 0, 7, 14, 21, and 28. On Day 14, the immune challenge groups received 10.5 x $10^9$ colony-forming units of *S. typhimurium.* Following administration of *S. typhimurium*, serum samples from 1 piglet/pen were collected every week on Days 14, 21, and 28 for measurements of haptoglobin, α1-acid glycoprotein, immunoglobulin M (IgM), and IgG concentrations. Serum samples were also collected on Days 14, 16, 18, and 20 for measurement of insulin-like growth factor 1 (IGF-1) concentration.

The average consumption of *Q. saponaria* extract by piglets was 6, 14, and 26 mg/kg body weight/day in the 125, 250, and 500 mg dose groups, respectively. No effects were reported with respect to average daily growth or average daily feed intake in the *Q. saponaria* extract groups. Piglets in the mid-dose group exhibited a trend towards a significant decrease in gain:feed intake ratio compared to all other groups ($P<0.06$). This decrease was not dose-related and was considered a minor effect by the study authors. There were no significant, compound-related effects on serum acute phase proteins, immunoglobulins, or *in vitro* phagocytic function reported compared to the controls. There were no treatment-related adverse effects on rectal temperature (1 piglet/pen) reported on Days 14 to 21.

Sen *et al*. (1998) reported on the effects of *Q. saponaria* saponins (obtained from 3 different suppliers; Sigma, Roth, and Nor-feed) on the growth of *E. coli*. Aqueous solutions of 0%, 0.05%, 0.1%, 0.25%, 0.5%, 0.75%, or 1.0% *Q. saponaria* saponins were added to a protein-free minimal medium prior to or after heat-sterilization. *E. coli* was then incubated in the medium at 37°C for 6 hours, plated, and incubated for another 16 to 20 hours. Incubation with 0.1%, 0.75%, or 0.5% saponins from Sigma, Roth, and Nor-fed, respectively, resulted in maximum growth of the bacteria. Total bacterial counts were increased by 31%, 151%, and 87% at 0.1%, 0.75%, and 0.5% *Q. saponaria* saponins, respectively, compared to controls. Growth of *E. coli* was significantly hindered by saponins (obtained from Sigma) at concentrations ≥0.25% compared to controls. A significant increase in growth of *E. coli* was exhibited in media containing saponins (obtained from Nor-feed and Roth) at concentrations of ≥0.25% and 0.1%, respectively, compared to controls. The study authors noted that the varying effects of the *Q. saponaria* saponins from different sources on microbial growth suggested that their biological activity varied. Additionally, heat-treatment of the saponins did not affect maximal growth concentrations.

## 6.5 Human Studies

Extracts of *Q. saponaria* have been investigated in 2 human studies, both of which support the tolerability of quillaia extract at doses up to 0.54 g/day (equivalent to 0.33 g *Q. saponaria* saponins/day or 4.7 mg *Q. saponaria* saponins/kg body weight/day for a 70-kg human) for periods of up to 4 weeks (Kim *et al.,* 2003; Naknukool *et al*., 2011).

[8] Saponin content of extract not reported.

In a single-arm intervention study conducted by Naknukool *et al*. (2011), effects of oral supplementation with *Q. saponaria* saponin preparation (containing 45% saponins) on liver function and inflammation biomarkers were investigated in human volunteers. Daily supplements consisting of 4 g sports drink powder and 15 mg *Q. saponaria* saponin powder (providing of 0.5 mg *Q. saponaria* saponin/kg body weight/day) were mixed with 100 mL of water and given to 8 healthy men (22 to 23 years of age) after breakfast for 7 consecutive days. Blood samples were obtained from the subjects at baseline and 24 hours following the last drink consumed for the determination of peripheral macrophage chemotactic and phagocytic activity and concentrations of albumin, immunoglobulin E (IgE), IgG, ALT, AST, γ-glutamyl transferase (GGT), cRP, interleukin-1α (IL-1α), and tumor necrosis factor-α (TNF-α). Compared to baseline, there were no adverse effects on peripheral macrophage chemotactic or phagocytic activity and no changes in plasma concentrations of ALT, AST, GGT, cRP, IL-1α, or TNF-α; or serum concentrations of IgG, IgE, or albumin following consumption of *Q. saponaria* saponins. Significant increases in α1-globulins and α2-globulins (biomarkers of inflammation) were reported compared to baseline ($P<0.05$). The authors noted that the increases reported were of no toxicological significance, as they were within historical control ranges. Based on the results, the study authors concluded that there were no effects on liver function, proinflammatory cytokines, or inflammatory responses resulting from supplementation with 0.5 mg *Q. saponaria* saponin/kg body weight/day.

In a randomized, double-blind study reported by Kim *et al.* (2003), the tolerability and effects of combined *Q. saponaria* and *Y. schidigera* extracts were examined in 86 adult subjects (sex not reported) that presented with high blood triglyceride concentrations (>220 mg/dl). The subjects consumed 0.9 g *Q. saponaria* (containing 61.8% saponins) and *Y. schidigera* (containing 21.4% saponins) extracts at a combined ratio of 6:4 or ingested a placebo, 3 times daily over a period of 4 weeks. Daily intakes of 0.54 and 0.33 g *Q. saponaria* extract and saponins, respectively, were consumed. Baseline and end of study measurements were obtained for total, HDL-, and LDL-cholesterol. In addition, a questionnaire was provided to the subjects to assess the severity of gastrointestinal symptoms such as abdominal bloating, gas distension, constipation, and diarrhea. The symptoms were graded on a subjective scale (*i.e.,* 0 = no symptoms; 1 = mild; 2 = moderate; 3 = severe; 4 = very severe). Blood lipids and gastrointestinal symptoms (reported as gas distension, belching, constipation, diarrhea, and hangover) were not adversely affected by consumption of *Q. saponaria* and *Y. schidigera* extracts.

## 6.6 Allergenicity

Only a single case report of sensitization to *Q. saponaria* bark dust through inhalation was identified (Raghuprasad *et al*., 1980). In the case-report, Raghuprasad *et al*. (1980) described a 24-year-old Caucasian male smoker with a history of rhinitis who developed sensitization to *Q. saponaria* bark dust. The sensitization emerged following the start of his position as a spray drier operator in a "saponin dust" manufacturing factory using *Q. saponaria* bark. Exposure to the raw bark dust for a few minutes resulted in the subject sneezing and developing dyspnea and wheezing. The symptoms continued despite wearing a protective mask. At the start, the subject reported that exposure to saponin dust only resulted in nasal symptoms. However, 3 months into working at the factory, the subject experienced symptoms of wheezing, rhinorrhea, and ocular lacrimation and itching. When the subject was not at work, significant improvements were reported in the experienced symptoms. Scattered wheezes heard on auscultation of the chest were the only physical observation reported at his initial examination. In a bronchial challenge test, the subject was exposed to *Q. saponaria* dust which resulted in immediate bronchoconstriction along with faintness, diffuse erythema, and hypotension. The patient was also subjected to skin prick and intradermal challenge tests. The immunoassay demonstrated that the subject was allergic to *Q. saponaria* bark dust and that there was cross-reactivity between gum acacia and gum tragacanth.

Quillaia extract type 1 has had FEMA GRAS status since 1965 (Hall and Oser, 1965) and has thus been present in the U.S. food supply for greater than 50 years. The lack of reports of oral allergenicity to quillaia extracts, despite the long history of use, supports the low allergenic potential of quillaia extracts.

## 6.7 Quillaia Extracts and Bile Metabolism

The chemical structures of quillaia saponins and bile acids are similar in that both are amphipathic. Quillaia saponins consist of a single hydrophobic fat-soluble triterpene structure and two hydrophilic water-soluble carbohydrate chains (Figure 6.7-1), while bile acids consist of a hydrophobic fat-soluble steroid structure and a hydrophilic water-soluble carbohydrate chain terminating in a carboxylic acid (Figure 6.7-2). The function of bile acids and quillaia saponins also are similar. Bile acids facilitate digestion and absorption of lipids in the small intestine as well as regulate cholesterol homeostasis. The amphipathic structure of bile acids makes them potent "digestive surfactants" that form micelles in the small intestine by surrounding lipids, in turn promoting then absorption of lipids (Figure 6.7-3). Under normal circumstances, enterohepatic circulation enables 95% of bile acids to be reabsorbed from the distal ileum and transported back to the liver *via* the portal circulation, and therefore only approximately 5% of bile acids are not reabsorbed and are eliminated in the feces (Staels and Fonseca, 2009). The similar amphipathic structure of quillaia saponins allow them to also act as emulsifiers and foaming agents when used as food additives (Oakenfull, 1981). Due to their chemical and functional similarities, the potential for interactions between quillaia saponins and bile acids, and the potential for an interaction to affect liver weight, were investigated.

**Figure 6.7-1 Chemical Structure of Quillaia Saponins**

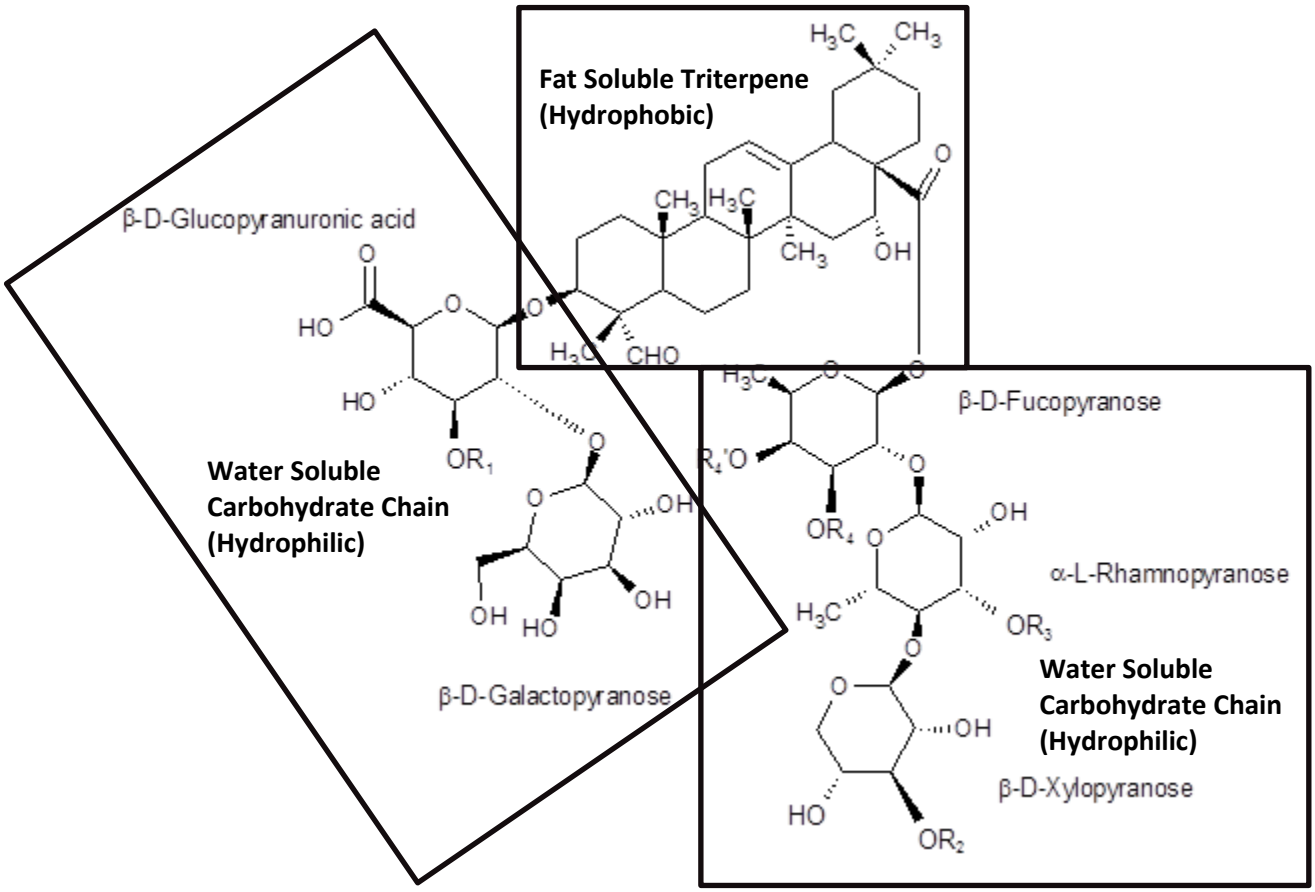


**Figure 6.7-2 Chemical Structure of Cholic Acid**

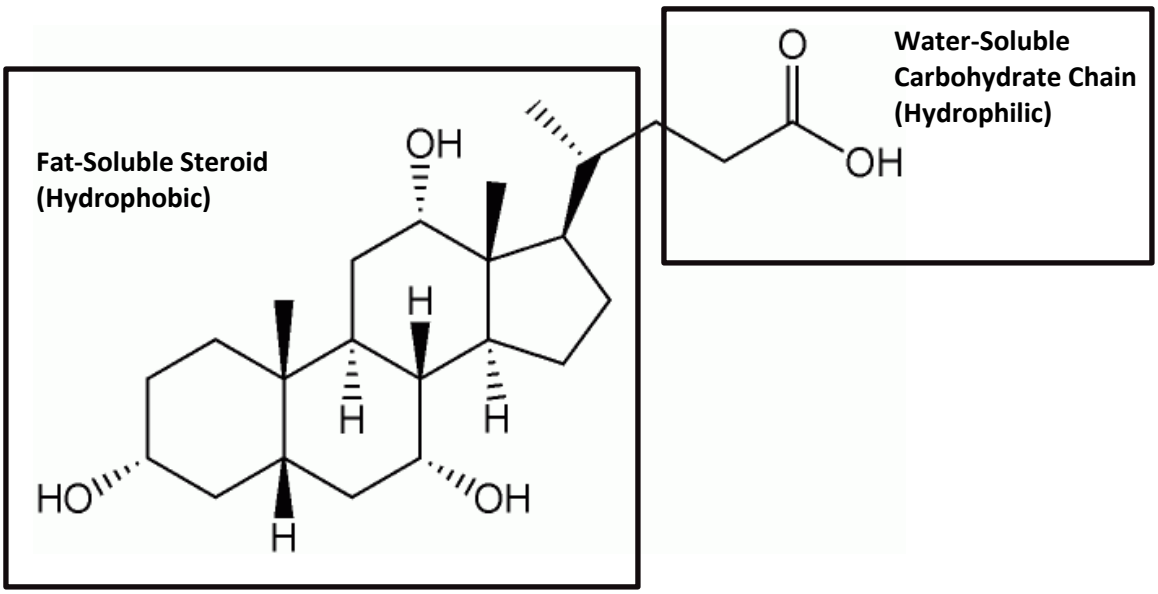
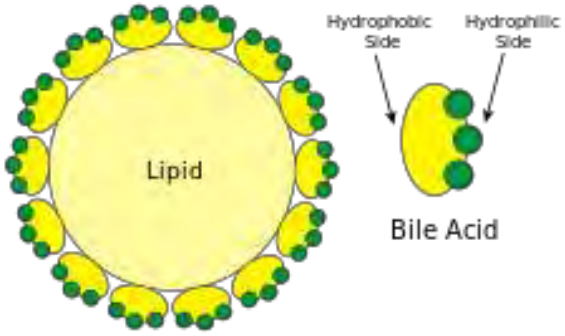



**Figure 6.7-3 Action of Bile Acid in Lipid Digestion**




Source: https://en.wikipedia.org/wiki/Bile

As their amphipathic structure suggests, with a hydrophobic and a hydrophilic end, saponins have been demonstrated to form insoluble micelle complexes with cholesterol and other sterols such as bile acids (Oakenfull, 1986; Cheeke, 2000). The hydrophobic fat-soluble triterpene of quillaia saponins has been demonstrated to associate *via* lipophilic bonding with the hydrophobic fat-soluble steroid structure of bile acids in a stacked micellar aggregation (Sidhu and Oakenfull, 1986).

In *in vitro* studies, purified triterpene saponins from soapwort (*Saponaria officinalis*), soya beans and quillaia (*Q. saponaria*), as well as an ethanol extract of defatted fenugreek (*Trigonella foenumgraecum*) seeds, containing steroidal saponins were reported to decrease the rate of or inhibit absorption of various bile acids, including cholate, taurocholate, and deoxycholate (bile acids), and cholesterol using perfused loops of rat small intestine or the rat everted-sac technique (Sidhu and Oakenfull, 1986; Stark and Madar, 1993). The effects on the rates of absorption varied between the saponins, with quillaia saponins having the least effect (Sidhu and Oakenfull, 1986).

In *in vivo* studies, the consumption of saponins has been reported to affect cholesterol and bile acid metabolism in hamsters, rats, chicks, laying hens, pigs, and monkeys (Oakenfull *et al.*, 1979; Topping *et al.*, 1980; Malinow *et al.*, 1981; Stark and Madar, 1993; Jenkins and Atwal, 1994; Lee *et al.*, 2005; Afrose *et al.*, 2009, 2010; Shi *et al.*, 2014). The dietary intake of saponins generally has a hypocholesterolemic effect, with significant reductions in plasma cholesterol and significant increases in fecal cholesterol and bile acids. Mechanistically, it is suggested that the interaction of saponins with cholesterol and bile acids creates large mixed micelles, which prevents their reabsorption and results in their excretion in the feces (Sidhu and Oakenfull, 1986; Cheeke 2001). As the reservoir of bile acids in the body (liver or gastrointestinal tract) is generally maintained at a constant level, the excess excretion of bile acids caused by saponins leads to increased production of bile acids in the liver. As bile acids are produced in the liver from cholesterol (Staels and Fonseca, 2009), increased production of bile acids in the liver leads to decreased serum and liver cholesterol, and therefore a hypocholesterolemic effect.

However, despite the effects of saponins on cholesterol and bile acids in the reviewed studies, there was no effect on liver weight in any of the studies at doses ranging from 6 mg quillaia saponins/kg body weight/day to 600 mg European soapwort saponins/kg body weight/day for triterpene glycoside saponins (Oakenfull *et al.*, 1979; Afrose *et al.*, 2009; Shi *et al.*, 2014) and at doses of 1,540 and 2,560 mg ethanol extract of defatted fenugreek seeds/kg body weight with unknown steroidal saponin content (Stark and Madar, 1993). Notably, the dose of European soapwort saponins with no effect on liver weight (*i.e.*, 600 mg/kg body weight/day) was twice as high as the NOAEL for quillaia saponins (*i.e.*, 300 mg/kg body weight/day) determined in the 2-year study in rats by Drake *et al.* (1982). Thus, although there is a plausible mechanism for how quillaia saponins may decrease liver weight, there is no evidence from the current studies to support this theory. Furthermore, there was no consistent effect on liver weight in the toxicology studies conducted with quillaia extracts (Gaunt *et al*., 1974; Phillips *et al*., 1979; Drake *et al*., 1982). If there were a true effect of quillaia extracts on liver weight, a consistent, statistically significant, dose-dependent change in liver weight would be expected. In the absence of a consistent, statistically significant, dose-dependent adverse change in liver weight in the subchronic and chronic toxicology studies (Gaunt *et al*., 1974; Phillips *et al*., 1979; Drake *et al*., 1982), it is concluded that there is no causal relationship between the consumption of quillaia extracts and decreased liver weight.

## 6.8 GRAS Panel Evaluation

Naturex has concluded that quillaia extract type 2 meeting appropriate food-grade specifications and manufactured consistent with the principles of cGMP is GRAS for use in food and beverages, as described in Part 1.3, on the basis of scientific procedures.

This GRAS conclusion is based on data generally available in the public domain pertaining to the safety of quillaia extract type 2, as discussed herein, and on consensus among a panel of experts (the GRAS Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The GRAS Panel consisted of the following qualified scientific experts: Professor Emeritus Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Dr. David J. Brusick, Ph.D., A.T.S. (Toxicology Consultant), and Professor Gary M. Williams, MD (New York Medical College). The GRAS Panel was selected and convened in accordance with the U.S. Food and Drug Administration (FDA) guidance for industry on *Best Practices for Convening a GRAS Panel* (U.S. FDA, 2017).

The GRAS Panel, convened by Naturex, independently and critically evaluated all data and information presented herein, and concluded that the proposed uses, as described in Part 1.3, of quillaia extract type 2, meeting appropriate food-grade specifications and produced consistent with current cGMP, are safe and suitable. The GRAS Panel also unanimously concluded that the proposed uses, as described in Part 1.3, of quillaia extract type 2, meeting appropriate food-grade specifications and manufactured consistent with the principles of cGMP, are GRAS, based on scientific procedures. A summary of data and information reviewed by the GRAS Panel, and evaluation of such data as it pertains to the proposed GRAS uses of quillaia extract type 2 is presented in Appendix B.

## 6.9 Conclusion

Based on the above data and information presented herein, Naturex has concluded that the intended uses, as described in Part 1.3, of quillaia extract type 2 meeting appropriate food-grade specifications and manufactured consistent with the principles of cGMP are safe and suitable. Furthermore, Naturex has concluded that the intended uses, as described in Part 1.3, of quillaia extract type 2 meeting appropriate food-grade specifications and manufactured consistent with the principles of cGMP are GRAS, on the basis of scientific procedures. General recognition of Naturex's GRAS conclusion is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of quillaia extract type 2 in food, who similarly concluded that the proposed uses of quillaia extract type 2 are GRAS on the basis of scientific procedures.

Quillaia extract type 2 therefore may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

# Part 7. §170.255 List of Supporting Data and Information


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**Table of CFR Sections Referenced (Title 21—Food and Drugs)**

| Part | Section § | Section Title |
|---|---|---|
| Subchapter B—Food for Human Consumption | | |
| 170—Food additives | 170.3 | Definitions |
| | 170.30 | Eligibility for classification as generally recognized as safe (GRAS) |

**Table of CFR Sections Referenced (Title 21—Food and Drugs)**

| Part | Section § | Section Title |
|---|---|---|
| 172—Food additives permitted for direct addition to food for human consumption | 172.510 | Natural flavoring substances and natural substances used in conjunction with flavors |


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# APPENDIX A
# Summary of Genotoxicity Study Results

# SUMMARY OF GENOTOXICITY RESULTS

## A.1 Bacterial Reverse Mutation Assay (OECD TG 471)

The results from the bacterial reverse mutation assay conducted with quillaia extract type 1 in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 471 and in compliance with GLP [OECD, 1997, 1998; Sequani Limited, 2018a (unpublished)] are summarized in Tables A.1-1 and A.1-2.

**Table A.1-1 Mean Number of Revertants per Plate – Plate Incorporation**

| Strain | Dose levels µL saponins/plate [µL quillaia extract type 1/plate] | | | | | | PC |
|---|---|---|---|---|---|---|---|
| | 0 | 0.05 [0.2155] | 0.15 [0.6465] | 0.5 [2.155] | 1.5 [6.465] | 5 [21.55] | |
| **Absence of S9** | | | | | | | |
| TA1535 | 14.3 | 15.7 | 20.0 | 13.7 | 13.7 | 16.3 | 605.7 |
| TA1537 | 5.0 | 7.7 | 4.7 | 8.7 | 9.3 | 11.7 | 404.3 |
| TA98 | 19.7 | 20.3 | 21.7 | 18.7 | 19.0 | 31.3 | 120.3 |
| TA100 | 78.0 | 95.3 | 98.0 | 86.0 | 88.0 | 106.7 | 545.3 |
| WP2 *uvrA* | 36.0 | 26.3 | 32.3 | 29.0 | 33.7 | 39.0 | 634.7 |
| **Presence of S9** | | | | | | | |
| TA1535 | 1.0 | 11.7 | 8.3 | 15.0 | 10.7 | 13.7 | 121.7 |
| TA1537 | 13.3 | 11.0 | 12.7 | 9.3 | 8.7 | 5.7 | 203.0 |
| TA98 | 31.7 | 32.3 | 25.7 | 19.3 | 26.7 | 25.0 | 430.7 |
| TA100 | 121.7 | 120.0 | 134.7 | 91.3 | 103.0 | 93.3 | 1,863.7 |
| WP2 *uvrA* | 37.7 | 40.3 | 39.3 | 37.7 | 28.0 | 40.0 | 224.3 |

PC = positive control.

**Table A.1-2 Mean Number of Revertants per Plate – Pre-Incubation**

| Strain | Dose levels µL saponins/plate [µL quillaia extract type 1/plate] | | | | | | PC |
|---|---|---|---|---|---|---|---|
| | 0 | 0.05 [0.2155] | 0.15 [0.6465] | 0.5 [2.155] | 1.5 [6.465] | 5 [21.55] | |
| **Absence of S9** | | | | | | | |
| TA1535 | 10.0 | 14.3 | 10.7 | 14.0 | 11.7 | 15.0 | 722.7 |
| TA1537 | 10.0 | 7.3 | 8.7 | 7.3 | 5.3 | 7.0 | 101.0 |
| TA98 | 24.0 | 29.0 | 32.7 | 23.0 | 29.3 | 36.0 | 126.0 |
| TA100 | 153.7 | 141.0 | 143.3 | 144.7 | 137.3 | 135.0 | 612.0 |
| WP2 *uvrA* | 33.0 | 30.0 | 32.7 | 21.3 | 36.7 | 31.0 | 1398.0 |
| **Presence of S9** | | | | | | | |
| TA1535 | 8.3 | 13.3 | 9.0 | 8.0 | 11.0 | 9.0 | 154.3 |
| TA1537 | 10.3 | 9.3 | 14.3 | 7.3 | 10.7 | 5.7 | 145.3 |
| TA98 | 39.7 | 30.7 | 30.3 | 28.7 | 30.7 | 29.7 | 1,060.0 |

**Table A.1-2 Mean Number of Revertants per Plate – Pre-Incubation**

| Strain | Dose levels µL saponins/plate [µL quillaia extract type 1/plate] | | | | | | PC |
|---|---|---|---|---|---|---|---|
| | 0 | 0.05 [0.2155] | 0.15 [0.6465] | 0.5 [2.155] | 1.5 [6.465] | 5 [21.55] | |
| TA100 | 172.7 | 164.3 | 155.7 | 133.3 | 137.0 | 124.7 | 1,864.3 |
| WP2 *uvrA* | 33.0 | 36.3 | 34.7 | 26.7 | 36.3 | 45.3 | 186.3 |

PC = positive control.

## A.2 *In Vitro* Mammalian Cell Micronucleus Test (OECD TG 487)

An *in vitro* mammalian cell micronucleus test was conducted with quillaia extract type 1 in accordance with the OECD TG 487 and in compliance with GLP [OECD, 1998, 2016a; Sequani Limited, 2018b (unpublished)]. The results of the 3-hour treatment in the presence and absence of S9 mix, with harvesting 44 hours after initiation of treatment are summarized below in Tables A.2-1 and A.2-2. Continuous treatment (27 hours) was not performed due to the positive result seen after the 3-hour treatment in the presence of S9 mix.

**Table A.2-1 Micronucleus Frequency – 3-Hour Treatment in the Presence of S9 Mix**

| Quillaia Extract Type 1 Dose µL saponins/mL [µL quillaia extract type 1/mL] | Sample | Nucleated Events | MN | Mean MN | % MN | Mean % MN |
|---|---|---|---|---|---|---|
| 0 | A | 20007 | 157 | 133.5 | 0.78 | 0.66 |
| | B | 20004 | 110 | | 0.55 | |
| 0.002 [0.00862] | A | 20000 | 96 | 89.5 | 0.48 | 0.45 |
| | B | 20000 | 83 | | 0.41 | |
| 0.01 [0.0431] | A | 20000 | 145 | 166.0 | 0.72 | 0.82 |
| | B | 20000 | 187 | | 0.93 | |
| 0.012 [0.05172] | A | 19991 | 501 | 422.5 | 2.44 | 2.07*** |
| | B | 20000 | 344 | | 1.69 | |
| CPA 2.0 µg/mL | A | 20101 | 601 | 522.0 | 2.90 | 2.53*** |
| | B | 20120 | 443 | | 2.15 | |
| CPA 2.5 µg/mL | A | 20171 | 978 | 857.0 | 4.62 | 4.07*** |
| | B | 20197 | 736 | | 3.52 | |

CPA = cyclophosphamide; MN = micronuclei.
*** significant at 0.1% level.

**Table A.2-2 Micronucleus Frequency – 3-Hour Treatment in the Absence of S9 Mix**

| Quillaia Extract Type 1 Dose µL saponins/mL [µL quillaia extract type 1/mL] | Sample | Nucleated Events | MN | Mean MN | % MN | Mean % MN |
|---|---|---|---|---|---|---|
| 0 | A | 19990 | 45 | 52.5 | 0.22 | 0.26 |
| | B | 20000 | 60 | | 0.30 | |
| 0.002 [0.00862] | A | 20027 | 67 | 87.0 | 0.33 | 0.43* |
| | B | 20012 | 107 | | 0.53 | |
| 0.01 [0.0431] | A | 20000 | 114 | 100.0 | 0.57 | 0.50*** |
| | B | 20000 | 86 | | 0.43 | |
| 0.012 [0.05172] | A | 19978 | 150 | 132.5 | 0.75 | 0.66*** |
| | B | 20000 | 115 | | 0.57 | |
| 0.014 [0.06034] | A | 20027 | 241 | 188.0 | 1.19 | 0.93*** |
| | B | 20208 | 135 | | 0.66 | |
| MMC 0.1 µg/mL | A | 20168 | 577 | 595.5 | 2.78 | 2.88*** |
| | B | 20030 | 614 | | 2.97 | |

MMC = mitomycin C; MN = micronuclei.
* = significant at 5% level.
*** = significant at 0.1% level.

## A.3 *In Vivo* Mammalian Erythrocyte Micronucleus Test (OECD TG 474)

An *in vivo* mammalian erythrocyte micronucleus test was conducted with quillaia extract type 1 in accordance with the OECD TG 474 and in compliance with GLP [OECD, 1998, 2016b; Sequani Limited, 2018c (unpublished)]. The results are summarized in Table A.3-1.

**Table A.3-1 Micronucleus Data for Quillaia Extract Type 2 compared to Negative and Positive Control**

| Parameter | Negative Control 0 mg/kg/day | Quillaia Extract 8620 mg/kg/day (2000 mg/kg/day | CPA 15 mg/kg |
|---|---|---|---|
| N | 6 | 5 | 6 |
| Mean RET | 18,552.50 | 19,397.40 | 18,506.67 |
| Mean MN-RET | 19.83 | 13.00 | 274.17 |
| Mean MN-RET frequency | 0.11 | 0.07 | $1.48^{+++}$ |
| Mean NCE | 468,950.17 | 1,384,912.60 | 1,402,416.83 |
| Mean % RET | 3.87 | $1.53^{WW}$ | $1.41^{WW}$ |

CPA = cyclophosphamide; N = number of animals.
$^{WW}$ = statistically significant (Wilcoxon's Test) p<0.01.
$^{+++}$ = statistically significant (Poisson test) p<0.001.

# APPENDIX B
# GRAS Panel Statement

# Report of the Generally Recognized as Safe (GRAS) Panel Concerning the GRAS Status of Quillaia Extract Type 2 for Use in Foods and Dietary Ingredients

### 27 November 2019

## INTRODUCTION

Naturex SA (Naturex) intends to market quillaia extract type 2 as an ingredient in traditional food products and as an excipient in dietary ingredients in the United States (U.S.). A critical and comprehensive evaluation of the available pertinent data and information concerning the safety and Generally Recognized as Safe (GRAS) status of the proposed uses of Naturex's quillaia extract type 2 was conducted by a panel of independent scientists (the "GRAS Panel"), qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, at the request of Naturex. The GRAS Panel was specifically asked to determine whether the intended uses of quillaia extract type 2 would be GRAS based on scientific procedures. For the purposes of the GRAS Panel's evaluation, "safe" or "safety" indicates that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i) (U.S. FDA, 2019).

The GRAS Panel consisted of the below-signed qualified scientific experts: Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine); David J. Brusick, Ph.D., A.T.S (Toxicology Consultant); and Gary M. Williams, MD (New York Medical College). The GRAS Panel was selected and convened in accordance with the U.S. Food and Drug Administration (FDA) guidance for industry on *Best Practices for Convening a GRAS Panel* (U.S. FDA, 2017). Naturex confirms that prior to convening the GRAS Panel, all reasonable efforts were made to identify and select a balanced GRAS Panel with expertise in appropriate scientific disciplines deemed necessary for the safety evaluation of quillaia extract type 2, and efforts were placed on identifying conflicts of interest or relevant appearance issues that would potentially bias the outcome of the deliberations of the GRAS Panel; no such conflicts of interest or appearance of conflicts were identified. The GRAS Panel received a reasonable honorarium as compensation for the GRAS Panel's time, and honoraria provided to the GRAS Panel were not contingent upon the outcome of the GRAS Panel deliberations.

The GRAS Panel, independently and collectively, critically evaluated a supporting dossier submitted by Naturex, "*Documentation Supporting the Evaluation of Quillaia Extract Type 2 as Generally Recognized as Safe (GRAS) for Use in Food*" dated September 2018. This dossier is a comprehensive package of data and information, including the method of manufacture, product specifications and analytical data, stability, intended conditions of use, estimated intake of saponins (reference component) from all permitted and proposed uses of quillaia extract type 1 and type 2, estimated intake of quillaia extract type 2 (ingredient) from all proposed uses, and a summary of the available scientific information and data pertinent to the safety of quillaia extract type 2 as it applies to potential consumer exposures. In addition, the GRAS Panel evaluated other information deemed appropriate or necessary.

Following independent, critical evaluation of such data and information, the GRAS Panel convened *via* teleconference on 11 September 2018. The GRAS Panel reviewed their findings and, following discussion, unanimously concluded that the intended uses described herein of quillaia extract type 2, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice (cGMP), are GRAS based on scientific procedures. In November 2019, the GRAS Panel reviewed a revised version of the dossier, dated November 2019, which included additional manufacturing information on alternative processing aids and final product state of quillaia extract type 2, information on the potential interaction between quillaia saponins and bile acids, and a summary of the European Food Safety Authority's (EFSA's) evaluation of quillaia extracts. The GRAS Panel unanimously concluded that the additional information did not change their previous conclusion that the intended uses described herein of quillaia extract type 2, meeting appropriate food-grade specifications and manufactured consistent with cGMP, are GRAS based on scientific procedures.

A summary of the basis for the GRAS Panel's conclusion is provided in the following section.

# SUMMARY AND BASIS FOR GRAS

Naturex intends to market quillaia extract type 2 (liquid and powder) as an ingredient in traditional food products and as an excipient in dietary ingredients in the U.S. (Table A-1). Quillaia extract type 2 is extracted from wood and/or bark of the Chilean tree *Quillaja saponaria* Molina (family *Rosaceae*), a large evergreen tree with shiny, leathery leaves and thick bark. Specifications for quillaia extract type 2 have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and quillaia extract type 2 liquid and powder manufactured by Naturex meets all limits within this specification (JECFA, 2014).

The GRAS Panel critically reviewed details of the manufacturing process for quillaia extract type 2. Quillaia extract type 2 is manufactured following a Food Safety Assurance Plan based on the Hazard Analysis and Critical Control Point (HACCP) system and consistent with cGMP. Quillaia extract type 2 is extracted from milled wood and/or bark chips of the Chilean tree *Quillaja saponaria* Molina (family *Rosaceae*) with hot water. The extract is stabilized with food-grade acid with or without the use of a food-grade enzyme preparation, reducing the pH to less than 4.0, and concentrated. The concentrated extract is purified and concentrated further until the saponins content reaches 65% (dry basis), and the concentrated extract is filtered. Then the product is pasteurized. For liquid products, the liquid extract can be formulated with 0.1 to 0.2% sodium benzoate as preservative. Sodium benzoate is a direct food substance that has been affirmed as GRAS for use as an antimicrobial agent at levels not to exceed cGMP with the stipulation that current use results in a maximum of 0.1% in food (21 CFR §184.1733 – U.S. FDA, 2019). For powder products, the liquid extract is spray-dried with inlet air temperature. The final quillaia extract type 2 is packaged and labeled after samples are obtained for retention, and a final quality control analysis of the product is conducted. Naturex confirms that all food contact articles, processing aids, and additives used in the manufacture of quillaia extract type 2 are food-grade and approved for their intended use in accordance with an appropriate federal regulation, effective food contact notification, or have previously been concluded to be GRAS.

Naturex has established physical, chemical, and microbiological specification and control plan limits for quillaia extract type 2 liquid and powder that are consistent with those established by JECFA (JECFA, 2014) to ensure the product is of food-grade quality. Compositionally, Naturex's quillaia extract type 2 consists of 65 to 75% saponins on a dry weight basis. Analysis of Naturex's quillaia extract type 2 using reverse phase high-performance liquid chromatography (HPLC) demonstrates that the chromatographic profile is consistent with the chromatographic standard in the JECFA specifications and that the major saponin present is QS-18, with lower levels of QS-7. The total quantity of saponins is calculated using reverse phase

HPLC. The GRAS Panel reviewed the results from 6 non-consecutive lots of quillaia extract type 2 liquid and 4 non-consecutive lots of quillaia extract type 2 powder that demonstrate that the manufacturing process produces a consistent product that meets all specification and control plan limits. The results also demonstrate that lead and potential microbiological contaminants are below levels of toxicological concern. Additional analytical data demonstrate that polyphenols comprise a minor component of quillaia extract type 2 (1.31 to 5.22% of the liquid extract), and that quillaia extract type 2 does not contain a significant level of starch, sugars, fiber, protein, calcium, or magnesium.

The GRAS Panel reviewed data supporting the bulk stability of quillaia extract type 2 liquid under real-time storage conditions in Naturex's Chilean facility (20 to 25°C and 50 to 75% relative humidity) for 22 to almost 30 months. The results demonstrate that quillaia extract type 2 liquid is stable for the duration of the storage periods, with no appreciable change in organoleptic properties, Brix, saponin content, or levels of microbiological contaminants. The GRAS Panel confirmed that the results support the shelf-life of 24 months for quillaia extract type 2 for both the liquid and powder products.

Extracts of quillaia (*i.e.*, type not differentiated) are food additives permitted for direct addition to food for human consumption under 21 CFR §172.510 (U.S. FDA, 2019), where they are listed as natural flavoring substances and natural substances used in conjunction with flavors. In addition, the use of quillaia extract type 1 as a foaming agent in semi-frozen carbonated and non-carbonated beverages at levels not to exceed 500 mg/kg (dried basis) was concluded to be GRAS by the American Beverage Association (U.S. FDA, 2005) and use of quillaia extract type 1 as a flavor in a number of food categories has been concluded to be GRAS by the Expert Panel of the Flavor and Extract Manufacturers Association of the United States (Cohen *et al.*, 2015).

Naturex intends to use quillaia extract type 2 in the following food groups: alcoholic beverages, beverages and beverage bases, chewing gum, coffee and tea, condiments and relishes, confections and frostings, dairy product analogues, fats and oils, frozen dairy desserts, fruit and water ices, hard candy, jams and jellies, soft candy, and dietary supplements (for a technological purpose) (Table A-1). These applications include direct use of quillaia extract type 2, as well as carry over from food flavors, colors, cloudy agents, and active delivery. Use-levels of quillaia extract type 2 are expressed on the basis of the saponin content (reference component) and range from 19.5 to 120 mg/100 g in traditional foods and 600 to 1,300 mg/100 g in dietary supplements. These use-levels were also converted to the equivalent level of the ingredient itself based on the minimum saponin content of 65% (dry basis), as established in Naturex's specification for quillaia extract type 2.

The GRAS Panel reviewed assessments of the anticipated intake of saponins (reference component) from all permitted and proposed uses of quillaia extract type 1 and type 2, as well as intake of quillaia extract type 2 (ingredient), from all proposed uses that were conducted using data available in the 2013-2014 cycle of the U.S. National Health and Nutrition Examination Survey (CDC, 2015, 2016; USDA, 2016). Among the total U.S. population (all ages) and on a consumer-only basis, the resulting mean and 90$^{th}$ percentile intakes of saponins from all permitted and proposed food-uses in the U.S. of quillaia extract types 1 and 2 were estimated to be 31 and 75 mg/person/day, respectively, equivalent to 0.5 and 1.0 mg/kg body weight/day. Among the individual population groups, the highest mean and 90$^{th}$ percentile consumer-only intakes of saponins on an absolute basis were determined to be 38 and 91 mg/person/day respectively (0.4 and 1.0 mg/kg body weight/day), as identified among male adults. When expressed on a body weight basis, infants and young children had the highest mean intakes of saponins, at 0.8 mg/kg body weight/day, while children aged 3 to 11 years were determined to have the highest 90$^{th}$ percentile consumer-only intakes of 1.5 mg/kg body weight/day.

When considering the intakes of quillaia extract type 2, on a consumer-only basis, the resulting mean and 90$^{th}$ percentile intakes of quillaia extract type 2 by the total U.S. population from proposed food-uses in the U.S. were estimated to be 45 and 109 mg/person/day, respectively, equivalent to 0.7 and 1.5 mg/kg body weight/day. Among the individual population groups, the highest mean and 90$^{th}$ percentile consumer-only intakes of quillaia extract type 2 on an absolute basis were determined to be 53 and 127 mg/person/day, respectively (0.6 and 1.4 mg/kg body weight/day) identified among male adults. When expressed on a body weight basis, infants and young children had the highest mean and 90$^{th}$ percentile consumer-only intakes of quillaia extract type 2 of 1.7 and 2.5 mg/kg body weight/day, respectively.

The GRAS Panel critically evaluated the published data and information characterizing the safety of saponins and quillaia extract type 2, including safety evaluations conducted by other expert panels and original research publications. The safety of quillaia extracts was evaluated by the Scientific Committee on Foods (SCF) (SCF, 1978), by JECFA at their 26$^{th}$, 29$^{th}$, 57$^{th}$, 61$^{st}$, and 65$^{th}$ meetings (JECFA, 1982, 1986, 2002, 2004, 2006) and by EFSA (2019). SCF (1978) evaluated the natural extract of quillaia bark as specified in the British Pharmacopoeia (BP, 1973) and reviewed the results of long-term studies in the mouse (later published as Phillips *et al.*, 1979) and rat (later published as Drake *et al.*, 1982). An acceptable daily intake (ADI) of 5 mg/kg body weight for the spray-dried extract was established by the SCF. The GRAS Panel noted that the SCF did not report how the ADI was derived and also noted that the ADI did not directly correspond to the no-observed-adverse-effect level (NOAEL) reported by the authors in either the mouse (Phillips *et al.*, 1979) or rat (Drake *et al.*, 1982) studies.

JECFA initially evaluated quillaia during their 26$^{th}$ meeting, where a 13-week toxicity study in rats (Gaunt *et al.*, 1974), an 84-week study in mice (Phillips *et al.*, 1979), and a 108-week study in rats (Drake *et al.*, 1982) were reviewed (JECFA, 1982). JECFA (1982) concluded that it was not possible to evaluate quillaia extract and could not establish an ADI due to the lack of specifications for quillaia extract. Following the preparation of tentative specifications for quillaia at JECFA's 29$^{th}$ meeting, JECFA established an ADI of 0 to 5 mg/kg body weight/day based on their selected NOAEL of 1.0% in the diet (0.5 g/kg body weight/day) from the long-term rat study (JECFA, 1986). The GRAS Panel noted that the NOAEL selected by JECFA was the mid-dose and was in contrast to the NOAEL that was reported by the study authors of 3.0% in the diet (1.5 g/kg body weight/day), the highest dose tested (Drake *et al*., 1982). The GRAS Panel noted that in the evaluation, JECFA (1982) commented that there were minor changes in body weight gain and some relative organ weights in lifetime studies in the rat (Drake *et al*., 1982). The GRAS Panel considered these changes to be JECFA's basis for selecting the mid-dose as the NOAEL. JECFA also commented that no compound-related histological changes were reported. The GRAS Panel noted that body weights were reduced only in male rats in the high-dose (3.0% in the diet) group, and that body weights of the high-dose female rats were not significantly different from controls. The GRAS Panel also noted that the decreases in body weight were considered by the authors of the study to be due to unpalatability of the diet, as in preliminary palatability tests, male rats consumed less diet containing 3% *Q. saponaria* extract compared to a control diet (21 *vs*. 25 g/day) and gained less weight (11 *vs*. 26 g/day) (Drake *et al.*, 1982). The GRAS Panel further noted that the significant differences in relative organ weights occurred in one sex only and that there were no corresponding histological effects. The GRAS Panel concluded that JECFA was conservative in their determination of an ADI for quillaia extract and that there were no consistent, statistically significant, dose-dependent adverse effects in the long-term rat study to justify the selection of the mid-dose as the NOAEL.

At JECFA's 57th meeting, revisions were made to the specifications previously determined at the 29th meeting to clarify the differences between unpurified and semi-purified extracts (JECFA, 2002). In addition, the ADI previously determined by JECFA was changed to a temporary ADI of 0 to 5 mg/kg body weight for unpurified extract, pending clarification of the specifications. At the 61st meeting of JECFA, the Committee concluded that separate specifications were required for the 2 types of quillaia, type 1 ('unpurified' – saponin content between 20% and 26%) and type 2 ('semi-purified' – saponin content between 65% and 90%) (JECFA, 2004). Four major saponins (*i.e.,* QS-7, QS-17, QS-18, and QS-21) were identified in the saponin fraction of type 1 quillaia extracts and were determined to be representative of the total saponin content. The assay for quantification of saponin content was based on these 4 saponins. The Committee reviewed a study characterizing the saponin profiles of extracts from quillaia trees and concluded that the data submitted for toxicological and dietary exposure assessment were specific to the material described as a type 1 extract by the Committee. Thus, the "temporary" designation on the ADI of 0 to 5 mg/kg body weight for quillaia extract type 1 was removed. An ADI for quillaia type 2 extracts could not be established due to the limited availability of data to characterize the saponin fraction of type 2 extracts. At its 65th meeting, the Committee reviewed chromatographic data that demonstrated that the saponin profile of type 2 extracts (prepared by membrane ultrafiltration or chromatography) and type 1 extract were similar and concluded that no further toxicity studies on quillaia type 2 extracts were required due to the similarity in saponin profiles (JECFA, 2006). Thus, the toxicological data on quillaia extracts type 1 are relevant to the safety assessment of quillaia extracts type 2. In addition, saponins were considered to be the reference component responsible for toxicity, as JECFA concluded that there were no differences in toxicity between the type 1 and type 2 extracts when assessed based on their saponin content. The Committee decided to express the ADI on the basis of the saponin content. Taking the lower end of the specified range of saponins in the type 1 extract (*i.e.*, 20%), a group ADI of 0 to 1 mg/kg body weight, expressed as quillaia saponins, was established and the previously determined ADI of 0 to 5 mg/kg body weight (expressed as quillaia extract type 1) was withdrawn (JECFA, 2006).

EFSA evaluated the safety of quillaia extracts taking into account the same subchronic and chronic toxicity studies as the SCF and JECFA and also considered new genotoxicity data (EFSA, 2019). EFSA concluded that the 2-year study in rats (Drake *et al.*, 1982) was the most comprehensive and robust study and established an ADI for quillaia extract of 3 mg saponins/kg body weight/day on the basis of a NOAEL of 1,500 mg quillaia extract/kg body weight/day, factoring in that quillaia extract Type 1 contains *ca.* 20% saponins which are the bioactive substances in quillaia extract considered responsible for toxicity on the basis that the median lethal dose values for the Type 1 and 2 extracts were about the same when expressed on a saponin basis.

The GRAS Panel critically evaluated the publications of the studies reviewed by the SCF, JECFA, and EFSA, namely a 13-week toxicity study in rats (Gaunt *et al.*, 1974), an 84-week study in mice (Phillips *et al.*, 1979), and a 108-week study in rats (Drake *et al.*, 1982). In the 13-week toxicity study conducted in CFE strain SPF rats (15/sex/group), there were no adverse effects reported with respect to behavior and general condition, hematological, serum biochemical, and urinary analyses, and gross or histopathology following consumption of diets containing up to 4.0% *Q. saponaria* extract[1] in the diet (Gaunt *et al.*, 1974). Significant differences in organ weights were reported, and the study authors concluded that "*until evidence to the contrary is produced, the dietary levels producing changes in both absolute and relative organ weights must be regarded as having a toxic effect*". These significant differences consisted of decreased absolute and relative liver weights and absolute kidney weights in the mid- and high-dose male groups; decreased relative kidney weights in the mid-dose male group; and decreased relative kidney weights in the high-dose female group. A NOAEL of 0.6% in the diet (*i.e.,* approximately 400 mg/kg body weight/day of the extract), the lowest concentration tested, was determined by the study authors. The GRAS Panel agreed that the

[1] Saponin content not reported; however, it was assumed to be 20% based on JECFA's conclusion that the test material in the toxicology studies was representative of a type 1 extract and the lower limit of 20% saponins in the type 1 extract.

significant differences in organ weights on which the NOAEL was based occurred in 1 sex only and did not result in any adverse effects on organ structure or function based on the lack of hematological, serum biochemical, urinary, or gross or histological abnormalities. The GRAS Panel concluded that a NOAEL of 4% *Q. saponaria* extract in the diet (*i.e.,* approximately 2,500 mg/kg body weight/day, providing 500 mg *Q. saponaria* saponins/kg body weight/day), the highest concentration tested, would be more appropriate based on the lack of consistent, statistically significant, dose-dependent adverse effects on any parameter reported.

In the 84-week study in TO strain, SPF mice (48/sex/group), there were no adverse effects on behavior, general condition, histopathology, or incidence of tumors following consumption of up to 1.5% *Q. saponaria* extract[2] in the diet (Phillips *et al.*, 1979). Significant differences from controls in hematological parameters were not considered to represent a safety concern due to their transient nature, lack of a dose-response relationship, and opposing direction of effect in males and females. The authors selected the mid-dose of 0.5% in the diet (*i.e.,* approximately 700 mg/kg body weight/day) as the NOAEL for *Q. saponaria* extract on the basis of significantly reduced terminal body weights in high-dose males and significant differences in organ weights in high-dose animals (*i.e.,* decreased absolute weights of the liver and kidney in high-dose males, increased relative [to body weight] weight of the brain and stomach in high-dose males, and increased absolute weight of the small intestine in high-dose females). The GRAS Panel noted that there were no significant differences in the terminal body weights of female high-dose mice compared to their respective controls and that the organ weight changes occurred in 1 sex only, changes in absolute organ weights were not paralleled by changes in the relative (to body weight) organ weights, and no histopathological abnormalities were reported. The GRAS Panel concluded that a NOAEL of the highest concentration tested, 1.5% *Q. saponaria* extract in the diet (*i.e.,* approximately 2,200 mg/kg body weight/day, providing 440 mg *Q. saponaria* saponins/kg body weight/day) would be more appropriate based on the lack of definitive compound-related adverse effects.

In the 108-week study in Wistar rats (48/sex/group), there were no significant differences compared to controls in cumulative deaths, and a lack of compound-related, dose-dependent adverse effects on body and organ weights, hematology, serum biochemistry, urinalysis, tumor incidence, and gross/histopathology following consumption of diets containing up to 3.0% *Q. saponaria* extract[3] (Drake *et al.*, 1982). Based on the results of the study, the highest concentration tested, 3% *Q. saponaria* extract in the diet (*i.e.,* approximately 1,500 mg *Q. saponaria* extract/kg body weight/day, providing 300 mg *Q. saponaria* saponins/kg body weight/day), was selected as the NOAEL by the study authors.

In addition to the data reviewed by the SCF and/or JECFA, the GRAS Panel also reviewed data on the metabolic fate of related saponins in light of the lack of data on the metabolic fate of quillaia extract or quillaia saponins, and reviewed the results of recently conducted, unpublished genotoxicity studies on quillaia extract type 1.

Based on data on the related saponins glycyrrhizinic acid (*i.e.*, glycyrrhizin), escin, anhuienoside C, and soybean saponins, quillaia saponins are expected to be metabolized *via* sequential deglycosylation by the gastrointestinal microflora following oral administration (Gestetner *et al.,* 1968; Takeda *et al.*, 1996; Okamura *et al.*, 2003; Hasegawa, 2004; Yang *et al.,* 2004; Qian and Cai, 2010; Wang *et al.*, 2011; He *et al.*, 2015; Zhao *et al.*, 2015). Saponins are reported to be "*not significantly absorbed after oral administration*" (EMEA, 1996). The oral bioavailability of the related saponins, escin saponins, glycyrrhizin, pulsatilla

[2] Saponin content not reported; however, it was assumed to be 20% based on JECFA's conclusion that the test material in the toxicology studies was representative of a type 1 extract and the lower limit of 20% saponins in the type 1 extract.
[3] Saponin content not reported; however, it was assumed to be 20% based on JECFA's conclusion that the test material in the toxicology studies was representative of a type 1 extract and the lower limit of 20% saponins in the type 1 extract.

saponin D, and DS-1 from *Dianthus superbus* in rats was reported to range from 0.16% for escin Ib to 4.0% for purified glycyrrhizin saponin (Wang *et al.*, 1995; Wu *et al.*, 2012, 2014; Ouyang *et al.*, 2015; Ren *et al.*, 2017). The oral bioavailability of glycyrrhizin following oral administration of a glycyrrhiza extract was 1.7% (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. Limited data were available from human studies; however, the area under the concentration-time curve (AUC) values for the escin saponins were low, ranging from 1.8 to 22.4 ng*h/mL (Wu *et al.*, 2010), which suggests that the escin saponins have low oral bioavailability in humans. Considering the similarities among the backbone structure of the related saponins and their glycosides compared to quillaia, it is expected that quillaia saponins also will have low oral bioavailability.

Quillaia extract type 1 was not mutagenic in a bacterial reverse mutation assay in *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* strain WP2 *uvrA* either in the presence or absence of S9 mix (Sequani Limited, 2018a [unpublished]). In an *in vitro* mammalian cell micronucleus test, quillaia extract type 1 was considered to be either clastogenic or aneugenic in the presence of S9 mix and results were equivocal in the absence of S9 mix (Sequani Limited, 2018b [unpublished]). In an *in vivo* mammalian erythrocyte micronucleus test conducted in rats, quillaia extract type 1 was neither clastogenic nor aneugenic (Sequani Limited, 2018c [unpublished]). The GRAS Panel noted that in each of the genotoxicity tests, the dose levels for quillaia extract type 1 were based on saponin levels. The positive response in the *in vitro* micronucleus test was not considered to be of toxicological concern due to the negative response in the *in vivo* micronucleus test, the lack of carcinogenic effects in the long-term mouse and rat studies, and the known potential for false positives in the *in vitro* micronucleus assay. The results of the recently conducted genotoxicity studies confirm the lack of genotoxic potential for quillaia extracts.

Two human studies that had been published since the SCF and JECFA reviews also were reviewed by the GRAS Panel. The results of these human studies support the tolerability of quillaia extracts at doses up to 0.54 g/day (equivalent to 0.33 g *Q. saponaria* saponins/day or 4.7 mg *Q. saponaria* saponins/kg body weight/day for a 70-kg human) for periods of up to 4 weeks (Kim *et al.,* 2003; Naknukool *et al*., 2011).

The GRAS Panel noted that only a single case report of sensitization to *Q. saponaria* bark dust through inhalation was identified and considered that the allergenic potential of quillaia extract type 2 is low.

Quillaia saponins and bile acids both have amphipathic structures and function as emulsifiers. Due to the potential for quillaia saponins and bile acids to associate and form stacked micelles (Sidhu and Oakenfull, 1986), the GRAS Panel reviewed data on interactions between various saponins, bile acids, and cholesterol, and the potential for effects on liver weight in hamsters, rats, chicks, laying hens, pigs, and monkeys (Oakenfull *et al.*, 1979; Topping *et al.*, 1980; Malinow *et al.*, 1981; Stark and Madar, 1993; Jenkins and Atwal, 1994; Lee *et al.*, 2005; Afrose *et al.*, 2009, 2010; Shi *et al.*, 2014). While the dietary intake of saponins generally has a hypocholesterolemic effect, with significant reductions in plasma cholesterol and significant increases in fecal cholesterol and bile acids reported at doses ranging from 6 mg quillaia saponins/kg body weight/day to 600 mg European soapwort saponins/kg body weight/day for triterpene glycoside saponins (Oakenfull *et al.*, 1979; Afrose *et al.*, 2009; Shi *et al.*, 2014) and at doses of 1,540 and 2,560 mg ethanol extract of defatted fenugreek seeds/kg body weight with unknown steroidal saponin content (Stark and Madar, 1993), which provides a plausible mechanism to decrease liver weight, the GRAS Panel noted that in none of the reviewed studies did a decrease in liver weight occur in conjunction with the hypocholesterolemic effects. The dose of European soapwort saponins with no effect on liver weight (*i.e.*, 600 mg/kg body weight/day) was twice as high as the NOAEL for quillaia saponins (*i.e.*, 300 mg/kg body weight/day) determined in the 2-year study in rats by Drake *et al.* (1982). Furthermore, there was no consistent effect on liver weight in the toxicology studies conducted with quillaia extracts (Gaunt *et al*.,

1974; Phillips *et al*., 1979; Drake *et al*., 1982). The GRAS Panel noted that if there were a true effect of quillaia extracts on liver weight, a consistent, statistically significant, dose-dependent change in liver weight would be expected. In the absence of a consistent, statistically significant, dose-dependent adverse change in liver weight in the subchronic and chronic toxicology studies (Gaunt *et al*., 1974; Phillips *et al*., 1979; Drake *et al*., 1982), the GRAS Panel concluded that there was no causal relationship between the consumption of quillaia extracts and decreased liver weight.

Following critical review of the toxicological studies previously reviewed by the SCF and JECFA and review of additional data, including the metabolic fate of related saponins, unpublished genotoxicity studies, and published human studies, the GRAS Panel concluded that the pivotal study on the safety of quillaia extract type 2 was the 108-week study in rats (Drake *et al.*, 1982). This same study was used by JECFA and EFSA to establish the current ADIs. The GRAS Panel noted that JECFA was conservative in their selection of the mid-dose as the NOAEL and determination of an ADI for quillaia saponins, as at the high dose-level, the cited decreases in body weights occurred in male rats only and were attributed by the study authors to unpalatability, and the differences in relative organ weights occurred in one sex only with no corresponding histological effects. The GRAS Panel noted that EFSA derived their ADI using the author's NOAEL, which was the highest dose tested. The GRAS Panel concluded that there were no consistent, statistically significant, dose-dependent adverse effects in this study to justify the selection of the mid-dose as the NOAEL, as selected by JECFA and used to establish their ADI. The GRAS Panel considered that the lack of adverse, compound-related effects was consistent with the low oral bioavailability of saponins. Using currently accepted scientific standards, the GRAS Panel concluded that the NOAEL that was determined by the authors of the study to be 3% *Q. saponaria* extract in the diet (*i.e.,* approximately 1,500 mg *Q. saponaria* extract/kg body weight/day, providing 300 mg *Q. saponaria* saponins/kg body weight/day) (Drake *et al.*, 1982) was the most appropriate NOAEL on which to establish an ADI for quillaia saponins and on which to base the safety of the intake of quillaia extract type 2. Applying a 100-fold safety factor, the GRAS Panel determined that the resultant ADI for quillaia saponins would be 3 mg/kg body weight/day. The GRAS Panel compared the proposed ADI of 3 mg *Q. saponaria* saponins/kg body weight/day determined from the NOAEL from Drake *et al.* (1982) to the highest 90th percentile consumer-only intakes of saponins resulting from the combined current uses of quillaia extract type 1 and the proposed uses of quillaia extract type 2 of 1.5 mg saponins/kg body weight/day that occurred in children aged 3 to 11 years and noted that the intakes did not exceed the proposed ADI. The GRAS Panel also noted that the estimated intakes of saponins were conservative in nature. Thus, using current scientific thinking, and supported by new data available, the GRAS Panel concluded that the safety of the estimated intakes of saponins resulting from the current uses of quillaia extract type 1 and the proposed uses of quillaia extract type 2 was supported. Following its independent and collective critical evaluation of the available information on quillaia extracts type 1 and 2 using appropriate scientific procedures, the GRAS Panel concluded that the proposed uses of quillaia extract type 2 are safe and GRAS based on scientific procedures.

## CONCLUSION

We, the GRAS Panel, have independently and collectively, critically evaluated the data and information summarized above and conclude that the proposed uses specified herein of quillaia extract type 2, meeting appropriate food-grade specifications and produced consistent with current Good Manufacturing Practice (cGMP), are safe and suitable.

We, the members of the GRAS Panel also unanimously conclude that the proposed uses specified herein of quillaia extract type 2, meeting appropriate food-grade specifications and manufactured consistent with cGMP, are Generally Recognized as Safe (GRAS), based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

[redacted]

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**Table of CFR Sections Referenced (Title 21—Food and Drugs)**

| Part | Section § | Section Title |
|---|---|---|
| ***Subchapter B—Food for Human Consumption*** | | |
| 170—Food additives | 170.3 | Definitions |
| 172—Food additives permitted for direct addition to food for human consumption | 172.510 | Natural flavoring substances and natural substances used in conjunction with flavors |
| 184—Direct food substances affirmed as generally recognized as safe | 184.1733 | Sodium benzoate |

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# APPENDIX A
# Summary of the Proposed Food-Uses and Use-Levels for Quillaia Extract Type 2 in the U.S.

**Table A-1 Summary of the Proposed Food-Uses and Use-Levels for Quillaia Extract Type 2 in the U.S.**

| Food Category (21 CFR §170.3) | Proposed Food-Uses | Quillaia Extract Use-Level, Type 2, as Saponins, (mg/100 g)[a] | Quillaia Extract Use-Level, Type 2 (mg/100 g)[b] |
|---|---|---|---|
| Beverages, Alcoholic | Cocktail Drinks[c] | 19.5 | 30 |
| | Distilled Liquors[c] | 19.5 | 30 |
| Beverages and Beverage Bases | Energy Drinks[c] | 19.5 | 30 |
| Chewing Gum | Chewing Gum | 39.0 | 60 |
| Coffee and Tea | Specialty Coffee Drinks (Lattes, Cappuccinos, Mochas)[c] | 19.5 | 30 |
| Condiments and Relishes | Mustard | 120 | 184.6 |
| Confections and Frostings | Frostings, Icings | 120 | 184.6 |
| | Coatings | 120 | 184.6 |
| Dairy Product Analogs | Coffee Whiteners[c] | 19.5 | 30 |
| | Non-Dairy Milk and Cream | 45 | 69 |
| Fats and Oils | Fat-Based Sauces | 90 | 138.5 |
| | Mayonnaise and Mayonnaise-Type Dressings | 120 | 184.6 |
| | Salad Dressings | 90 | 138.5 |
| Frozen Dairy Desserts | Ice Cream * | 26 | 40 |
| | Other Frozen Milk Desserts | 65 | 100 |
| Fruit and Water Ices | Edible Ices, Sherbet, and Sorbet | 60 | 92.3 |
| Hard Candy | Hard Candy | 39 | 60 |
| Jams and Jellies | Jams, Jellies, Preserves, and Marmalades[c] | 19.5 | 30 |
| Soft Candy | Nougat and Toffees | 20 | 30.8 |
| | Gummies | 20 | 30.8 |
| | Soft Candy | 20 | 30.8 |
| Dietary Supplements | Solid Dietary Supplements | 600; 3 mg/500 mg serving[d] | 923; 4.6 mg/500 mg serving |
| | Liquid Dietary Supplements | 600; 30 mg/5 g serving[d] (~1 tsp) | 923; 46 mg/5 g serving |
| | Botanical Supplements, Powdered | 1,300; 6.5 mg/500 mg serving[d] | 2,000; 10 mg/500 mg serving |

CFR = Code of Federal Regulations; tsp = teaspoon; U.S. = United States.

[a] Use-levels 'as saponins' (reference component) were used in the intake assessment described in Section 5.2.1, considering both current and proposed use-levels. When there was overlap in the 'current' (as Table 4.3-1) and 'proposed' uses, the proposed use-levels were utilized. In cases where a single food-use within a food category was proposed at a higher use-level than current uses, the proposed use-level was used for the identified food-use and the current use-level was used for all remaining food-uses within the food category.

[b] Use-levels converted from a saponin basis (reference component) to the ingredient itself using a minimum specification of 65%. These values were used in the intake assessment described in Section 5.2.2, considering proposed use-levels only.

[c] Use of quillaia extract is present at specified use-level in final food through carry-over, including food flavors, colors, cloudy agent, actives delivery.

[d] Values for an average serving were based on typical products in which quillaia extract are proposed for use.

* Quillaia is intended for use in unstandardized products when standards of identity do not permit its addition.