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GRAS Notification for Quillaia Extract Type 1 in Semi-frozen Carbonated and Non-carbonated Beverages

GRAS Notification for Quillaia Extract Type 1 in Semi-frozen Carbonated and Non-carbonated Beverages

Submitted by

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U.S. Food and Drug Administration
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Summary

The American Beverage Association (ABA) is submitting this Notification that Quillaia Extract Type 1 ("Quillaia"), prepared according to food grade specifications, is Generally Recognized as Safe (GRAS) for use as a foaming agent in semi-frozen carbonated and non-carbonated beverages (FCBs) when used at levels up to 500 mg/kg (dried basis). This GRAS Notification has been developed in accordance with the FDA policy set forth in the Federal Register Notice dated April 17, 1997 (62 FR 18938).

Quillaia is obtained by aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaia saponaria* Molina (family Rosaceae), followed by clarification and purification. The unpurified extracts (Type I) are used in food applications, primarily for their foaming properties.

Semi-frozen carbonated and non-carbonated beverages are essentially similar in composition to corresponding unfrozen beverages (i.e. water-based flavored drinks) except for the use of foaming agents and the use of carbonation or air to increase the volume, thus reducing the specific gravity of the dispensed beverage. They are made only in retail establishments from beverage concentrates using unique equipment that resembles ice cream machines, which inject water and the carbon dioxide or air. The use level for Quillaia in the proposed beverages is 500 mg/kg on a dried basis in unexpanded FCBs, prior to the incorporation of the air or carbon dioxide gas. The amount of Quillaia present in the product as consumed will be lower due to expansion and the use level corresponds to 295 mg Quillaia per liter of dispensed FCB on a volume basis.

Quillaia has been approved for many uses by organizations within the US and globally. The Flavor and Extract Manufacturer's Association (FEMA) has previously determined Quillaia to be GRAS as a flavor ingredient in non-alcoholic beverages (GRAS 3.[2973]) at an average maximum use level of ~103 ppm. The 27th Session of the Codex Alimentarius Commission adopted the recommendation of the 36th Session of the Codex Committee on Food Additives and Contaminants (CCFAC) for the use of Quillaia Extract, Type 1, INS 999 ("quillaia") as a foaming agent at the level of 100 mg/kg in food category 14.1.4, "water-based flavoured drinks, including "sport" or "electrolyte" drinks and particulated drinks." Quillaia extract has also been approved for use in "water-based flavoured non-alcoholic drinks" in the European Union (additive E 999, Directive 95/2/EC, Annex IV). Specifications have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO Food Additives Series 52, 2004) and the European Commission (Directive 96/77/EC, 2 December 1996).

The 57th meeting of JECFA (2001) reviewed the literature pertaining to the safety of quillaia and established a temporary Acceptable Daily Intake, ADI, of 0-5mg/kg bw/day (WHO Food Additives Series 48, 2002; Appendix A) that was later confirmed at the Committee's 61st meeting in 2003 (WHO Food Additives Series 52, 2004). A search of the more recent scientific literature by Exponent, Inc. (Exponent) revealed no new information indicative of any adverse effects associated with the consumption of Quillaia. At the request of the ABA, Exponent

critically evaluated and summarized information relevant to the GRAS status of the proposed use of Quillaia in FCBs, including both generally available and unpublished company information on consumption of Quillaia in beverages; the composition, specifications and safety of Quillaia; and information and data from ABA about proposed uses of Quillaia in FCBs.

Exponent estimated the consumer exposure associated with the use of quillaia in FCBs at a use level of 500 mg/kg (dried basis) in the unexpanded FCB. Quillaia intakes among consumers of FCBs in the United States are well below the established ADI of 5 mg/kg bw. Estimated daily intake (EDIs) based on FCB consumption data from the 1994-96, 1998 CSFII (as represented by consumption of frozen novelties) were 1.4 mg/kg bw per day, or 27% of the ADI, for average consumers and 2.2 mg/kg bw per day, or 44% of the ADI, for the 90th percentile consumer.

Dr. Barbara J. Petersen, an expert qualified by training and experience, has reviewed this report and concluded that Quillaia, meeting appropriate specifications and used according to Good Manufacturing Practice, is GRAS by scientific procedures for semi-frozen carbonated and non-carbonated beverages at the proposed levels. The Expert's statement is included as Exhibit 1 of this document.

Identity of Substance and Production

The identity and production of Quillaia Extract Type 1 ("Quillaia") has been previously described in the Joint FAO/WHO Expert Committee on Food Additives (JECFA's) safety evaluation of quillaia extracts (WHO Food Additives Series 48, 2002), included here in Appendix A. Quillaia extracts are obtained by aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaia saponaria* Molina (family Rosaceae). The term 'quillaia' refers to the dried inner bark of the tree, which is a large evergreen with shiny, leathery leaves and a thick bark, native to China and several South American countries, principally Bolivia, Chile, and Peru.

Following extraction of Quillaia from the milled inner bark or of the wood of pruned stems and branches of *Quillaia saponaria* Molina (family Rosaceae), the quillaia extracts are treated with 'stabilizing agents' such as egg albumin and polyvinyl-pyrrolidone and then filtered through diatomaceous earth. The stabilizing agents remove substances that would likely precipitate during storage, such as protein-polyphenol complexes. After filtration, the liquid is concentrated, and the concentrate may be sold as such or be spray-dried and sold as a powder containing carriers such as lactose and maltodextrin. The unpurified extracts (Type I) are used in food applications, primarily for their foaming properties (WHO Food Additives Series 48, 2002).

Chemical Identity and Specifications

Specifications for Quillaia extracts (Type I) have been established by JECFA and the EU and are provided in Appendix B. The JECFA specifications were established at the Committee's 61st meeting; these specifications replaced the tentative specifications that had been prepared at the Committee's 57th meeting pending additional data. The EU specifications are listed in "Commission Directive 96/77/EC of 2 December 1996 laying down specific purity criteria on food additives other than colours and sweeteners" as amended by M2 Commission Directive 2000/63/EC of 5 October 2000.

History of Use

History of Consumption

1. Flavoring Agent

Quillaia has been approved for many uses by organizations within the US and globally. The Flavor and Extract Manufacturer's Association (FEMA) has determined quillaia to be GRAS as a flavor ingredient in the following uses (GRAS 3.[2973]):

Product	Average Usual (ppm)	Average Maximum (ppm)	Mean Daily Consumption (g)	Updated
Alcoholic Beverage	90	100	32.5	21-Jul-88
Baked Goods	24	30	137.2	21-Jul-88
Frozen Dairy	5	7	25.6	21-Jul-88
Gelatin Pudding	5	7	20.4	21-Jul-88
Non-alcoholic Beverage	91.5	102.93	104	21-Jul-88
Soft Candy	16	20	5.8	21-Jul-88

Source: RIFM – FEMA Database (accessed 1/12/2005).

Based on the Research Institute for Fragrance Materials (RIFM) – FEMA database, the historical consumption of quillaia as a flavor is as follows:

Year	Country	Flavor consumption (kg)
1995	USA	80994
1987	USA	17500
1982	USA	19600
1975	USA	6990
1970	USA	15400

Source: RIFM – FEMA Database (accessed 1/12/2005).

2. Foaming Agent

The 27th Session of the Codex Alimentarius Commission adopted the recommendation of the 36th Session of the Codex Committee on Food Additives and Contaminants (CCFAC) for the use of Quillaia Extract, Type 1, INS 999 ("quillaia") as a foaming agent at the level of 100 mg/kg in food category 14.1.4, "water-based flavoured drinks, including "sport" or "electrolyte" drinks and particulated drinks". The JECFA evaluation included estimated daily intakes of quillaia in the U.S. that were based on the 1989-92 Continuing Survey of Food Intakes of Individuals (CSFII) (WHO Food Additives Series 48, 2002). Exponent has updated the US intake estimates to reflect the more recent consumption data available from the 1994-96, 1998 CSFII (USDA, 2000). These are presented in Table 1. Based on this analysis, the average intake of quillaia from these beverages is 0.4 mg/kg bw/day. Intake by the highly exposed consumer (90th percentile) is 0.7 mg/kg bw/day. The 90th percentile intake amounts to only 14% of the ADI.

Quillaia extract has also been approved for use in "water-based flavoured non-alcoholic drinks" in the European Union (E 999, Directive 95/2/EC, Annex IV).

Intended Use and Use Level in Beverage

Quillaia is intended for use as a foaming agent in semi-frozen carbonated and non-carbonated beverages (FCBs) at a usage level not to exceed 500 mg/kg (dried basis).

Semi-frozen carbonated and non-carbonated beverages are essentially similar in composition to corresponding unfrozen beverages (i.e. water-based flavored drinks) except for the use of foaming agents and the use of carbonation or air to increase the volume, thus reducing the specific gravity of the dispensed beverage. They are made in retail establishments from beverage concentrates using unique equipment that resembles ice cream machines, which inject water and the carbon dioxide or air (see Appendix C for a photograph of the machine used to dispense these beverages and instructions for dispensing). Quillaia extract is added to create a soft or creamy-textured beverage that is light and fluffy. It is also the key factor in the proper mixing of ingredients, in the formation of the foam and in the dispersion of the foam throughout the beverage. Furthermore, quillaia assists in keeping the beverage from forming a solid block of ice in the machine, and maximizing the smooth mouth-feel of the ice crystals in the beverage. Carbonation or air increases the volume of the original unfrozen beverage up to 180%, essentially doubling the melted volume. The typical density of the final dispensed beverage product ranges from 530 to 590 g/L (ABA, personal communication).

FCBs are offered for sale in the same range of container sizes as those that are used for dispensed non-frozen beverages (e.g. soft drinks) in the same establishment. Therefore the volume purchased will be similar to non-frozen beverages, but the amount of dried quillaia extract present in the product as consumed will be lower due to the expansion. The concentration of quillaia in the consumed product on a volume basis can be estimated by dividing the quillaia concentration of the unexpanded FCB by 1.8.

Acceptable Daily Intake (ADI)

The toxicological effects of quillaia were reviewed by the JECFA Committee at its 26th meeting (1982) and it was determined that there were adequate lifetime studies in mice and rats that established a NOEL of 0.5% of the diet (700 mg/kg bw/day) in mice and 1% of the diet (500 mg/kg bw/day) in rats. However, no specifications for quillaia extract type I were available and the Committee could not establish an ADI. At the Committee's 29th meeting (1985), new tentative specifications were prepared and an ADI of 0-5 mg/kg bw was established. This ADI was made temporary in the Committee's safety evaluation at its 57th meeting in 2001 (WHO Food Additives Series 48, 2002) pending clarification of the specifications for quillaia extracts and questions regarding the relevance of a 90-day study in rats that had become available to the Committee after the ADI was established. At the Committee's 61st meeting (2003), new data suggested that the saponin content of trees is genetically determined and therefore assumed to not change significantly over time. In addition, the manufacturing process had not been significantly changed since 1975. Therefore, the Committee argued that the test material was toxicologically representative of quillaia extract type I and the "temporary" classification for the ADI of 0-5 mg/kg bw was removed (WHO Food Additives Series 52, 2004). The report of the 57th meeting is included in Appendix A. The report of the 61st meeting is over 600 pages long and for this reason is not included as an appendix but can be accessed on-line at: http://whqlibdoc.who.int/publications/2004/924166052X.pdf.

A search by Exponent of the scientific literature published subsequent to the latest JECFA review revealed no new information indicative of any adverse effects associated with the consumption of Quillaia.

Estimated Daily Intake (EDI) of Quillaia from FCBs

Concentration of Quillaia in FCBs as Consumed

Volume Basis

The intake assessment presented in this report uses the 500 mg/kg concentration of quillaia extract (dried basis) in the unexpanded FCB adjusted to reflect the quillaia extract concentration in the dispensed (expanded) beverage (i.e., the finished product as consumed). Because the volume expansion is somewhat variable due to machine differences, to be conservative we used the upper end of the range of typical density values for FCBs in this intake assessment (i.e. 0.590 kg/L). The 0.590 kg/L FCB is derived from the measured density of the unexpanded FCB divided by the expansion constant of 1.8. This value corresponds to approximately 17.4 g solids/fluid ounce dispensed product (ABA, personal communication). Therefore, a use level of 500 mg/kg in the unexpanded FCB corresponds to 295 mg quillaia extract/L FCB as consumed. The concentration of quillaia extract in FCBs as consumed was calculated as follows:

$$\frac{500 \text{ mg dried quillaia extract}}{\text{kg unexpanded FCB}} \times \frac{0.590 \text{ kg unexpanded FCB}}{\text{L expanded FCB}} = \frac{\mathbf{295 \text{ mg quillaia extract}}}{\text{L } \mathbf{\text{expanded FCB}}}$$

Note that approximately 80% of the fluid volume of dispensed FCB is air or gas from carbonation.

Weight Basis

According to information provided by ABA, containers for FCBs are typically available in the following sizes: 8 fl oz, 12 fl oz, 24 fl oz., 32 fl oz., 44 fl oz., or 64 fl oz. The amount of quillaia in each of these container sizes (assuming the container is filled to capacity) is given in Table 2. At the use level of 500 mg/kg, total quillaia content ranges from 70 mg for 8 fl oz to 558 mg for 64 fl oz.

Available Data and Methods

Exponent is not aware of any individual dietary record data specific to US consumption of FCBs; thus, the use of surrogate data was necessary. We therefore present here three different approaches to estimation of intake using available consumption data.

1. Industry survey data: ABA (personal communication) provided U.S. market survey information containing data about the frequency and amount of purchase of FCBs by consumers. Exponent used these data and the concentration of quillaia in FCBs as consumed (295 mg/L expanded FCB) to estimate the average daily quillaia intake of consumers of FCBs. The distributions of frequency of consumption and amounts consumed by eating occasion were combined using a Monte Carlo approach. Multiplying the distribution of the amount of FCBs consumed per eating occasion by the distribution of the number of eating occasions per day resulted in a final distribution of the daily intake of FCBs and was used to estimate mean and 90th percentile daily intakes.

2. Surrogate data approach #1: In the USA, there are consumption data available for "frozen novelties" (CSFII 1994-96, 98) (USDA, 2000). Based on market and survey data provided by ABA and information in the CSFII, certain frozen novelties were deemed by Exponent to be appropriate surrogates for FCBs. Frozen novelties differ from FCBs in that they are more dense, i.e., contain less air. We estimated an average daily intake of quillaia extract based on the 2 days of individual dietary records for consumption of selected frozen novelties. To the extent that these frozen novelties are denser than FCBs, the exposures to quillaia in FCBs generated in this way are overestimates.

3. Surrogate data approach #2: Exponent estimated average daily intake using market and survey data provided by ABA on the frequency of purchase of FCBs and amount of surrogate frozen novelties consumed per eating occasion (g/EO) from the 1994-96, 1998 CSFII (USDA, 2000). The distributions of frequency of consumption and amounts consumed by eating occasion were combined using a Monte Carlo approach. Multiplying the distribution of the amount of frozen novelties consumed per eating occasion by the distribution of the number of eating occasions per day resulted in a final distribution of the daily intake of FCBs and was used to estimate mean and 90th percentile daily intakes.

Industry Consumption Data

ABA provided 2 sets of survey data concerning the frequency of purchase of FCBs by consumers (personal communication, summarized in Tables 3 and 4). Due to the following differences in the two frequency datasets provided by ABA, Exponent believes it is not appropriate to combine them into a single distribution:

- Survey design: The survey designs were different between the two datasets. Dataset 1 was a national internet panel survey while Dataset 2 was collected through a stratified random nationally representative telephone survey.

- Season: Dataset 1 was conducted in November 2002 while Dataset 2 was conducted in the Spring of 2002. Marketing data suggests that there are seasonal consumption trends (ABA, personal communication).

- Duration of recall: Dataset 1 asked about the frequency of purchase and consumption within the last three months and Dataset 2 focused on the frequency of purchase within the last six months.

- Products surveyed: Different products (including products that might not contain quillaia due to formulation differences) may have been included in each survey.

Exponent used these data and the concentration of quillaia in FCBs as consumed (295 mg/L) to estimate the average daily intake of consumers of FCBs.

FCB Surrogates: Frozen Novelties

The market for FCBs is well established in the USA where these beverages have been widely available for more than 20 years. The ABA member companies provided market and survey data to Exponent indicating that the consumption pattern of FCBs is typically more similar to that of selected "frozen novelties" than to regular carbonated and non-carbonated beverages (i.e., these drinks are generally not consumed regularly with meals, for example). This pattern of lower frequency of consumption is also corroborated by the proportion of individuals in the US population consuming soft drinks, 64.5%, as compared to 1.1% of individuals that consume frozen novelties (USDA, 2000).

The following "frozen novelties" codes from the 1994-96, 1998 CSFII were deemed by Exponent to be similar enough to be appropriate surrogates for FCBs ("FCB-surrogate frozen novelties"). Although FCBs are not dairy-based, we included the codes for milk-based fruit drinks and smoothies in order that the number of respondents would be sufficient to generate statistically reliable estimates[1].

Food Code	Food Description
11552200	MILK-BASED FRUIT DRINK (INCL ORANGE JULIUS)
11553000	FRUIT SMOOTHIE DRINK, W/ FRUIT AND DAIRY PRODUCTS
91601000	ICE, FRUIT
91621000	SNOW CONE, SLURPS

[1] Minimum sample size criteria, adjusting for design effect, for the 90th percentile estimates is n=200 as set by the National Centers for Health Statistics. For upper percentile (>0.75), the minimum sample size (n) satisfies the following rule: $n \geq [8/(1-p)]$. http://www.cdc.gov/nchs/about/major/nhanes/nhanes3/nh3gui.pdf

Exponent assumed that the quillaia content of these FCB surrogate products was the same as the quillaia content of FCBs on a weight basis (500 mg/kg). It is our understanding that the consumption values in the CSFII have already been adjusted for the specific gravity of the food.

FCB Surrogates and Consumption Frequency

In this approach, we used Monte Carlo analysis (Crystal Ball® 4.0 with 10,000 iterations) to determine mean and 90th percentile daily intake estimates resulting from the multiplication of two consumption distributions. One distribution represents the frequency of consumption of FCBs based on ABA data described above (Tables 3 and 4). The second distribution represents the amount of FCB-surrogate frozen novelties consumed per eating occasion, as derived from the 1994-96, 98 CSFII (presented in Table 5). Due to differences in the two frequency datasets discussed above, it was not appropriate to combine them into a single distribution; we ran 2 separate analyses instead, one with each dataset.

Note that frozen novelties differ from FCBs in that they are more dense, i.e., contain less air. We estimated an average daily intake of quillaia extract based on the 2 days of individual dietary records for consumption of selected frozen novelties. To the extent that these frozen novelties are more dense than FCBs, the exposures to quillaia in FCBs generated in this way are overestimates and are therefore very conservative.

Results

Estimates of the intake of quillaia extracts in the US using the approaches discussed above are presented in Tables 6 and 7.

Intake estimates based solely on industry consumption data from Dataset 1 (ICBA, 2002a) with quillaia use levels of 500 mg/kg unexpanded FCB (corresponding to 295 mg quillaia/L expanded FCB) were 1.5 mg/kg bw per day, or 30% of the ADI, for average consumers and 4.65 mg/kg bw per day, or 93% of the ADI, for the 90th percentile consumer. Intake estimates based on Dataset 2 (ABA2002b) were 0.30 mg/kg bw, or 6% of the ADI for average consumers and 0.64 mg/kg bw, or 13% of the ADI, for the 90th percentile consumer. See Table 6 for a summary of these results.

Intake estimates based only on consumption of FCBs (as represented by consumption of frozen novelties in CSFII) with quillaia extract use levels at 500 mg/kg unexpanded FCB were 1.4 mg/kg bw per day, or 27% of the ADI, for average consumers and 2.2 mg/kg bw per day, or 44% of the ADI, for the 90th percentile consumer. See Table 7 for a summary of these results.

Daily intakes estimated using both the amount of FCB-surrogate frozen novelties consumed per eating occasion (1994-96, 1998 CSFII) and ABA member companies' market data on frequency of consumption (ICBA, 2002a; ICBA, 2002b) are also presented in Table 7. Intake estimates based on Dataset 1 (ICBA, 2002a) were 0.82 mg/kg bw per day, or 16% of the ADI, for consumers at the mean level and 2.39 mg/kg bw per day, or 48% of the ADI, for consumers at the 90th percentile of consumption. Intake estimates based on Dataset 2 (ICBA, 2002b) were 0.18 mg/kg bw per day, or 4% of the ADI, for consumers at the mean level and 0.46 mg/kg bw per day, or 9% of the ADI, for consumers at the 90th percentile of consumption.

The intakes presented in this assessment are conservative to the extent that the density of the surrogate frozen novelties is greater than that of FCBs. Using frozen novelties as surrogates for FCBs overstates the amount of solids consumed per serving and therefore also overestimates the intake of quillaia in those solids. Furthermore, all FCBs were assumed to contain quillaia at the maximum proposed level. According to ABA (personal communication), the formulation of some frozen uncarbonated beverages does not necessitate the use of quillaia yet this was not taken into account in our analysis.

Basis for the GRAS Determination

Summary of Technical Evidence of Safety

The basis of this GRAS notification is scientific procedures.

Cumulative exposure: existing and proposed use

The proposed use of quillaia extract is as a foaming agent in semi-frozen carbonated and non-carbonated beverages (FCBs) at levels up to 500 mg/kg (dried basis). Estimated daily intakes of quillaia extract as a foaming agent in FCBs at the mean and high levels (90th percentile) of consumption are well below the ADI (Tables 6 and 7).

Exponent also estimated the cumulative daily intake of quillaia from the existing use as a foaming agent in brewed soft drinks and the proposed use as a foaming agent in FCBs. The total EDI is presented in Table 8.

The estimated total daily intakes from the combined consumption of FCBs (proposed use) <u>and</u> soft drinks (approved use) were 0.56 mg/kg/bw per day, or 11% of the JECFA ADI, for the average consumers and 1.20 mg/kg bw per day, or 24% of the ADI, for the 90th percentile consumer.

The margin of exposure is actually higher because of the conservatism built into the estimate of total EDI:

1. The EDI estimate for the proposed use employed frozen novelties as surrogates for FCBs. This approach overstates the amount of solids consumed per serving and therefore also overestimates the intake of quillaia in those solids.

2. All FCBs were assumed to contain quillaia at the maximum proposed level. It is important to note the formulation of some frozen uncarbonated beverages does not necessitate the use of quillaia yet this was not taken into account in our analysis.

3. All brewed sodas were assumed to contain the quillaia extract as a foaming agent at the maximum level specified by Codex.

We may conclude that the conservatively estimated exposures associated with the proposed use of quillaia as a foaming agent in FCBs do not pose any hazard to consumers. The proposed use meets the GRAS standard for safety.

Discussion of Information Inconsistent with GRAS Determination

Exponent is aware of no information that would be inconsistent with a finding that the proposed use of quillaia extracts, in FCBs, meeting appropriate specifications and used according to Good Manufacturing Practice, is GRAS. A recent search of the scientific literature resulted in no new safety concerns. The EDIs and cumulative EDIs presented in the above sections are all well below the established ADI of 0-5 mg/kg bw, even considering several conservative assumptions underlying the calculations.

References

American Beverages Associations (ABA), 2002a. Dataset #1.

American Beverages Associations (ABA), 2002b. Dataset #2.

U.S. Department of Agriculture (USDA) (2000). CSFII Data Set and Documentation: The 1994-96, 1998 Continuing Surveys of Food Intakes by Individuals. Food Surveys Research Group. Beltsville Human Nutrition Research Center. Agricultural Research Service. April 2000.

World Health Organization Food Additives Series 48, WHO (2002). WHO Food Additive Series 48, Safety Evaluation of certain Food Additives and Contaminants (Quillaia Extracts); Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives.

World Health Organization Food Additives Series 52, WHO (2004). Evaluation of Certain Food Additives and Contaminants; Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO technical report series 922. Geneva 2004. http://whqlibdoc.who.int/publications/2004/924166052X.pdf.

Table 1. Updated intake of quillaia extract from foaming agents used in beverages in the US.

	Soft drink consumption (g/day)	EDI (mg/kg bw/day)	% ADI[a]	Assumptions	Survey	Date of survey
Total US Population – Consumers Only						
Mean	233	0.4	8	Extract in limited number of soft drinks[b], 100 mg/kg use level, consumers 5.7% of population	CSFII; 2-day intake; sample, 20,607; assumed body weight, 60 kg	1994-96, 1998
90th Percentile	429	0.7	14			

[a] JECFA ADI, 0–5 mg/kg bw.

[b] Soft drinks likely to contain the additive are "brewed sodas," e.g. ginger beer, root beer and cream soda.

Table 2. Typical FCB container sizes offered for sale and total quillaia content of each (typical concentration 295 mg/L) (source of data: ABA)

Size of FCB (fluid ounces)	Amount of Quillaia in purchased FCB (Total mg Quillaia)
8	70
12	105
24	209
32	279
44	384
64	558

Table 3. Frequency of Consumption of FCBs (Dataset 1; n = 463; ABA, 2002a)

Frequency (EO/year)	% of consumers
≥1x/day	15
4-6x/week	9
1-3x/week	13
1x/week	12
2-3x/month	23
≤1x/month	28

Table 4. Frequency of Consumption of FCBs (Dataset 2; n = 400; ABA, 2002b)

Frequency (EO/year)	% of consumers
>1x/week	10
1x/week	15
1x/2-3 weeks	19
1x/month	27
<1x/month	29

Table 5. Distribution of the Amount of FCB-Surrogate "Frozen Novelties" Consumed Per Eating Occasion Among the Total US Population[2] (USDA, 2000)

Amount FCB Consumed (g/EO)	Percentile
97	10
145	20
191	25
192	30
194	40
276	50
290	60
384	70
385	75
387	80
524	90
719	95
963	98
1062	99
1066	99.5
1158	99.9

[2] The unweighted total number of eating occasions and associated person-days of consumption was 253 and 244, respectively.

Table 6. Estimated daily intakes of quillaia extracts from consumption of FCBs, based on ABA data

Reference	Population group	Consumption (L/day)		Quillaia extract intake (mg/kg bw per day)		% ADI[a]	Assumptions	Survey	Date of survey
USA (ABA, 2002a)	Consumers only	Mean	0.304	Mean	1.50	30	Extract in FCBs; GFSA level, 500 mg/kg[b]	Marketing survey; ages 12-49; nationally representative internet panel; frequency of consumption of FCBs within the last three months; sample 716 (463 consumers). Assumed body weight, 60kg.	November 2002
	Consumers only	90th percentile	0.95	90th percentile	4.65	93			
USA (ABA, 2002b)	Consumers only	Mean	0.061	Mean	0.30	6	Extract in FCBs; GFSA level, 500 mg/kg[b]	Marketing survey; ages 14-44; stratified random nationally representative telephone survey of FCB consumers; frequency of consumption over past 6 months; sample 400. Assumed body weight, 60kg.	Spring 2002
	Consumers only	90th percentile	0.13	90th percentile	0.64	13			

[a] JECFA ADI, 0–5 mg/kg bw

[b] 500 mg/kg is the level of anhydrous quillaia extract in the unexpanded beverage. Based on data provided by ABA, the actual amount of quillaia in the dispensed beverage is calculated to be 295 ppm [(500 mg anhydrous quillaia extract/kg unexpanded beverage) x (0.590 kg unexpanded beverage/L FCB) = 295 mg quillaia extract/L FCB].

Table 7. Estimated daily intakes of quillaia extracts from consumption of frozen carbonated beverages

Reference	Population group	Consumption (g/day)		Quillaia extract intake (mg/kg bw per day)		% ADI[a]	Assumptions	Survey	Date of survey
USA (United Sates Department of Agriculture, 2000)	Consumers only	Mean	164	Mean	1.4	27	Extract in frozen carbonated beverages (FCBs)[b]; GFSA level, 500 mg/kg; consumers 1.1% of population.	National survey; 2-day intake; sample, 20,607; assumed body weight, 60 kg.	1994-96,98 (combined surveys)
	Consumers only	90th percentile	261	90th percentile	2.2	44			
USA (ABA, 2002a; United States Department of Agriculture, 2000)	Consumers only	Mean[c]	98	Mean	0.82	16	Extract in FCBs; GFSA level, 500 mg/kg. Frequency of consumption based on data from ABA, 2002a; amount consumed per eating occasion based on USDA, 2000.	Marketing survey; ages 12-49; nationally representative internet panel; frequency of consumption of FCBs within the last three months; sample 716 (463 consumers). National survey; 2-day intake; sample 20,607. Assumed body weight, 60kg.	November 2002
	Consumers only	90th percentile[c]	287	90th percentile	2.39	48			
USA (ABA, 2002b; United States Department of Agriculture, 2000)	Consumers only	Mean[c]	22	Mean	0.18	4	Extract in FCBs; GFSA level, 500 mg/kg Frequency of consumption based on data from ABA, 2002b; amount consumed per eating occasion based on USDA, 2000.	Marketing survey; ages 14-44; stratified random nationally representative telephone survey of FCB consumers; frequency of consumption over past 6 months; sample 400. National survey; 2-day intake; sample 20,607. Assumed body weight, 60kg.	Spring 2002
	Consumers only	90th percentile[c]	55	90th percentile	0.46	9			

[a] JECFA ADI, 0–5 mg/kg bw

[b] Frozen carbonated beverage food codes in USDA 2000 include: Milk-based fruit drink (incl orange julius); Fruit smoothie drink, w/ fruit and dairy products; ice, fruit; snow cone, slurps.

[c] Daily intake was estimated by combining frequency of consumption of FCBs as determined by market data collected by members of the ABA with g/eating occasion data collected from a national survey (USDA, 2000).

Table 8. Total estimated daily intake of quillaia extract from existing and proposed uses.

	EDI (mg/kg bw/day)	% ADI[a]	Assumptions	Survey	Date of survey
Total US Population – Consumers Only					
Mean	0.56	11	Extract in FCBs and limited number of soft drinks[b]; use level of 100 mg/kg for soft drinks and 500 mg/kg for FCBs; consumers 6.7% of population.	CSFII; 2-day intake; sample, 20,607; assumed body weight, 60 kg	1994-96, 1998
90th Percentile	1.20	24			

[a] JECFA ADI, 0–5 mg/kg bw.
[b] Soft drinks likely to contain the additive are "brewed sodas," e.g. ginger beer, root beer and cream soda.

Exhibit I – Expert Statement

Based on a review of generally available and unpublished company information relating to the potential GRAS status of Quillaia Type I used as a foaming agent in semi-frozen carbonated and noncarbonated beverages, I have concluded that the proposed use is GRAS. This determination is based on scientific procedures and publicly available data and information. Specifically, intakes of Quillaia Type I associated with both existing and proposed uses were conservatively estimated using food consumption data from the 1994-96 CSFII. Intakes from the proposed uses were estimated using surrogate consumption data from similar foods in the CSFII, frozen novelties. The conservative estimates of total Quillaia intake are well within the Acceptable Daily Intake established by JECFA, 0-5 mg/kg bw/day. These analyses are conservative and meet widely used and generally accepted scientific standards for estimating exposures to food ingredients. I conclude that the proposed use of Quillaia Type 1 as a foaming agent in FCBs, if it meets appropriate specifications and is used according to Good Manufacturing Practice, is Generally Recognized as Safe.

(b)(6) 9/27/2005

Barbara J. Petersen, Ph.D. Date

Barbara Petersen, Ph.D., M.P.H.
Practice Director and Principal Scientist, Food & Chemicals Practice

Professional Profile

Dr. Barbara Petersen is a Principal Scientist and serves as Director of Exponent's Food and Chemicals practice and is based in Washington, D.C. Dr. Petersen is internationally recognized for her expertise in risk assessment, exposure assessment methodology, food consumption and nutrient profile modeling, and applications of Monte Carlo techniques to conduct risk assessments. Dr. Petersen has pioneered the technical methods for incorporating information about food composition, dietary practices, actual agricultural practices and commercial food processing technologies into regulatory and health issues. Dr. Petersen has successfully applied these approaches to develop software that maximizes the utility of data and provides realistic risk assessments that allow the user to understand the sources of potential intakes of nutrients and contaminants. Applications include international assessments for food safety, food additives and contaminants, obesity related projects, intake calculations to support new pesticides, GRAS self affirmations and preparation of food additive petitions, nutrition labeling justifications, new food product designs and marketing strategies, and product stewardship programs. She has developed a software tool for evaluating the impact of food product modifications on trans fatty acid intake including the ability to understand the range of intakes across different population groups.

Dr. Petersen has directed the design and conduct of seven statistically based national market basket studies. These studies were designed for different purposes, including acute and chronic assessments for pesticides, compliance assessments under Proposition 65, and market research.

Dr. Petersen served on the National Academy of Sciences Institute of Medicine, Board on Health Promotion and Disease Prevention, Committee on the Review of the USDA E. Coli 0157:H7 Farm-to-Table Process Risk Assessment, the EPA Science Advisory Board's Integrated Exposure Committee and as an Expert Advisor to WHO/FAO for their 2002 meeting on acrylamide and several session of JECFA and for numerous consultations on risk assessment. Also she served as Principal Investigator for the National Cancer Institute's International FOODBASE project, a major effort to collect and computerize descriptive and summary information on food consumption surveys conducted in more than 40 countries and was the P.I. responsible for developing a new microbial risk assessment tool for the US Food and Drug Administration.

Credentials and Professional Honors

Ph.D., Biochemistry (minors: nutrition, microbial physiology, organic chemistry), George Washington University, 1976; thesis topic: metabolism of poly-unsaturated fatty acids
M.P.H., Nutrition (minor: biochemistry), University of California at Los Angeles, 1972
B.S., Nutrition (minor: chemistry), New Mexico State University, 1970

Relevant Experience

Dietary Intake Assessment: United States. Designed Novigen and TAS proprietary food intake systems, including microcomputer-based food consumption, nutrient and exposure analysis databases and the systems for integrating dietary intake of foods or components of foods with biological activity parameters for those foods/constituents including food additives, functional ingredients, nutraceuticals and nutrients. Responsible for overall systems design for dietary components of US EPA's Tolerance Assessment System, including the integration of: (1) USDA food consumption databases; (2) US EPA tolerance listings; (3) US EPA toxicology summary information; and (4) statutory requirements into a single analysis system. Created methodology/project specifications that for the first time permitted national and international dietary intake data to be transported from the mainframe to microcomputer format and subsequent methods to permit sensitivity analyses for ingredients. Directed the development of sophisticated and powerful software to permit rapid analysis on the microcomputer for aggregate exposure analysis. Directed the development of Novigen's line of commercial exposure assessment software, DEEM™ and Calendex™ and while at TAS, EXPOSURE 1-4®, including the modules for distributional/Monte Carlo analysis. Participant in the peer review of the EPA Exposure Factors Handbook and the NAS peer review of the USD E. coli 01l57:H7 Farm-to-Table Process Risk Assessment.

Dietary Intake Assessment: International. Principal Investigator for International Food Consumption/Nutrition Project for the National Cancer Institute. Participated in the European Commission FLAIR/ENFANT project from 1989-1995. Member WHO/FAO Joint Expert Consultations for Guidelines for Dietary Exposure to Pesticides and Member of faculty for TNO/WHO workshop, Risk Assessment in Europe. Expert to the WHO Nutrition group for Global Monitoring. Conducted risk assessments for populations in the United Kingdom, Germany, Canada and other European Union (EU) countries.

Environmental Modeling: Designed, developed and installed exposure modeling projects, including major and innovative conversion of the results of food consumption surveys to forms compatible with US EPA, FDA and California EPA regulatory mandates and models and systems to assess exposure to contaminants from water. Evaluation of compliance for dietary contributions under California regulations. Conducted more than 5000 exposure/risk assessments for private and government clients. Developed statistical approach for assessing chronic exposure to contaminants in fish and for treatment of the Limit of Detection (LOD) in exposure assessment.

National Food Survey Design: Responsible for directing the statistical design, field collection and analysis of more than ten national food monitoring studies, including database design for automated data collection and analysis. Applied expertise in modern food technology for the design of US EPA's program for assessing residues in processed foods and for USDA's Food Safety and Inspection's Services monitoring/surveillance results to assess sulfonamides. Designed and conducted a survey of the school breakfast program for the USDA.

Compliance and Regulatory Assessments: Seventeen years of experience producing strategies and technical support to corporate clients for assessing compliance with state and federal regulations product safety, litigation, and vulnerability assessments. (Developed exposure assessment tools for the California government's risk assessment mandate.)

Pesticide Usage Research: Directed local and national surveys of pesticide usage, including pesticide use on California tomatoes (fresh market and processed), celery, citrus, and all U.S. apples. Worked with farmers and their associations to design, implement and interpret surveys to quantify pesticide use practices. Developed procedures for quantifying the food byproducts used in animal feeds and for assessing pesticide levels in those feeds. Directed the utilization of federal and state pesticide usage data for improving risk assessments.

Proposition 65: Exposure modeling to evaluate compliance with Proposition 65.

Dietary Exposure Evaluation Modules (DEEM™): Designed and developed modules of the DEEM-US and DEEM-United Kingdom Systems including:

- cumulative and aggregate intake and exposure assessments
- ingredient analysis options
- nutrient analysis options
- recipe modification module that allows the user to assess the impact of changes in formulations
- user-friendly menus
- contaminant analysis module
- food additive analysis options
- subgroup analysis options, including menus for user-defined subgroups and income subgroups
- customized system upgrades to accommodate user requests
- Monte Carlo assessments

Joint Distribution Methodology: Designed a computerized adaptation of Monte Carlo analysis for computing the probability of exposure to food contaminants and nutrients that now provides the basis for DEEM™ and Calendex™.

Designed technical background documents in anticipation of the National Academy of Sciences report entitled, "Pesticides in the Diets of Infants and Children."

Nutrition Labeling: Criteria for the design of nutrient databases and related issues.

Served as the Principal Investigator and provided project management for more than 1000 separate projects, conducted at multiple locations and involving scientists and other technical and non-technical personnel. Developed corporate management structure, to assure that all projects receive direct corporate oversight and quality control and quality assurance, and that needed resources are available in a timely and cost-efficient manner.

Provided oversight for new computer software for modeling exposure and for databases for exposure and risk assessment.

Twenty years' experience as the Corporate Officer for technical direction of Food and Exposure Assessment projects and as the Principal in charge of Novigen (and TAS) business and operations. As Corporate treasurer, developed and directed cost efficiency control procedures, personnel policy and procedures, and corporate financial controls.

Selected Activities

Chemical defense strategies for international and U.S. regulatory needs, Canadian defenses and California issues.

Food additive and ingredient petitions, GRAS Notifications, and product safety assessments.

Provided Expert advice and assessment for litigation involving food and water contamination including Proposition 65 related litigation.

Principal investigator for the design of the FDA's LANGUAL interface.

Market Basket Survey Protocol design strategies.

FOODBASE project design and direction - international dietary practices including information for more than 40 countries.

Principal investigator, FDA Statistical Methodology support contract, nationwide market basket studies and risk-based assessments.

Participant in the EC FLAIR Eurofoods-Enfant Conference. Presentations in 1990, 1991, 1992, 1993, 1994, and follow-on work in 1995.

Participant in IARC Subgroup for the European Prospective Study on Nutrition, Cancer and Health, Lyon France, June 1991.

Expert Advisor to WHO/FAO for meeting on acyrlamide, for several sessions of JECFA and for numerous consultations on risk assessment.

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Fellowships

NIH Post-Doctoral Fellow, NIAAA, Biochemistry Laboratory. 1978-1980. Specialization: genetics of alcoholism, basic enzymology.

Post-Doctoral Fellow, Georgetown University, Department of Biochemistry. 1976-1978. Specialization: mechanisms of action of insulin and glucagon hormone receptors, particularly in patients with cancer and other disorders affecting taste.

Science Advisory Boards/Panels

- Advisor, WHO/FAO Joint Expert Committee on Food Additives and Contaminants (1997–2001) and to numerous consultations
- Member, EPA Science Advisory Board, IHEC (1995-2001)

Professional Affiliations

- Councilor, International Society for Exposure Assessment (1995-1997)
- GAO Expert Panel member for the evaluation of the National Surveys of Food Consumption (1993–1994)
- 1997 Eastern Food Science Conference Planning Committee
- Institute of Food Technologists
- International Society of Exposure Analysis
- Society of Risk Analysis
- Program Co-Chair, Exposure Specialty Committee, Society for Risk Analysis, Exposure Section, 1991 and 1992
- Chair, Toxicology and Safety Evaluation Section, Institute of Food Technologists, 1997; and CODEX, 1999-2000, Chair, Special Task Force, Biotechnology
- Invited participant, EPA National Human Exposure Assessment Survey Workshop
- Invited participant in the Joint FAO/WHO Expert Consultation on Food Consumption Methodologies and risk assessments (1997, 1998, 2001, 2002)

Appendix A – WHO Food Additives Series: 48, Safety Evaluation of Certain Food Additives and Contaminants – Quillaia Extracts

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WHO FOOD ADDITIVES SERIES: 48

SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

QUILLAIA EXTRACTS

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1. EXPLANATION

Quillaia extracts (synonyms, soapbark extracts, Quillay bark extracts, bois de Panama, Panama bark extracts, quillai extracts) are obtained by aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaja saponaria* Molina (family Rosaceae). The term 'quillaia' refers to the dried inner bark of the tree, which is a large evergreen with shiny, leathery leaves and a thick bark, native to China and several South American countries, principally Bolivia, Chile, and Peru.

Unpurified extracts contain over 60 triterpenoid saponins, consisting predomi-nantly of glycosides of quillaic acid. Polyphenols and tannins are major components. Some simple sugars and calcium oxalate are also present. The saponin concentration of freshly prepared, unpurified extracts is 190–200 g/kg of solids (about 20%). The extracts are treated with 'stabilizing agents' such as egg albumin and polyvinyl-pyrrolidone and then filtered through diatomaceous earth. The stabilizing agents remove substances that would probably precipitate during storage, such as protein–polyphenol complexes. After filtration, the liquid is concentrated, and the concentrate may be sold as such (solids constituting about 550 g/L) or be spray-dried and sold as a powder containing carriers such as lactose and maltodextrin. The unpurified extracts are used in food applications, primarily for their foaming properties.

Semi-purified powdered extracts are produced by subjecting unpurified extracts to ultra-filtration or affinity chromatography to remove most non-saponin solids, such as polyphenols. These semi-purified extracts have higher saponin concentrations (750–800 g/kg of solids; about 80%) and better emulsifying properties than unpurified extracts.

Highly purified extracts are produced for use as adjuvants in the production of animal and human vaccines and not for food use. These products generally contain more than 90% saponins.

In previous evaluations, the Committee considered data that related to unpurified quillaia extracts. Quillaia extract was reviewed toxicologically by the Committee at its twenty-sixth meeting (Annex 1, reference 59). The available toxicological data included adequate lifetime studies in mice and rats, from which a NOEL was identified. However, in the absence of data, no specifications were prepared, and, hence, no ADI could be allocated. At its twenty-ninth meeting (Annex 1, reference 70), the Committee prepared new tentative specifications and established an ADI of 0–5 mg/kg bw. The Codex Committee on Food Additives and Contaminants (CCFAC) (2000) at its thirty-second session requested the Expert Committee to re-evaluate all relevant information on the toxicity and, in particular, the intake of quillaia extracts. No new data were submitted to the Committee. Published reports on quillaia extracts or specific saponins that provided information relevant to a toxicological assessment of quillaia extracts were evaluated at the present meeting.

Quillaia extracts are mixtures of biologically active compounds, including saponins, tannins, polyphenols, and calcium oxalate. The saponins present in quillaia extract have a variety of biological activities: haemolytic, cytotoxic, immune-enhancing (adjuvant), mucosal irritation and inflammation, and anti-hypercholesterolaemic. The biological activities and the potency of individual saponins vary widely and depend in vivo primarily on the route of administration.

2. BIOLOGICAL DATA

2.1 Biochemical aspects: Effects on enzymes and other biochemical parameters

Saponins extracted with ethanol from soapbark trees and administered orally to rabbits with experimental atherosclerosis resulted in an increased ratio of plasma lecithin to cholesterol, normalized

blood cholesterol levels, and decreased elevated blood pressure. Subcutaneous injection of the saponin extract did not affect the atherosclerotic symptoms (Efimova et al., 1966).

Groups of 12 Swiss mice (strain and sex unstated) were injected into the footpad with aliquots of seven different 5% extracts of *Q. saponica* bark. Animals that lived longer than 24 h and controls were killed. The degree of oedema in the mice, haemolysis of rabbit erythrocytes, and the adjuvant strength of the various extracts in stimulating immunity to staphylococcal toxin were measured with the same quillaia extracts. No relationship was found between these parameters (Richou et al., 1965).

The effect of a range of saponins, including crude quillaia saponin on gut permeability was assessed by monitoring the steady-state glucose transfer potential in vitro in sections of jejunal mucosa from male Wistar rats. The individual saponins elicited widely different responses in the small gut and these were significantly affected by pH, concentration, chemical formula, and the presence of other materials in the solution. Quillaia extract caused a reduction in transmural potential difference comparable to that observed with the basic glycoalkaloids in potato and tomato and the complex bisdesmosides from Gypsophilia and alfalfa. These saponins were all more potent than the saponins from soya, which showed only weak activity. The reduction in transmural potential difference has been associated with increased uptake of both passively permeable sugars and large compounds and with a loss of the ability of the mucosa to accumulate actively transported organic species(Gee et al., 1989).

When 'pure' saponin from Q. saponaria was administered subcutaneously to mice that had been immunized with bovine serum albumin or Crotalus durissus (South American rattlesnake) venom, a significantly higher antibody titre against the antigens was found in the sera of immunized mice than in animals receiving the antigens alone. Tumour necrosis factor activity was increased in mice immunized with bovine serum albumin and/or saponin, but interferon-*gamma* was produced only in mice immunized with both bovine serum albumin and saponin (Gebara et al., 1995).

The effect of the Quillaia saponin fractions QH-A, QH-B, and QH-C and a crude Quillaia saponin extract (Spikoside) on haemolytic activity, cytotoxicity, and macromolecular synthesis was studied in vitro. A concentration of 5 µg/ml of QH-B or QH-C caused haemolysis of chicken erythrocytes after 1 h of incubation at 37 °C. QH-A was haemolytic at an approximately 10-fold higher concentration, 50 µg/ml. The crude extract caused haemolysis at a concentration of 20 µg/ml. No haemolytic activity was observed at concentrations ≤100 µg/ml when these preparations were incorporated into an immunostimulating complex matrix. Cytotoxicity was assessed by measuring intracellular dehydrogenase activity by a colorimetric method. Seeded WEHI 164 cells clone 13^9 were incubated with various concentrations of the extracts for 2 h before analysis. QH-B and QH-C inhibited enzyme activity at a concentration of approximately 10 µg/ml and the crude extract at a concentration of approximately 20 µg/ml. QH-A was tolerated at concentrations ≤100 µg/ml. When QH-A, QH-B, QH-C, and the crude extract were incorporated into the immunostimulating complex matrix, the cells tolerated approximately 10-fold higher concentrations. Macromolecular synthesis was assessed by measuring incorporation of [^{3}H]leucine and [^{3}H]uridine into protein and RNA, respectively, in WEHI 164 cultured cells. Treatment with QH-B or QH-C at concentrations ≤10 µg/ml for 30 min had no affect on protein or RNA synthesis (Rönnberg et al., 1995).

2.2 Toxicological studies

Saponins vary widely in the kind and intensity of their biological activity. Some of the more important effects included haemolysis (strong in vitro, much weaker in vivo), local irritation, inflammation (intestine), anti-inflammatory and antimicrobial activity, cytotoxicity, and anti-hypercholesterolaemic in

laboratory animals. Severe toxic effects reported after large oral doses were liver damage, respiratory failure, gastric pain, diarrhoea, haemolysis of erythrocytes, convulsions, and coma (Leung, 1980).

2.2.1 Acute toxicity

In mice, saponins extracted from the soapbark tree were less acutely toxic when administered orally (LD50, 1600 mg/kg bw) than when administered subcutaneously (650 mg/kg bw), intraperitoneally (280 mg/kg bw), or intravenously (280 mg/kg bw) (Efimova et al., 1966).

The acute toxicity of a Q. saponaria extract and of QS-7, QS-18, and QS-21 saponins isolated from the extract was investigated in groups of five CD-1 mice (sex not stated), 8–10 weeks of age given an intradermal concentration of 125, 250, or 500 µg of each saponin or the quillaia extract and monitored for 72 h. QS-18 was the most lethal of the substances tested, with 4/5, 5/5, and 5/5 deaths at the three doses, respectively. The authors stated that deaths occurred at a dose of QS-18 as low as 25 µg. QS-7 was not lethal at doses up to 500 µg, and QS-21 was lethal only at 500 µg, at which 1/5 mice died. The quillaia extract resulted in the deaths of 1/5, 2/5, and 4/5 mice at the three doses, respectively (Kensil et al., 1991).

The acute toxicity of subcutaneously administered QH-A, QH-B, QH-C, and crude Quillaia saponin (Spikoside) was tested in groups of 10 female ICR mice at doses of 50–400 µg. All mice survived after the highest dose of QH-A and QH-C, with no visible signs of toxicity. All mice given 400 µg QH-B died within 12 h, while seven mice survived injection with 200 µg. When the crude extract was administered, doses of 200 and 400 µg resulted in 6 and 10 deaths, respectively. All mice survived the lower doses of QH-B or the crude extract (Rönnberg et al., 1995).

A purified, toxic Quillaia triterpenoid fraction with strong adjuvant activity, designated QH-B, was used to study whether modification of the carbohydrate moiety with sodium periodate would alter the toxicity without harming the adjuvant activity and the cholesterol-binding capacity. Groups of 10 ICR female mice received single increasing subcutaneous doses of modified QH-B and were observed for 3 weeks. Unmodified QH-B and QH-B modified by treatment with 2.5–10 mmol/L sodium periodate had similar LD50 values, ranging from 29 to 70 µg per mouse. Treatment of the fraction with 25 or 50 mmol/L sodium periodate increased the LD50 of QH-B to 197 and 132 µg, respectively. The difference in the toxicity of the periodate-modified QH-B may have been due to alterations in the structure of the sugars galactose and xylose (Rönnberg et al., 1997).

2.2.2 Short-term studies of toxicity

Rats

Groups of 15 male and 15 female weanling CFE rats were housed five per cage and fed diets containing 0 (control), 0.6, 2, or 4% quillaia extract for 13 weeks. Groups of five male and five female rats from the same lot of animals were fed diets containing 0 (control), 2.0%, or 4.0% quillaia extracts for 2 or 6 weeks. Body weights and food intake were measured at the beginning and weekly throughout the study. Urine was analysed during the final week of the study. At sacrifice, the absolute and relative organ weights were determined, and tissues and organs from the group given 4% were analysed histologically. Haematological and serum chemical parameters were tested in all groups.

No abnormalities of behaviour or condition were seen in the rats receiving quillaia extract. The body weights of those at the highest concentration (4%) were significantly lower than those of controls up to day 78 in males, but only for the first 2 weeks in females. Food and water consumption were reduced in

animals of each sex at all dietary levels, but by the end of the study the weights of the treated rats did not differ significantly from those of the controls. Feeding quillaia saponin did not affect the results of haematological examinations, serum or urine analyses, renal concentrating ability, or urinary cell excretion. The relative liver weight was reduced in males given 2 or 4% quillaia extract, and the relative stomach weight was increased in animals of each sex at the same concentrations. No histopathological effects attributable to treatment were found. The NOEL was 0.6% in the diet, equivalent to 400 mg/kg bw per day (Gaunt et al., 1974).

In a 90-day study to assess the safety of a saponin extract from Thea sinensis L., quillaia saponin (approximately 8.5% sapogenin; not clear whether this product meets current specifications) was used for comparison. Although the Committee examined this study, it was considered 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 (Kawaguchi et al., 1994).

2.2.3 Long-term studies of toxicity

Mice

Groups of 48 male and 48 female TO strain mice were fed diets containing 0, 0.1, 0.5, or 1.5% quillaia extract for 84 weeks. The mice were observed regularly for abnormal condition or behaviour, and some males were weighed at intervals. Haematological parameters were measured at weeks 24, 56, and 84. No compound-related effects were reported. At the highest dose, male mice showed decreased weight gain. Quillaia at these concentrations had no adverse effect on condition, behaviour, or death rate. A detailed autopsy and histopathological examination of tissues and organs at the end of the study showed no compound-related effects. No carcinogenic effects were seen. The slightly lower body-weight gain of mice at the highest dietary concentration and some changes in organ weights, albeit of doubtful significance, resulted in a NOEL of 0.5%, equivalent to 700 mg/kg bw per day (Phillips et al., 1979).

Rats

Groups of 48 male and 48 female Wistar-derived rats from a specific pathogen-free breeding colony were housed in groups of four and fed diets containing 0 (control), 0.3, 1, or 3% quillaia extract for 108 weeks. Haematological examinations were made at weeks 15, 25, 52, and 108, and urine was analysed at weeks 13, 24, and 78. At the end of the study, a complete autopsy was carried out, including histological examination of tissues and organs.

Male rats at the highest dietary concentration had lower body weights than controls throughout the experiment, the differences being statistically significant between 10 and 22 months. Females at the lowest dietary concentration had significantly higher body weights than controls during the first 6 months of the study. The lower body weights and reduced incidence of glomerulonephrosis in male rats fed 3% quillaia were considered to be due to reduced food consumption. Preference tests run before the start of the 2-year study showed that rats avoided the diet containing quillaia extract. Haematological and urinary parameters were within the normal ranges.

In general, the incidence of histopathological lesions was similar in treated and control animals, only those of fibrosis of the heart and dilatation of the glands of the gastric mucosa in females at the lowest dietary concentration being greater than those of controls. These effects were considered to be fortuitous, as there was no dose–response relationship and no similar occurrence in males. A variety of benign and malignant tumours was found. The incidences of haemangiomas and haemangio-sarcomas in the lymph nodes were similar in control and treated animals. The only tumour for which the incidence

was statistically significantly different from that in controls was thyroid adenoma, which occurred more frequently in females fed 1% quillaia in the diet. This finding was not considered to be related to treatment, as the incidence did not increase with the dietary concentration of quillaia extract, and the total incidence in rats fed 1% quillaia extract was not statistically significantly different from the total control incidence (Drake et al., 1982).

3. INTAKE

Quillaia extracts may be used as a foaming agent in soft drinks, such as ginger beer, root beer, and cream soda, in cocktail mixes, and as an emulsifier in other foods, such as baked goods, candies, frozen dairy products, gelatine, and puddings. The major food use is in soft drinks. Quillaia extracts are currently proposed for use in the Codex draft General Standard for Food additives (GSFA) at 500 mg/kg in food group 14.1.4 'Water-based flavoured drinks', including 'sport' or 'electrolyte' drinks and particulated drinks.

Data on intake of quillaia extracts were submitted to the Committee by Australia and New Zealand, the United Kingdom, and the USA.

3.1 Screening for additives by the budget method

The budget method was used to decide whether the intake of quillaia extracts should be assessed further. The calculations indicated that the theoretical maximum level of use of quillaia extracts is 100 mg/kg, assuming that it is used in only half the beverages in a food supply and that the ADI is 0–5 mg/kg bw. As this theoretical level is lower than the proposed level of 500 mg/kg in the draft GSFA, further assessment of intake is needed. The draft GSFA proposes use of quillaia extract in one food group only, 14.1.4 'Water-based flavoured drinks'.

As quillaia extracts are proposed for use in a single food group, a reverse budget method was used to indicate the maximum amount of the food group that can be consumed before the ADI is exceeded. In this case, up to 600 g/day of water-based flavoured drinks could be consumed if quillaia extracts were used at a concentration of 500 mg/kg, assuming an ADI of 0–5 mg/kg bw and an average body weight of 60 kg, while a child of 15 kg can drink only 150 g/day before exceeding the ADI.

If quillaia extracts were used at 100 mg/kg (the maximum manufacturers' use levels are 95 mg/kg in the United Kingdom and 100 mg/kg in the USA), up to 3000 g/day of water-based flavoured drinks could be consumed before the ADI was exceeded, assuming an ADI of 0–5 mg/kg bw, an average body weight of 60 kg, and up to 750 g/day for a 15-kg child.

3.2 Poundage data

Poundage (disappearance) data were available from the USA, based on information reported to the National Academy of Sciences (1989): 38 600 pounds (17 500 kg) of quillaia extracts were reported to have been used in food applications in 1987. The intake of quillaia extracts per capita was calculated to be 0.0055 mg/kg bw per day (0.1% ADI), assuming a body weight of 60 kg and 60% response to the survey (raw poundage data divided by 0.6 to account for underreporting). The per capita intake at the 90th percentile was calculated by multiplying by a factor of 2, to give 0.011 mg/kg bw per day or 0.2% ADI (use of factor discussed in WHO, 1987).

3.3 Individual dietary records

Estimates of the intake of quillaia extracts based on individual dietary records from national surveys in Australia and New Zealand, the United Kingdom, and the USA were based on consumption of the whole water-based flavoured drinks category or those soft drinks likely to contain the additive. The estimates and the assumptions made in deriving the estimates are summarized in Table 1.

Table 1. Estimated intakes of quillaia extracts from individual dietary records

Country and reference	Population group	Soft drink consumption (g/day)	Quillaia extract intake(mg/kg bw per day)	% ADI[a]	Assumptions	Survey	Date o survey
Australia (Australia–New Zealand Food Authority, 2001a)	All respondents Consumers only Consumers only	Mean, 240 Mean, 590 Median, 410 95th percentile, 1600	Mean, 2.3 Mean, 5.5 Median, 3.7 95th percentile, 16	47 110 74 320	Extract in all water-based drinks; GSFA level, 500 mg/kg; consumers, 41% of population	National survey, single 24-h recall, 13 858 sample; ≥ 2 years; mean body weight, 67 kg; individual body weights used in calculations	1995
	All respondents Consumers only Consumers only	Mean, 9 Mean, 380 Median, 310 95th percentile, 800	Mean, 0.07 Mean, 3.0 Median, 2.2 95th percentile, 7.2	1.4 59 45 140	Extract in limited number of soft drinks only; GSFA level, 500 mg/kg[b]; consumers, 2% of population		
New Zealand (Australia–New Zealand Food Authority, 2001b)	All respondents Consumers only Consumers only	Mean, 180 Mean, 510 Median, 370 95th percentile, 1600	Mean, 1.1 Mean, 3.4 Median, 2.5 95th percentile, 9.8	23 69 49 200	Extract in all water-based drinks; GSFA level, 500 mg/kg; consumers 35% of population	National survey, 4636 sample; single 24-h recall; ≥ 15 years; mean body weight, 71 kg, individual body weights used	1997
United Kingdom (Food Standards Agency, 2001)	Adult respondents Adult consumers	Mean, 120 97th percentile, 640	Mean, 1.0 97th percentile, 5.3	19 110	GSFA, 500 mg/kg; extract in all water-based drinks[c]; consumers 22%	National survey; 7-day records; adults 16–64 years; sample, 2197; assumed body weight, 60 kg	1986–
	Adult respondents Adult consumers	Mean, 120 97th percentile, 640	Mean, 0.2 97th percentile, 0.1	4 20	Maximum manufacturers' use level, 95 mg/kg; extract in all water-based drinks		
	Child respondents Child consumers	Mean, 260 97th percentile, 800	Mean, 8.8 97th percentile, 28	180 550	GSFA, 500 mg/kg; extract in all water-based	National survey; 7-day records; children 1.5–	1992

	Child respondents Child consumers	Mean, 260 97th percentile, 800	Mean, 1.7 97th percentile, 5.2	34 100	drinks[c]; consumers 87% Maximum manufacturers' use level, 95 mg/kg; extract in all water-based drinks	4.5 years; sample, 1675; assumed body weight, 14.5 kg	
USA (Food & Drug Administration, 2001)	Consumers only Consumers only All respondents Consumers only Consumers only	Mean, 180 90th percentile, 330 Mean, 7 Mean, 180 90th percentile, 330	Mean, 1.5 90th percentile, 2.7 Mean, 0.01 Mean, 0.3 90th percentile, 0.5	30 54 0.2 6 11	Extract in brewed soft drinks only; GSFA level, 500 mg/kg; consumers 3.8% of population Extract in limited number of soft drinks only, maximum level of use, 100 mg/kg[b]; consumers 3.8% of population	National survey; 3-day intake (one 24-h record plus self-reported daily intake, weighted data); sample, 11 912; assumed body weight, 60 kg	1989–(comb survey

[a] JECFA ADI, 0–5 mg/kg bw

[b] Soft drinks likely to contain the additive are, e.g. ginger beer, root beer and cream soda.

[c] Calculated from data given in submission from the United Kingdom

If use of quillaia extracts is assumed to be at the GSFA level (500 mg/kg) in all water-based drinks, the intake would exceed the ADI for consumers in Australia at the mean level (5.5 mg/kg bw per day or 109% of the ADI) and at the high level (16 mg/kg bw per day or 316% of the ADI) and for consumers at the high level in New Zealand (9.8 mg/kg bw per day or 196% of the ADI), for child respondents in the study in the United Kingdom (8.8 mg/kg bw per day or 177% of the ADI), and for consumers at the high level among both child (28 mg/kg bw per day or 550% of the ADI) and adult consumers (5.3 mg/kg bw per day or 106% of the ADI) in the United Kingdom.

Estimates of intake based only on soft drinks likely to contain quillaia extracts and the level of use stated in the draft GSFA were submitted by Australia and the USA. The mean intake of quillaia extracts by

consumers in Australia was below the ADI (3 mg/kg bw per day, 59% of the ADI), but that of consumers of large amounts of soft drinks likely to contain the additive exceeded the ADI (7.2 mg/kg bw per day, 145% of the ADI). The estimated intakes of quillaia extracts in the USA were 1.5 mg/kg bw per day (30% of the ADI) for consumers at the mean level and 2.7 mg/kg bw per day (54% of the ADI) for those at the 90th percentile of consumption.

Estimates of intake based only on consumption of water-based drinks and national levels of use were submitted by the United Kingdom, where the maximum level of use of quillaia extracts is 95 mg/kg, although 200 mg/kg is permitted in the European Union. The estimated mean intakes of quillaia extracts were 0.18 mg/kg bw per day, or 4% of the ADI, by adult respondents and 1.7 mg/kg bw per day, or 34% of the ADI, by child respondents. The estimated intake of quillaia extracts by adult consumers of large amounts was below the ADI, but that for children who were high consumers exceeded the ADI (5.2 mg/kg bw per day or 105% of the ADI).

Estimates of intake based only on soft drinks likely to contain the additive and national levels of use were submitted by the USA, where the maximum manufacturers' level of use of quillaia extracts is 100 mg/kg. The estimated intakes were 0.3 mg/kg bw per day, or 6% of the ADI, for consumers at the mean level and 0.54 mg/kg bw per day, or 11% of the ADI, for consumners at the 90th percentile of consumption.

3.4 Evaluation of intake estimates

Screening by application of the budget method showed that further assessment of the intake of quillaia extracts was required. The reverse budget method indicated that up to 600 g of water-based flavoured soft drinks could be consumed by a 60-kg person, or 150 g by a 15-kg child, before the ADI of 5 mg/kg bw was exceeded, if quillaia extracts were used at a concentration of 500 mg/kg, as proposed in the draft GSFA. Intake estimates based on poundage data from the USA indicated low per capita intakes of quillaia extracts (< 1% ADI), although this type of estimate tends to result in underestimates of the intake by high consumers.

Intake estimates based on individual records in national surveys tend to provide more accurate estimates of the actual intake of food additives. The issue of the poor absorption of quillaia extracts was not considered in this evaluation. Data on food consumption submitted to the Committee indicated that consumers of large volumes of soft drinks likely to contain the additive (95th percentile) in Australia and children aged 1.5–4 years in the United Kingdom who are drink large volumes of all soft drinks (97.5th percentile) may exceed these amounts, although these may be overestimates of long-term consumption because they are derived from short-term surveys. Estimated intakes at the 95th percentile of consumption in Australia and New Zealand, based on a single 24-h recall, tend to overestimate the habitual intake of quillaia extracts by these consumers, as evidenced by the much higher reported levels of consumption at that level in those countries. In the surveys in the United Kingdom and the USA, the amounts of food consumed were averaged over a number of days (3 and 7, respectively), which would tend to decrease the reported daily consumption of all foods but in particular foods consumed occasionally (Gibney, 1999; Lambe et al., 2000).

The use of food consumption data for all water-based drinks, as in the submissions from New Zealand and the United Kingdom, would result in overestimates of the actual intake of quillaia extracts, which are used in a limited number of drinks as a foaming agent. Nevertheless, the estimated intakes based on these data and national levels of use did not exceed the ADI for the population of the United Kingdom, as quillaia extracts are permitted for use at lower maximum levels (95 mg/kg). Young children are an exception, as their relatively heavy consumption of water-based drinks and low body weight resulted in

an estimated intake of quillaia extract that exceeded the ADI for consumers at the high level. This estimate is still conservative in that it was assumed that all water-based drinks contained quillaia extracts at the maximum manufacturers' level of use. In addition, data from short-term nutritional surveys do not permit estimation of the frequency or duration of exceedence over the ADI.

The most accurate estimates of intake were those from the USA, where information on consumption of soft drinks likely to contain the additive and national levels of use were available. The estimated intakes of quillaia extracts were well below the ADI for consumers at both mean and high levels. Quillaia extracts are not currently permitted for use in Australia or New Zealand; however, were the additive to be permitted at levels of use similar to those in the USA and used in only a limited number of soft drinks (such as ginger beer, root beer, and cream soda), the estimated intake would also be below the ADI.

4. COMMENTS

Toxicological data

Studies of acute toxicity showed that quillaia extracts are less toxic when administered orally than when administered systemically. Fractions isolated from Q. saponaria differed widely in acute toxicity as well as in adjuvant activity and cholesterol-binding capacity. QS-18, the major saponin of quillaia extracts, was more acutely toxic to mice than two other saponins that were isolated and was more toxic than the extract itself when administered intradermally.

In a 90-day study, rats fed diets containing 4% quillaia extract (equivalent to 2000 mg/kg bw per day; specifications conformed to the Emulsifiers and Stabilisers in Food Regulations 1975 of the United Kingdom, but information on the actual composition of the material tested was not available) showed decreased body-weight gain, decreased relative liver weight, and increased stomach weight, with no treatment-related histological changes. The NOEL was a dietary concentration of 0.6%, equivalent to 400 mg/kg bw per day.

In a more recent 90-day study, rats were given quillaia saponins in deionized water by gavage at a dose of 1200 mg/kg bw. Severe and lethal toxic effects were observed during the study. In the surviving animals, the weights of several organs were increased, and several haematological and clinical parameters were changed. Histopathological examination showed inflammatory changes in the forestomach, larynx, trachea, and lungs.

Minor changes in body-weight gain and the relative weights of some organs were reported in lifetime studies in mice and rats given quillaia extract (with specifications conforming to the Emulsifiers and Stabilisers in Food Regulations 1975 of the United Kingdom), at dietary concentrations up to 1.5% in rats and 3% in mice. No compound-related histopathological changes were reported. The NOELs for quillaia extract in the diet were 0.5% (700 mg/kg bw per day) for mice and 1% (500 mg/kg bw per day) for rats.

The Committee noted that the differences in toxicity observed in the 90-day studies in rats treated orally, outlined above, may have been due to differences in the test material, i.e., the concentrations and types of saponins present, and/or in the method of administration, i.e., in the diet and by gavage in water.

The existing specifications for quillaia extracts were revised in order to clarify further the differences between unpurified and semi-purified extracts. As additional information on composition was determined to be necessary, the specifications were designated as tentative. Once the requested information has been received, the Committee will consider whether separate specifications for

unpurified and semi-purified extracts are required.

Intake

Quillaia extracts can be used as foaming agents in soft drinks and in cocktail mixes and as emulsifiers in foods such as baked goods, candies, frozen dairy products, gelatine, and puddings. Their major food use is in soft drinks such as ginger beer, root beer, and cream soda.

A reverse budget method based on the temporary ADI of 0–5 mg/kg bw and use of quillaia extracts in soft drinks at a level of 500 mg/kg indicated that a person weighing 60 kg could consume up to 600 g of drink per day before exceeding the ADI, while a child weighing 15 kg could consume only 150 g of drink per day before exceeding the ADI. Data on food consumption submitted to the Committee indicated that consumers of soft drinks likely to contain the additive at the 95th percentile in Australia and children aged 1.5–4 years in the United Kingdom who consume soft drinks at the 97.5th percentile could exceed these amounts, although the data may overestimate long-term consumption because they are derived from short-term surveys.

Estimates of intake based on consumption of soft drinks likely to contain this food additive and the levels of use of quillaia extract in the draft GSFA were submitted by Australia and the USA. Estimates of the mean intakes of quillaia extracts by respondents in the United Kingdom were available which were based on consumption of all water-based flavoured drinks and are therefore more conservative. For Australia, the mean intakes were 3 mg/kg bw per day (60% of the ADI) for consumers on the basis of the draft GSFA level (500 mg/kg) and 7.2 mg/kg bw per day (145% of the ADI) for high consumers. For the USA, the estimated mean intakes of quillaia extracts were 1.5 mg/kg bw per day (30% of the ADI) for consumers on the basis of the draft GSFA level and 2.7 mg/kg bw per day (54% of the ADI) for consumers at the 90th percentile.

Estimates of intake based only on consumption of soft drinks likely to contain the food additive and national levels of use were submitted by the USA. The maximum level of use of quillaia extracts by manufacturers in the USA is 100 mg/kg. The estimated mean intake of quillaia extracts by consumers was 0.3 mg/kg bw per day (6% of the ADI), and that for consumers at the 90th percentile was 0.54 mg/kg bw per day (11% of the ADI). Data from the United Kingdom based on a use level by manufacturers of 95 mg/kg indicated that children who consumed soft drinks at the 97.5th percentile level had an intake of quillaia extracts of 5.2 mg/kg bw per day (105% of the ADI), but this value may be an overestimate of intake as it is based on consumption of all water-based flavoured drinks.

Use at the maximum level of 95–100 mg/day reported by the manufacturers, as in the United Kingdom and the USA, appeared to be adequate for the technological function of quillaia extracts as foaming agents in soft drinks and did not appear to result in intakes that exceed the ADI. Young children are a possible exception, but as the results of a short-term nutritional survey were used the frequency or duration of their potential exceedence of the ADI was unknown.

The Committee recommended that the Codex Committee on Food Additives and Contaminants review the use of quillaia extracts at 500 mg/kg proposed in the draft GSFA in the category 14.1.4 'Water based flavoured drinks' (Annex 1, Annex 4 of reference 137).

5. EVALUATION

The Committee made the previously established ADI of 0–5 mg/kg bw for unpurified extract temporary and extended it until 2003, pending clarification of the specifications for quillaia extracts. The

Committee emphasized that the temporary ADI is not applicable to the semi-purified or any other product derived from Q. saponaria or from other species of Quillaia.

The Committee will reconsider the subject when the specifications for quillaia extracts have been clarified; further studies of toxicity with specified quillaia products that reflect the nature of the product consumed by humans may be required.

6. REFERENCES

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See Also:
Toxicological Abbreviations
Quillaia extracts (WHO Food Additives Series 17)
QUILLAIA EXTRACTS (JECFA Evaluation)

Appendix B – Quillaia Extract (Type I) Specifications

QUILLAIA EXTRACT (TYPE 1)

Specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). The previous tentative specifications for Quillaia extracts prepared at the 57th JECFA (2001), published in FNP 52 Add 9 (2001), are replaced by these and by separate specifications for "Quillaia extract (Type 2)". An ADI of 0-5mg/kg mg/kg bw was established at the 29th JECFA (1985).

SYNONYMS	Quillaja extract, Soapbark extract, Quillay bark extract, Bois de Panama, Panama bark extract, Quillai extract; INS No. 999
DEFINITION	Quillaia extract (Type 1) is obtained by aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaja saponaria* Molina (family *Rosaceae*). It contains triterpenoid saponins (quillaia saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are major components and some sugars and calcium oxalate will be present. Quillaia extract (Type 1) is available commercially as liquid product or as spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol.
C.A.S. number	68990-67-0
Formula weight	Monomeric saponins range from ca. 1800 to ca. 2300, consistent with a triterpene with 8-10 monosaccharide residues
ASSAY	Saponin content: not less than 20 % and not more than 26 % on the dried basis ✓
DESCRIPTION	Red-brownish liquid or light brown powder with a pink tinge
FUNCTIONAL USES	Emulsifier, foaming agent
CHARACTERISTICS	
IDENTIFICATION	
Solubility (FNP 5)	Very soluble in water, insoluble in ethanol, acetone, methanol and butanol
Foam	Dissolve 0.5 g of powder extract in 9.5 g of water or 1 ml of liquid extract in 9 ml of water. Add 1 ml of this mixture to 350 ml of water in a 1000-ml graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam level (ml) after 30 min. Typical values are 150 ml of foam
Chromatography	Determine as in METHOD OF ASSAY. The retention time of major peak of the sample corresponds to the major saponin peak (QS-18) of the standard.
Colour and turbidity	Powder form only: Dissolve 0.5 g in 9.5 g of water. The solution is not turbid. Determine the absorbance of the solution against water at 520 nm. The absorbance is less than 1.2.
PURITY	
Water (FNP 5)	Powder form: not more than 6% (Karl Fischer Method)
Loss on drying (FNP 5)	Liquid form: 50 to 80% (2 g, 105°, 5 h)
pH (FNP 5)	3.7 -5.5 (4 % solution)
Ash (FNP 5)	Not more than 14% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from loss on drying)

$$C_{sap} = (A_{sample}/A_{standard})C_{Standard}$$

where $C_{Standard}$ (mg/ml) is the saponins concentration of the standard injected (e.g., $C_{Standard}$ = 13.5 mg/ml if the saponin content of 1.5 g of standard sample is 90 %) and A_{sample} and $A_{standard}$ are the sums of the peak areas attributed to the four principle saponins in the sample preparation and in the standard preparation, respectively, as noted in the figure. (Tannins and Polyphenols will elute before the saponins. The peaks due to the saponins will appear after the major peak due to the polyphenols - see figure.)

The percentage of saponins in the test sample is:

$$\% \text{ Saponins} = 100 \times C_{sap}/(0.1 W_{sample})$$

where W_{sample} is the weight of the sample (mg) taken for the sample preparation and 0.1 is the inverse of the sample volume, 10 ml.

EN

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►B

COMMISSION DIRECTIVE 96/77/EC

of 2 December 1996

laying down specific purity criteria on food additives other than colours and sweeteners

(Text with EEA relevance)

(OJ L 339, 30.12.1996, p. 1)

Amended by:

		Official Journal		
		No	page	date
►M1	Commission Directive 98/86/EC of 11 November 1998	L 334	1	9.12.1998
►M2	Commission Directive 2000/63/EC of 5 October 2000	L 277	1	30.10.2000
►M3	Commission Directive 2001/30/EC of 2 May 2001	L 146	1	31.5.2001
►M4	Commission Directive 2002/82/EC of 15 October 2002	L 292	1	28.10.2002
►M5	Commission Directive 2003/95/EC of 27 October 2003	L 283	71	31.10.2003

▼B

COMMISSION DIRECTIVE 96/77/EC

of 2 December 1996

laying down specific purity criteria on food additives other than colours and sweeteners

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 89/107/EEC of 21 December 1988 on the approximation of the laws of the Member States concerning food additives authorized for use in foodstuffs intended for human consumption [1], as amended by European Parliament and Council Directive 94/34/EC [2], and in particular Article 3 (3) (a) thereof,

After consulting the Scientific Committee for Food,

Whereas it is necessary to establish purity criteria for all additives other than colours and sweeteners mentioned in European Parliament and Council Directive 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners [3];

Whereas it is necessary to replace the purity criteria set out in Council Directive 65/66/EEC of 26 January 1965 laying down specific criteria of purity for preservatives authorized for use in foodstuffs intended for human consumption [4], as last amended by Directive 86/604/EEC [5];

Whereas it is necessary to replace the purity criteria set out in Council Directive 78/664/EEC of 25 July 1978 laying down specific criteria of purity for antioxidants which may be used in foodstuffs intended for human consumption [6], as amended by Directive 82/712/EEC [7];

Whereas Directives 65/66/EEC and 78/664/EEC should be repealed accordingly;

Whereas it is necessary to take into acount the specifications and analytical techniques for additives as set out in the *Codex Alimentarius* as drafted by the Joint FAO/WHO Expert Committee on Food Additives (Jecfa);

Whereas food additives, if prepared by production methods or starting materials significantly different from those included in the evaluation of the Scientific Committee for Food, or if different from those mentioned in this Directive, should be submitted for evaluation by the Scientific Committee for Food for the purposes of a full evaluation with emphasis on the purity criteria;

Whereas, the measures provided for in this Directive are in accordance with the opinion of the Standing Committee for Foodstuffs,

HAS ADOPTED THIS DIRECTIVE:

Article 1

The purity criteria referred to in Article 3 (3) (a) of Directive 89/107/EEC for food additives other than colours and sweeteners, as mentioned in Directive 95/2/EC, are set out in the Annex hereto.

[1] OJ No L 40, 11. 2. 1989, p. 27.
[2] OJ No L 237, 10. 9. 1994, p. 1.
[3] OJ No L 61, 18. 3. 1995, p. 1.
[4] OJ No 22, 9. 2. 1965, p. 373.
[5] OJ No L 352, 13. 12. 1986, p. 45.
[6] OJ No L 223, 14. 8. 1978, p. 30.
[7] OJ No L 297, 23. 10. 1982, p. 31.

▼M2

Nitrogen dioxide and nitrogen oxide	Not more than 10 µl/l

E 948 OXYGEN

Definition	
Chemical name	Oxygen
EINECS	231-956-9
Chemical formula	O_2
Molecular weight	32
Assay	Not less than 99 %
Description	Colourless, odourless, non-flammable gas
Purity	
Water	Not more than 0,05 %
Methane and other hydrocarbons calculated as methane	Not more than 100 µl/l

E 999 QUILLAIA EXTRACT

Synonyms	Soapbark extract, Quillay bark extract, Panama bark extract, Quillai extract, Murillo bark extract, China bark extract
Definition	Quillaia extract is obtained by aqueous extraction of *Quillai saponaria Molina*, or other *Quillaia* species, trees of the family *Rosaceae*. It contains a number of triterpenoid saponins consisting of glycosides of quillaic acid. Some sugars including glucose, galactose, arabinose, xylose, and rhamnose are also present, along with tannin, calcium oxalate and other minor components
Description	Quillaia extract in the powder form is light brown with a pink tinge. It is also available as an aqueous solution
Identification	
A. pH of a 2,5 % solution	Between 4,5 and 5,5
Purity	
Water	Not more than 6,0 % (Karl Fischer method) (powder form only)
Arsenic	Not more than 2 mg/kg
Lead	Not more than 5 mg/kg
Mercury	Not more than 1 mg/kg

E 1103 INVERTASE

Definition	Invertase is produced from *Saccharomyces cerevisiae*
Systematic name	β-D-Fructofuranoside fructohydrolase
Enzyme Commission No	EC 3.2.1.26
EINECS	232-615-7
Purity	
Arsenic	Not more than 3 mg/kg
Lead	Not more than 5 mg/kg
Cadmium	Not more than 0,5 mg/kg
Total bacterial count	Not more than 50 000/g
Salmonella spp.	Absent by test in 25 g

Appendix C – Photograph of dispensing machine and instruction for dispensing

DIET COLA
CONCENTRATE COLA
ORANGE
ORANGE

How to Dispense a Frozen Carbonated Beverage (FCB):

1 **Select the appropriate cup size**

2 **Snap on the dome lid**

3 **Put the nozzle inside the hole of the dome lid (about ½”) under the correct flavor**

4 **Pull the dispensing lever down toward you and fill up the cup until the product is about ½’ from the top of the dome lid**

5 **Serve the frozen treat to the customer with a sealed spoon straw**

Submission End

AM

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(formerly Novigen Sciences, Inc.)

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Reply to the U.S. Office

www.exponent.com

RECD JUN 14 2005

May 31, 2005

Susan J. Carlson, Ph.D.
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-255
5100 Paint Branch Parkway
College Park, MD 20740

Subject: Response to FDA questions on Quillaia Extract Type I GRAS notification
Project No. WD00878.000

Dear Dr. Carlson:

Based on our discussion with you on April 26, 2005, Exponent is providing the following responses to two questions posed by FDA regarding the notification and supporting documentation that Quillaia Extract Type 1 ("Quillaia"), prepared according to food grade specifications, is Generally Recognized as Safe (GRAS) for use as a foaming agent in semi-frozen carbonated and non-carbonated beverages (FCBs) when used at levels up to 500 mg/kg (dried basis), submitted by the American Beverage Association (ABA). The questions from FDA (in bold) and Exponent's and ABA's responses are explained below:

1. **Supporting documentation for the Quillaia GRAS notification states that polyvinyl-pyrollidone (PVP) is used as a stabilizing agent in the extraction of Quillaia (WHO Food Additive Series 48, 2002). FDA asked for clarification as to the use of PVP in Quillaia production and the regulatory status of this use of PVP.**

Exponent and ABA have inquired with the major suppliers of Quillaia and have received documentation (see enclosures) that PVP is either not used in the production of Quillaia or, if used as a processing aid in the extraction process, is removed in the filtration step. PVP is not found in the finished Quillaia product.

2. **FDA requested that intake of Quillaia from flavoring and foaming agents be considered in the EDI calculations, using data from the Flavor and Extract Manufacturing Association (FEMA) for all uses of Quillaia.**

According to data from FEMA's 1995 Poundage and Technical Effects Update Survey, 178,403 pounds (80,922 kg) of Quillaia extract was reported to have been used in food applications in 1995 (Lucas et al., 1999). Exponent calculated the daily per capita intake (PCI) for "eaters only" according to the following formula (Smith et al., 2003):

$$PCI_{Quillaia} = \frac{(80{,}922 \text{ kg/year})\ (10^6 \text{ mg/kg})}{(260 \times 10^6 \text{ people})\ (10\% \text{ eaters})\ (80\% \text{ reporting})\ (365 \text{ days/year})\ (60 \text{ kg bw})}$$

$$= 0.018 \text{ mg/kg bw/day}$$

The daily PCI of Quillaia extract among "eaters only" is 0.018 mg/kg bw/day from its use in all food applications. This intake corresponds to 0.36% of the ADI of 5 mg Quillaia/kg bw/day.

The PCI is based on 1995 poundage data for Quillaia extracts, the US population in 1995 (260 million people), an average bodyweight of 60 kg, and the assumptions that 10% of the population consumes foods containing Quillaia and only 80% of the volume was reported in the survey (accounts for underreporting as described in Lucas et al., 1999). This is a highly conservative estimate since we are assuming that the total volume of Quillaia that is produced in a year is consumed by only 10% of the US population. Based on the approved uses of Quillaia as a flavoring agent in highly consumed foods such as "baked goods" and "non-alcoholic beverages", this is most likely not the case and in fact, the percent of the population consuming Quillaia extracts is most likely closer to 100%.

Please direct any additional questions or information requests concerning this response to Dr. Nancy Rachman at Exponent, (202) 772-4907, or nrachman@exponent.com.

Sincerely,

(b)(6)

Nancy Rachman, Ph.D.
Senior Managing Scientist
Exponent, Inc.

Enclosures

DESERT KING
INTERNATIONAL

May 2, 2005

Mr. G. Bradley Curtis, P.E.
Global Purchasing Manager
The Coca-Cola Company
P.O. Box 1734
Atlanta, GA 30301

Dear Bradley,

Please be advised that we do not use PVP in the production of our Quillaja or Yucca extract products.

If you should need any further clarification pertaining to this matter, please do not hesitate to contact me directly.

Sincerely,

(b)(6)

Paul Hiley,
President

7024 Manya Circle San Diego, CA 92154 USA Tel 619 429 5222 800.YUCCA.DK Fax 619.429.5001 info@desertking.com www.desertking.com
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Ex

BERGHAUSEN CORPORATION
www.berghausen.com
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4524 Este Avenue
Cincinnati, OH 45232
513.541.5631
Fax 513.541.1169

Brad Curtis
Purchasing Manager
The Coca-Cola Company
Coca-Cola Plaza
Atlanta Georgia

Dear Brad,

Please be assured that no residual Polyvinylpyrrolidone (PVP) is found in the finished Quillaia Extract Liquid product produced by Berghausen Corporation. PVP is a polymer made up of very large molecules. It used as a processing agent early in the extraction process to help stabilize the product and then gets filtered out. Let me know if I can be of any further assistance in this matter.

Regards,

(b)(6)

Thomas Davlin
Quality Manager
Berghausen Corporation

EX

AM

Carlson, Susan

From: Carolyn Scrafford [cscrafford@exponent.com]
Sent: Monday, June 20, 2005 3:11 PM
To: Carlson, Susan
Cc: Orstan, Aydin; Nancy Rachman
Subject: RE: Quillaia Extract Gras Notice

Dear Susan,

Attached is a revised version of our amendment to GRN 000165 with the corrected PCI calculation. I apologize for any inconvenience this may have caused.

Best regards,
Carolyn

Carolyn Scrafford, M.P.H.
Managing Scientist, Food and Chemicals
Exponent, Inc.
Direct Line: 202-772-4928
Fax: 202-772-4979
cscrafford@exponent.com

From: Carlson, Susan [mailto:Susan.Carlson@cfsan.fda.gov]
Sent: Wednesday, June 15, 2005 9:51 AM
To: 'cscrafford@exponent.com'
Orstan, Aydin
Subject: Quillaia Extract Gras Notice

Dear Carolyn,
We have been reviewing your recent amendment to GRN 000165 and have noticed that the PCI calculation on page 2 seems to be off by a factor of 10. Would you please confirm this calculation?

Thank you,
Susan

Susan J. Carlson, Ph.D.
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
New telephone number: 301-436-1321

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Reply to the U.S. Office

June 17, 2005

Susan J. Carlson, Ph.D.
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-255
5100 Paint Branch Parkway
College Park, MD 20740

Subject: Revised Response to FDA questions on Quillaia Extract Type I GRAS notification
Project No. WD00878.000

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2. **FDA requested that intake of Quillaia from flavoring and foaming agents be considered in the EDI calculations, using data from the Flavor and Extract Manufacturing Association (FEMA) for all uses of Quillaia.**

According to data from FEMA's 1995 Poundage and Technical Effects Update Survey, 178,403 pounds (80,922 kg) of Quillaia extract was reported to have been used in food applications in 1995 (Lucas et al., 1999). Exponent calculated the daily per capita intake (PCI) for "eaters only" according to the following formula (Smith et al., 2003):

$$PCI_{Quillaia} = \frac{(80{,}922\ kg/year)\ (10^6\ mg/kg)}{(260 \times 10^6\ people)\ (10\%\ eaters)\ (80\%\ reporting)\ (365\ days/year)\ (60\ kg\ bw)}.$$

$$= 0.18\ mg/kg\ bw/day$$

The daily PCI of Quillaia extract among "eaters only" is 0.18 mg/kg bw/day from its use in all food applications. This intake corresponds to 3.6% of the ADI of 5 mg Quillaia/kg bw/day.

The PCI is based on 1995 poundage data for Quillaia extracts, the US population in 1995 (260 million people), an average bodyweight of 60 kg, and the assumptions that 10% of the population consumes foods containing Quillaia and only 80% of the volume was reported in the survey (accounts for underreporting as described in Lucas et al., 1999). This is a highly conservative estimate since we are assuming that the total volume of Quillaia that is produced in a year is consumed by only 10% of the US population. Based on the approved uses of Quillaia as a flavoring agent in highly consumed foods such as "baked goods" and "non-alcoholic beverages", this is most likely not the case and in fact, the percent of the population consuming Quillaia extracts is most likely closer to 100%.

Please direct any additional questions or information requests concerning this response to Dr. Nancy Rachman at Exponent, (202) 772-4907, or nrachman@exponent.com.

Sincerely,

(b)(6)

Nancy Rachman, Ph.D.
Senior Managing Scientist
Exponent, Inc.

Enclosures

DESERT KING
INTERNATIONAL

May 2, 2005

Mr. G. Bradley Curtis, P.E.
Global Purchasing Manager
The Coca-Cola Company
P.O. Box 1734
Atlanta, GA 30301

Dear Bradley,

Please be advised that we do not use PVP in the production of our Quillaja or Yucca extract products.

If you should need any further clarification pertaining to this matter, please do not hesitate to contact me directly.

Sincerely,

(b)(6)

Paul Hiley,
President

7024 Manya Circle San Diego, CA 92154 USA Tel 619 429 5222 800.YUCCA.DK Fax 619 429 5001 info@desertking.com www.desertking.com
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BERGHAUSEN CORPORATION
www.berghausen.com
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4524 Este Avenue
Cincinnati, OH 45232
513.541.5631
Fax 513.541.1169

Brad Curtis
Purchasing Manager
The Coca-Cola Company
Coca-Cola Plaza
Atlanta Georgia

Dear Brad,

Please be assured that no residual Polyvinylpyrrolidone (PVP) is found in the finished Quillaia Extract Liquid product produced by Berghausen Corporation. PVP is a polymer made up of very large molecules. It used as a processing agent early in the extraction process to help stabilize the product and then gets filtered out. Let me know if I can be of any further assistance in this matter.

Regards

(b)(6)

Thomas Davlin
Quality Manager
Berghausen Corporation

Exponent®
(formerly Novigen Sciences, Inc.)

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October 25, 2005

RECD OCT 26 2005

OCT 26 2005

Richard E. Bonnette
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-255
5100 Paint Branch Parkway
College Park, MD 20740

Subject: Response to FDA inquiries on Quillaia Extract Type I GRAS notification
Project No. WD00878.000

Dear Mr. Bonnette:

Based on our discussion with you on August 23, 2005, Exponent is providing the following response to the question posed by FDA regarding the notification and supporting documentation that Quillaia Extract Type 1 ("Quillaia"), prepared according to food grade specifications, is Generally Recognized as Safe (GRAS) for use as a foaming agent in semi-frozen carbonated and non-carbonated beverages (FCBs) when used at levels up to 500 mg/kg (dried basis), submitted by the American Beverage Association (ABA). The question from FDA (in bold) and Exponent's and ABA's response is explained below:

1. **Supporting documentation for the Quillaia GRAS notification states that egg albumin is used as a stabilizing agent in the extraction of Quillaia (WHO Food Additive Series 48, 2002). FDA asked for clarification as to the use of egg albumin in Quillaia production.**

Exponent and ABA have inquired with the major suppliers of Quillaia and have received documentation (see enclosures) that egg albumin is not used in the production of Quillaia.

Please direct any additional questions or information requests concerning this response to Dr. Nancy Rachman at Exponent, (202) 772-4907, or nrachman@exponent.com.

Sincerely,

(b)(6)

Nancy Rachman, Ph.D.
Senior Managing Scientist
Exponent, Inc.

Enclosures (3)

Ex

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DESERT KING
INTERNATIONAL

August 25, 2005

Mr. G. Bradley Curtis
Global Purchasing Manager
The Coca-Cola Company
P.O. Box 1734
Atlanta, GA 30301

Dear Mr. Curtis,

Please be advised that we do not use any egg albumin in the processing of our Yucca extracts or our Quillaja extracts.

If I can be of any further assistance in this matter, please do not hesitate to contact me.

Sincerely,

(b)(6)

Paul Hiley
President

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August 29, 2005

Mary Cash
The Coca-Cola Company
Coca-Cola Plaza
Atlanta, Georgia 30301

Dear Mary,

Please be assured that Berghausen Corporation does not use any Egg Albumin when manufacturing Quillaia Liquid Extract. It used to be used years ago but was taken out because it is an allergen. Please feel to contact me if you have any questions.

Regards,

(b)(6)

Thomas Davlin
Quality Manager
Berghausen Corporation

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NORTHBROOK, ILLINOIS 60062

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September 14, 2005

Mr. Bradley Curtis
The Coca-Cola Company
P.O. Box 1734
Atlanta, GA 30301

Dear Brad,

According to analytical results conducted on Bell's Quillaia Extract # 3476B, Egg Albumin is not detected in this product and does not need to be included in the list of ingredients.

Attached is Bell's allergen assessment on Quillaia Extract # 3476B.

Sincerely,

(b)(6)

James Heinz
President & CEO

cc: Tim Cambias