APPENDIX A : Use of Quillaia extract for high oil load emulsion

Summary

In order to flavor beverages, citrus oils have to be prepared into emulsions before they can be added into beverages. Traditionally, these beverage emulsions contain flavor oils up to 12% w/w. These emulsions are typically prepared w ith either gum Arabic or modified starches. Q-Naturale (quillaia extract) can be used to prepare high oil load emulsions, up to 50% w/w oil content while retaining good emulsion stability. Existing approved emulsifiers such as gum Arabic and modified starches cannot be used to prepare 50% oil emulsions. Preparation of high oil load emulsions can bring cost savings to emulsion manufacturers.

Objective

To demonstrate the capability of Q-NATURALE 200 (purified quillaia extract) to prepare high oil load emulsion and to compare its performance with gum Arabic and modified starch.

Experimental Design

3 different orange oil emulsions were prepared according to Table 1. For Trial A1 and A2, the emulsifier used, namely Gum Arabic and modified starch (starch sodium octenyl succinate) were dispersed and mixed till fully dissolved before use as water phase. For Trial A3, the liquid quillaia extract is added directly to water and ready for use as water phase. The oil blend is added to water phase with high shear mixing and passed through the homogenizer to achieve mean particle size of 0.4-0.45um. The oil droplet size distribution governs the stability of emulsion.

After the emulsion is prepared, it is checked for stability by applying in beverage. This beverage along with the emulsion is subjected to prolonged storage at ambient conditions for 6months to determine its stability.

Table A1 Composition of 50% oil emulsions prepared with different emulsifiers

Emulsion Composition (%)	Trial A1	Trial A2	Trial A3
Orange oil blend	50	50	50
Gum Arabic	20		
Modified starch	-	20	
Q-Naturale (purified quillaia extract)	-		10
Citric acid	0.35	0.35	0.35
Sodium benzoate	0.15	0.15	0.15
Water	29.5	29.5	39.5

Results

The stability of the emulsions prepared with different emulsifiers is reflected in Table A2. Emulsions prepared with gum Arabic and modified starch (Trial A1 and A2) were paste-like and not flowable. This reduces the ease of handling. Furthermore, these two emulsions (Trial A1 and A2) were considered unstable as ringing was observed in beverage after 1 month, and creaming and ringing were also found in emulsion after 6months. For the emulsion prepared with quillaia extract (Trial A3), both the beverage and emulsion are stable.

Table A2 Evaluation results of 50% oil emulsion prepared with different emulsifiers

Emulsion characterization	**Trial A1**	**Trial A2**	**Trial A3**
Viscosity (cP)	> 2000	> 2000	50
Stability in end application (beverage) after 1month	Ringing	Ringing	No ringing
Emulsion after 6months	Distinct creaming and oil layer on surface of emulsion	Distinct creaming and oil layer on surface of emulsion	No creaming or oiling

Discussion

It has been demonstrated here that quillaia extract can be used to prepare 50% oil emulsion with good stability and this cannot be achieved with existing food additives approved by Malaysia such as gum Arabic and modified starch.

APPENDIX A : Use of Quillaia extract for clear beverage emulsion

Background

There is growing popularity of clear beverages among consumers. The use of emulsion for application in beverage requires the oil droplet size to be very fine. Thus, the emulsifier used has to possess strong emulsification properties.

Objective

To demonstrate the use of quillaia extract in clear beverage application

Experimental Design

The emulsions were prepared according to Table B1. The emulsions were processed at high homogenization pressures at multiple passes in order to create very fine oil droplet size and finally applied to beverages to check the beverage clarity.

Table B1Composition of 10% oil emulsions for clear beverage application

Emulsion Composition (%)	Trial B1	Trial B2
Orange oil blend	10	10
Modified starch	10	-
Q-Naturale (purified quillaia extract)	-	10
Citric acid	0.35	0.35
Sodium benzoate	0.15	0.15
Water	29.5	29.5

Result

From Figure B1, it was observed that the beverage prepared with the starch sample (Trial B1) was slightly opaque while the beverage prepared with quillaia extract was transparent (Trial B2).

(A) Starch sample (B) Quillaia extract sample

Figure B1 Picture illustrating the beverage clarity difference between quillaia extract sample and starch sample

Discussion

Quillaia extract can be used to prepare very fine emulsions for clear beverage application. It has been demonstrated here that this cannot be achieved with existing approved emulsifier, starch sodium octenyl succinate.

CODEX GENERAL STANDARD FOR FOOD ADDITIVES

CODEX STAN 192-1995

PREAMBLE

1. SCOPE

1.1 FOOD ADDITIVES INCLUDED IN THIS STANDARD

Only the food additives listed herein are recognized as suitable for use in foods in conformance with the provisions of this Standard.[1] Only food additives that have been assigned an Acceptable Daily Intake (ADI) or determined, on the basis of other criteria, to be safe[2] by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[3] and an International Numbering System (INS) designation by Codex will be considered for inclusion in this Standard. The use of additives in conformance with this standard is considered to be technologically justified.

1.2 FOODS IN WHICH ADDITIVES MAY BE USED

This Standard sets forth the conditions under which food additives may be used in all foods, whether or not they have previously been standardized by Codex. The use of additives in foods standardized by Codex is subject to the conditions of use established by the Codex commodity standards and this standard. The General Standard for Food Additives (GSFA) should be the single authoritative reference point for food additives. Codex commodity committees have the responsibility and expertise to appraise and justify the technological need for the use of additives in foods subject to a commodity standard. The information given by the commodity committees may also be taken into account by the Codex Committee on Food Additives (CCFA) when considering food additive provisions in similar non-standardized foods. When a food is not covered by a commodity committee, CCFA will appraise the technological need.

1.3 FOODS IN WHICH ADDITIVES MAY NOT BE USED

Food categories or individual food items in which the use of food additives is not acceptable, or where use should be restricted, are defined by this Standard.

1.4 MAXIMUM USE LEVELS FOR FOOD ADDITIVES

The primary objective of establishing maximum use levels for food additives in various food groups is to ensure that the intake of an additive from all its uses does not exceed its ADI.

The food additives covered by this Standard and their maximum use levels are based in part on the food additive provisions of previously established Codex commodity standards, or upon the request of governments after subjecting the requested maximum use levels to an appropriate method for verifying the compatibility of a proposed maximum level with the ADI.

Annex A of this Standard may be used as a first step in this regard. The evaluation of actual food consumption data is also encouraged.

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1 Notwithstanding the provisions of this Section of the General Standard, the lack of reference to a particular additive or to a particular use of an additive in a food in the General Standard as currently drafted, does not imply that the additive is unsafe or unsuitable for use in food. The Commission shall review the necessity for maintaining this footnote on a regular basis, with a view to its deletion once the General Standard is substantially complete.

2 For the purpose of this standard "determined, on the basis of other criteria, to be safe" means that the use of a food additive does not pose a safety concern under conditions of use described by JECFA as being of no toxicological concern (e.g. use levels defined circumstances).

3 A data base of food additive specifications with their current ADI status, the year of their most recent JECFA evaluation, their assigned INS numbers, etc., are available in English at the JECFA website at FAO http://www fao.org/ag/agn/jecfa-additives/search.html?lang=en . The database has a query page and background information in English, French, Spanish, Arabic and Chinese. The reports of JECFA are available at the JECFA website at WHO http://www.who.int/ipcs/food/jecfa/en/

Adopted in 1995. Revision 1997, 1999, 2001, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010.

2. DEFINITIONS

a) ***Food additive*** means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities.[4]

b) ***Acceptable Daily Intake (ADI)*** is an estimate by JECFA of the amount of a food additive, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk.[5]

c) ***Acceptable Daily Intake "Not Specified" (NS)***[6] is a term applicable to a food substance of very low toxicity for which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance, arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food, does not, in the opinion of JECFA, represent a hazard to health.

For the above reason, and for reasons stated in individual JECFA evaluations, establishment of an acceptable daily intake expressed in numerical form is not deemed necessary by JECFA. An additive meeting the above criterion must be used within the bounds of good manufacturing practice as defined in section 3.3 below.

d) ***Maximum Use Level*** of an additive is the highest concentration of the additive determined to be functionally effective in a food or food category and agreed to be safe by the Codex Alimentarius Commission. It is generally expressed as mg additive/kg of food.

The maximum use level will not usually correspond to the optimum, recommended, or typical level of use. Under GMP, the optimum, recommended, or typical use level will differ for each application of an additive and is dependent on the intended technical effect and the specific food in which the additive would be used, taking into account the type of raw material, food processing and post-manufacture storage, transport and handling by distributors, retailers, and consumers.

3. GENERAL PRINCIPLES FOR THE USE OF FOOD ADDITIVES

The use of food additives in conformance with this Standard requires adherence to all the principles set forth in Sections 3.1 – 3.4.

3.1 FOOD ADDITIVE SAFETY

a) Only those food additives shall be endorsed and included in this Standard that, so far as can be judged on the evidence presently available from JECFA, present no appreciable health risk to consumers at the use levels proposed.

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4 Codex Alimentarius Procedural Manual.

5 Principles for the Safety Assessment of Food Additives and Contaminants in Food, World Health Organization, (WHO Environmental Health Criteria, No. 70), p. 111 (1987). For the purposes of this Standard, the phrase "without appreciable health risk" means that there is a reasonable certainty of no harm to consumers if an additive is used at levels that do not exceed those in this Standard. The provisions of this Standard do not sanction the use of an additive in a manner that would adversely affect consumer health.

6 For purposes of this Standard, the phrase acceptable daily intake (ADI) "not limited" (NL) has the same meaning as ADI "not specified". The phrase "acceptable ADI" refers to an evaluation by JECFA, which established safety on the basis of an acceptable level of treatment of food, limited numerically or by GMP, rather than on a toxicologically established ADI.

b) The inclusion of a food additive in this Standard shall have taken into account any ADI, or equivalent safety assessment established for the additive by JECFA and its probable daily intake[7] from all food sources. Where the food additive is to be used in foods eaten by special groups of consumers (e.g., diabetics, those on special medical diets, sick individuals on formulated liquid diets), account shall be taken of the probable daily intake of the food additive by those consumers.

c) The quantity of an additive added to food is at or below the maximum use level and is the lowest level necessary to achieve the intended technical effect. The maximum use level may be based on the application of the procedures of Annex A, the intake assessment of Codex members or upon a request by the CCFA to JECFA for an independent evaluation of national intake assessments.

3.2 JUSTIFICATION FOR THE USE OF ADDITIVES

The use of food additives is justified only when such use has an advantage, does not present an appreciable health risk to consumers, does not mislead the consumer, and serves one or more of the technological functions set out by Codex and the needs set out from (a) through (d) below, and only where these objectives cannot be achieved by other means that are economically and technologically practicable:

a) To preserve the nutritional quality of the food; an intentional reduction in the nutritional quality of a food would be justified in the circumstances dealt with in sub-paragraph (b) and also in other circumstances where the food does not constitute a significant item in a normal diet;

b) To provide necessary ingredients or constituents for foods manufactured for groups of consumers having special dietary needs;

c) To enhance the keeping quality or stability of a food or to improve its organoleptic properties, provided that this does not change the nature, substance or quality of the food so as to deceive the consumer;

d) To provide aids in the manufacture, processing, preparation, treatment, packing, transport or storage of food, provided that the additive is not used to disguise the effects of the use of faulty raw materials or of undesirable (including unhygienic) practices or techniques during the course of any of these activities.

3.3 GOOD MANUFACTURING PRACTICE (GMP)[8]

All food additives subject to the provisions of this Standard shall be used under conditions of good manufacturing practice, which include the following:

a) The quantity of the additive added to food shall be limited to the lowest possible level necessary to accomplish its desired effect;

b) The quantity of the additive that becomes a component of food as a result of its use in the manufacturing, processing or packaging of a food and which is not intended to accomplish any physical, or other technical effect in the food itself, is reduced to the extent reasonably possible; and,

c) The additive is of appropriate food grade quality and is prepared and handled in the same way as a food ingredient.

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7 Codex members may provide the CCFA with intake information that may be used by the Committee in establishing maximum use levels. Additionally, the JECFA, at the request of the CCFA, will evaluate intakes of additives based on intake assessments submitted by Codex members responding to a call for data. The CCFA will consider the JECFA evaluations when establishing the maximum use levels for additives.

8 For additional information, see the Codex Alimentarius Commission Procedural Manual. Relations Between Commodity Committees and General Committees- Food Additives and Contaminants.

3.4 SPECIFICATIONS FOR THE IDENTITY AND PURITY OF FOOD ADDITIVES

Food additives used in accordance with this Standard should be of appropriate food grade quality and should at all times conform with the applicable Specifications of Identity and Purity recommended by the Codex Alimentarius Commission[9] or, in the absence of such specifications, with appropriate specifications developed by responsible national or international bodies. In terms of safety, food grade quality is achieved by conformance of additives to their specifications as a whole (not merely with individual criteria) and through their production, storage, transport, and handling in accordance with GMP.

4. CARRY-OVER OF FOOD ADDITIVES INTO FOODS

4.1 CONDITIONS APPLYING TO CARRY-OVER OF FOOD ADDITIVES

Other than by direct addition, an additive may be present in a food as a result of carry-over from a raw material or ingredient used to produce the food, provided that:

a) The additive is acceptable for use in the raw materials or other ingredients (including food additives) according to this Standard;

b) The amount of the additive in the raw materials or other ingredients (including food additives) does not exceed the maximum use level specified in this Standard;

c) The food into which the additive is carried over does not contain the additive in greater quantity than would be introduced by the use of raw materials, or ingredients under proper technological conditions or manufacturing practice, consistent with the provisions of this standard.

An additive may be used in a raw material or other ingredient if the raw material or ingredient is used exclusively in the preparation of a food that is in conformity with the provisions of this standard.

4.2 FOODS FOR WHICH THE CARRY-OVER OF FOOD ADDITIVES IS UNACCEPTABLE

Carry-over of a food additive from a raw material or ingredient is unacceptable for foods belonging to the following food categories, unless a food additive provision in the specified category is listed in Tables 1 and 2 of this standard.

a) 13.1 - Infant formulae, follow-up formulae, and formulae for special medical purposes for infants.

b) 13.2 - Complementary foods for infants and young children.

5. FOOD CATEGORY SYSTEM[10]

The food category system is a tool for assigning food additive uses in this Standard. The food category system applies to all foodstuffs.

The food category descriptors are not to be legal product designations nor are they intended for labelling purposes.

The food category system is based on the following principles:

a) The food category system is hierarchical, meaning that when an additive is recognized for use in a general category, it is recognized for use in all its sub-categories, unless otherwise stated. Similarly, when an additive is recognized for use in a sub-category, its use is recognized in any further sub-categories or individual foodstuffs mentioned in a sub-category.

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9 An index (CAC/MISC 6) of all specifications adopted by the Codex Alimentarius Commission, as well as the year of adoption, is available at the Codex website (http://www.codexalimentarius net). These specifications, prepared by the JECFA, are also being published in 2006 in the "Combined Compendium of Food Additive Specifications," FAO JECFA Monographs No. 1, which consists of four volumes and in subsequent JECFA Monographs. The specifications are also available at the JECFA website (http://www fao.org/ag/agn/jecfa-additives/search html?lang=en). Although specifications for flavouring agents are not included in the printed compendium, with the exception of those few which have an additional non-flavour technological function, they are included in an online searchable database at the JECFA website at FAO. http://apps3 fao.org/jecfa/flav_agents/flavag-q.jsp?language=en.

10 Annex B to this Standard.

b) The food category system is based on product descriptors of foodstuffs as marketed, unless otherwise stated.

c) The food category system takes into consideration the carry-over principle. By doing so, the food category system does not need to specifically mention compound foodstuffs (e.g., prepared meals, such as pizza, because they may contain, *pro rata*, all the additives endorsed for use in their components), unless the compound foodstuff needs an additive that is not endorsed for use in any of its components.

d) The food category system is used to simplify the reporting of food additive uses for assembling and constructing this Standard.

6. DESCRIPTION OF THE STANDARD

This Standard consists of three main components:

a) Preamble

b) Annexes

i. Annex A is a guideline for considering maximum use levels for additives with numerical JECFA ADIs.

ii. Annex B is a listing of the food category system used to develop and organize Tables 1, 2, and 3 of the standard. Descriptors for each food category and sub-category are also provided.

iii. Annex C is a cross-reference of the food category system and Codex commodity standards.

c) Food Additive Provisions

i. Table 1 specifies, for each food additive or food additive group (in alphabetical order) with a numerical JECFA ADI, the food categories (or foods) in which the additive is recognized for use, the maximum use levels for each food or food category, and its technological function. Table 1 also includes the uses of those additives with non-numerical ADIs for which a maximum use level is specified.

ii. Table 2 contains the same information as Table 1, but the information is arranged by food category number.

iii. Table 3 lists additives with Not Specified or Not Limited JECFA ADIs that are acceptable for use in foods in general when used at *quantum satis* levels and in accordance with the principles of good manufacturing practice described in Section 3.3 of this preamble.

The Annex to Table 3 lists food categories and individual food items excluded from the general conditions of Table 3. The provisions in Tables 1 and 2 govern the use of additives in the food categories listed in the Annex to Table 3.

Unless otherwise specified, maximum use levels for additives in Tables 1 and 2 are set on the final product as consumed.

Tables 1, 2, and 3 do not include references to the use of substances as processing aids.[11]

[11] Processing Aid means any substance or material, not including apparatus or utensils, and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods or its ingredients to fulfill a certain technological purpose during treatment or processing and which may result in the non-intentional but unavoidable presence of residues or derivatives in the final product: Codex Alimentarius Commission Procedural Manual.

Table One

PROPYLENE GLYCOL ESTERS OF FATTY ACIDS

FoodCatNo	FoodCategory	MaxLevel	Notes	Year Adopted
11.4	Other sugars and syrups (e.g., xylose, maple syrup, sugar toppings)	5000 mg/kg		2001
13.3	Dietetic foods intended for special medical purposes (excluding products of food category 13.1)	5000 mg/kg		2001
13.4	Dietetic formulae for slimming purposes and weight reduction	5000 mg/kg		2001
14.1.4	Water-based flavoured drinks, including "sport," "energy," or "electrolyte" drinks and particulated drinks	500 mg/kg		2001

PROTEASE

INS 1101(i) Protease Functional Class: Flavour enhancer, Flour treatment agent, Stabilizer

FoodCatNo	FoodCategory	MaxLevel	Notes	Year Adopted
06.2.1	Flours	GMP		1999

QUILLAIA EXTRACTS

INS 999(i) Quillaia extract type I Functional Class: Emulsifier, Foaming agent

INS 999(ii) Quillaia extract type 2 Functional Class: Emulsifier, Foaming agent

FoodCatNo	FoodCategory	MaxLevel	Notes	Year Adopted
14.1.4	Water-based flavoured drinks, including "sport," "energy," or "electrolyte" drinks and particulated drinks	50 mg/kg	132 & 168	2007

RIBOFLAVINS

INS 101(i) R boflavin, synthetic Functional Class: Colour

INS 101(ii) R boflavin 5'-phosphate sodium Functional Class: Colour

INS 101(iii) R boflavin from Bacillus subtilis Functional Class: Colour

FoodCatNo	FoodCategory	MaxLevel	Notes	Year Adopted
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g., chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)	300 mg/kg	52	2008
01.3.2	Beverage whiteners	300 mg/kg		2005
01.5.2	Milk and cream powder analogues	300 mg/kg		2005
01.6.1	Unripened cheese	300 mg/kg		2005
01.6.2.1	Ripened cheese, includes rind	300 mg/kg		2005
01.6.2.2	Rind of ripened cheese	300 mg/kg		2005
01.6.4	Processed cheese	300 mg/kg		2005
01.6.5	Cheese analogues	300 mg/kg		2005
01.7	Dairy-based desserts (e.g., pudding, fruit or flavoured yoghurt)	300 mg/kg		2005

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EUROPEAN PARLIAMENT AND COUNCIL DIRECTIVE No 95/2/EC
of 20 February 1995
on food additives other than colours and sweeteners

(OJ L 61, 18.3.1995, p. 1)

Amended by:

		Official Journal		
		No	page	date
►**M1**	Directive 96/85/EC of the European Parliament and of the Council of 19 December 1996	L 86	4	28.3.1997
►**M2**	Directive 98/72/EC of the European Parliament and of the Council of 15 October 1998	L 295	18	4.11.1998
►**M3**	Directive 2001/5/EC of the European Parliament and of the Council of 12 February 2001	L 55	59	24.2.2001
►**M4**	Directive 2003/52/EC of the European Parliament and of the Council of 18 June 2003	L 178	23	17.7.2003
►**M5**	Regulation (EC) No 1882/2003 of the European Parliament and of the Council of 29 September 2003	L 284	1	31.10.2003
►**M6**	Directive 2003/114/EC of the European Parliament and of the Council of 22 December 2003	L 24	58	29.1.2004

Corrected by:

►**C1** Corrigendum, OJ L 248, 14.10.1995, p. 60 (95/2/EC)

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EUROPEAN PARLIAMENT AND COUNCIL DIRECTIVE No 95/2/EC

of 20 February 1995

on food additives other than colours and sweeteners

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty establishing the European Community, and in particular Article 100a thereof,

Having regard to the proposal from the Commission ([1]),

Having regard to the opinion of the Economic and Social Committee ([2]),

Acting in accordance with the procedure laid down in Article 189b of the Treaty ([3]),

Having regard to the 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 ([4]), and in particular Article 3 (2) thereof,

Whereas differences between national laws relating to preservatives, antioxidants and other additives and their conditions of use hinder the free movement of foodstuffs; whereas this may create conditions of unfair competition;

Whereas the prime consideration for any rules on these food additives and their conditions of use should be the need to protect the consumer;

Whereas it is generally recognized that unprocessed foodstuffs and certain other foodstuffs should be free from food additives;

Whereas, having regard to the most recent scientific and toxicological information on these substances, some of them are to be permitted only for certain foodstuffs and under certain conditions of use;

Whereas it is necessary to lay down strict rules for the use of food additives in infant formulae, follow-on formulae and weaning foods, as referred to in Council Directive 89/398/EEC of 3 May 1989 on the approximation of the laws of the Member States relating to foodstuffs intended for particular nutritional uses ([5]), and in particular Article 4 (1) (e) thereof;

Whereas this Directive is not intended to affect rules relating to sweeteners and colours;

Whereas, pending specific provisions pursuant to Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market ([6]), and pursuant to Council Directive 90/642/EEC of 27 November 1990 on the fixing of maximum levels for pesticide residues in and on certain products of plant origin, including fruit and vegetables ([7]), certain substances belonging to this category are provisionally covered by this Directive;

Whereas the Commission is to adapt Community provisions to accord with the rules laid down in this Directive;

([1]) OJ No C 206, 13. 8. 1992, p. 12, and OJ No C 189, 13. 7. 1993, p. 11.
([2]) OJ No C 108, 19. 4. 1993, p. 26.
([3]) Opinion of the European Parliament of 26 May 1993 (OJ No C 176, 28. 6. 1993, p. 117), confirmed on 2 December 1993 (OJ No C 342, 20. 12. 1993), common position of the Council of 10 March 1994 (OJ No C 172, 24. 6. 1994, p. 4) and decision of the European Parliament of 16 November 1994 (OJ No C 341, 5. 12. 1994)
([4]) OJ No L 40, 11. 2. 1989, p. 27.
([5]) OJ No L 186, 30. 6. 1989, p. 27.
([6]) OJ No L 230, 19. 8. 1991, p. 1. Directive as last amended by Commission Regulation (EEC) No 3600/92 (OJ No L 366, 15. 12. 1992, p. 10).
([7]) OJ No L 350, 14. 12. 1990, p. 71.

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Whereas the Scientific Committee for Food has been consulted for those substances which are not yet the subject of a Community provision;

Whereas it is necessary to include in this Directive specific provisions concerning additives referred to in other Community provisions;

Whereas it is desirable that when a decision is taken on whether a particular foodstuff belongs to a certain category of foods, the consultation of the Standing Committee for Foodstuffs procedure is followed;

Whereas modifications of existing purity criteria for food additives other than colours and sweeteners and new specifications for those where no purity criteria exist will be adopted in accordance with the procedure laid down in Article 11 of Directive 89/107/EEC;

Whereas the Scientific Committee for Food has not yet given an opinion on flour treatment agents; whereas those agents will be the subject of a separate Directive;

Whereas this Directive replaces Directives 64/54/EEC ([1]), 70/357/EEC ([2]), 74/329/EEC ([3]) and 83/463/EEC ([4]); whereas those Directives are hereby repealed,

HAVE ADOPTED THIS DIRECTIVE:

Article 1

▼M2

1. This Directive is a specific Directive forming a part of the comprehensive Directive, within the meaning of Article 3 of Directive 89/107/EEC, and applies to additives other than colours and sweeteners. It does not apply to enzymes other than those mentioned in the Annexes,

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2. Only additives which satisfy the requirements laid down by the Scientific Committee for Food may be used in foodstuffs.

3. For the purpose of this Directive:

(a) 'preservatives' are substances which prolong the shelf-life of foodstuffs by protecting them against deterioration caused by micro-organisms;

(b) 'antioxidants' are substances which prolong the shelf-life of foodstuffs by protecting them against deterioration caused by oxidation, such as fat rancidity and colour changes;

(c) 'carriers', including carrier solvents, are substances used to dissolve, dilute, disperse or otherwise physically modify a food additive without altering its technological function (and without exerting any technological effect themselves) in order to facilitate its handling, application or use;

(d) 'acids' are substances which increase the acidity of a foodstuff and/or impart a sour taste to it;

(e) 'acidity regulators' are substances which alter or control the acidity or alkalinity of a foodstuff;

(f) 'anti-caking agents' are substances which reduce the tendency of individual particles of a foodstuff to adhere to one another;

(g) 'anti-foaming agents' are substances which prevent or reduce foaming;

(h) 'bulking agents' are substances which contribute to the volume of a foodstuff without contributing significantly to its available energy value;

([1]) OJ No 12, 27. 1. 1964, p. 161/64.
([2]) OJ No L 157, 18. 7. 1970, p. 31.
([3]) OJ No L 189, 12. 7. 1974, p. 1.
([4]) OJ No L 255, 15. 9. 1983, p. 1.

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(i) ‘emulsifiers’ are substances which make it possible to form or maintain a homogenous mixture of two or more immiscible phases such as oil and water in a foodstuff;

(j) ‘emulsifying salts’ are substances which convert proteins contained in cheese into a dispersed form and thereby bring about homogenous distribution of fat and other components;

(k) ‘firming agents’ are substances which make or keep tissues of fruit or vegetables firm or crisp, or interact with gelling agents to produce or strengthen a gel;

(l) ‘flavour enhancers’ are substances which enhance the existing taste and/or odour of a foodstuff;

(m) ‘foaming agents’ are substances which make it possible to form a homogenous dispersion of a gaseous phase in a liquid or solid foodstuff;

(n) ‘gelling agents’ are substances which give a foodstuff texture through formation of a gel;

(o) ‘glazing agents’ (including lubricants) are substances which, when applied to the external surface of a foodstuff, impart a shiny appearance or provide a protective coating;

(p) ‘humectants’ are substances which prevent foodstuffs from drying out by counteracting the effect of an atmosphere having a low degree of humidity, or promote the dissolution of a powder in an aqueous medium;

(q) ‘modified starches’ are substances obtained by one or more chemical treatments of edible starches, which may have undergone a physical or enzymatic treatment, and may be acid or alkali thinned or bleached;

(r) ‘packaging gases’ are gases other than air, introduced into a container before, during or after the placing of a foodstuff in that container;

(s) ‘propellants’ are gases other than air which expel a foodstuff from a container;

(t) ‘raising agents’ are substances or combinations of substances which liberate gas and thereby increase the volume of a dough or a batter;

(u) ‘sequestrants’ are substances which form chemical complexes with metallic ions;

▼M6

(v) ‘stabilisers’ are substances which make it possible to maintain the physico-chemical state of a foodstuff; stabilisers include substances which enable the maintenance of a homogenous dispersion of two or more immiscible substances in a foodstuff, substances which stabilise, retain or intensify an existing colour of a foodstuff and substances which increase the binding capacity of the food, including the formation of cross-links between proteins enabling the binding of food pieces into re-constituted food;

▼B

(w) ‘thickeners’ are substances which increase the viscosity of a foodstuff.

4. Flour treatment agents other than emulsifiers are substances which are added to flour or dough to improve its baking quality.

5. For the purposes of this Directive the following are not considered as food additives:

(a) substances used for treatment of drinking water as provided for in Directive 80/778/EEC [1];

[1] OJ No L 229, 30. 8. 1980, p. 11. Directive as last amended by Directive 91/692/EEC (OJ No L 377, 31. 12. 1991, p. 48).

▼B

(b) products containing pectin and derived from dried apple pomace or peel of citrus fruits, or from a mixture of both, by the action of dilute acid followed by partial neutralization with sodium or potassium salts (‘liquid pectin’);

(c) chewing gum bases;

(d) white or yellow dextrin, roasted or dextrinated starch, starch modified by acid or alkali treatment, bleached starch, physically modified starch and starch treated by amylolitic enzymes;

(e) ammonium chloride;

(f) blood plasma, edible gelatin, protein hydrolysates and their salts, milk protein and gluten;

(g) amino acids and their salts other than glutamic acid, glycine, cysteine and cystine and their salts and having no additive function;

(h) caseinates and casein;

(i) inulin.

Article 2

▼M2

1. Only substances listed in Annexes I, III, IV and V may be used in foodstuffs for the purposes mentioned in Article 1(3) and Article 1(4),

2. Food additives listed in Annex I are permitted in foodstuffs, for the purposes mentioned in Article 1(3) and Article 1(4), with the exception of those foodstuffs listed in Annex II, following the ‘*quantum satis*’ principle,

▼B

3. Except where specifically provided for, paragraph 2 does not apply to:

(a) — unprocessed foodstuffs,

— honey as defined in Directive 74/409/EEC ([1])

— non-emulsified oils and fats of animal or vegetable origin,

— butter,

▼M2

— pasteurised and sterilised (including UHT) milk (including plain, skimmed and semi-skimmed) and plain pasteurised cream,

▼B

— unflavoured, live fermented milk products,

— natural mineral water as defined in Directive 80/777/EEC ([2]) and spring water,

— coffee (excluding flavoured instant coffee) and coffee extracts,

— unflavoured leaf tea,

— sugars as defined in Directive 73/437/EEC ([3]),

▼M2

— dry pasta, excluding gluten-free and/or pasta intended for hypoproteic diets, in accordance with Directive 89/398/EEC,

▼B

— natural unflavoured buttermilk (excluding sterilized buttermilk).

Within the meaning of this Directive, the term ‘unprocessed’ means not having undergone any treatment resulting in a substantial change in the original state of the foodstuffs; however, the foodstuffs may have been, for example, divided, parted, severed, boned, minced, skinned, pared, peeled, ground, cut, cleaned, trimmed, deep-frozen or frozen, chilled, milled or husked, packed or unpacked;

([1]) OJ No L 211, 12. 8. 1974, p. 10.
([2]) OJ No L 229, 30. 8. 1980, p. 1.
([3]) OJ No L 356, 27. 12. 1973, p. 71.

▼B

(b) foods for infants and young children as referred to in Directive 89/398/EEC, including foods for infants and young children not in good health; these foodstuffs are subject to the provisions of Annex VI;

(c) the foodstuffs listed in Annex II, which may contain only those additives referred to in that Annex and those additives referred to in Annexes III and IV under the conditions specified therein.

4. Additives listed in Annexes III and IV may only be used in the foodstuffs referred to in those Annexes and under the conditions specified therein.

5. Only those additives listed in Annex V may be used as carriers or carrier solvents for food additives and must be used under the conditions specified therein.

6. The provisions of this Directive shall also apply to the corresponding foodstuffs intended for particular nutritional uses in accordance with Directive 89/398/EEC.

7. Maximum levels indicated in the Annexes refer to foodstuffs as marketed, unless otherwise stated.

8. In the Annexes to this Directive, '*quantum satis*' means that no maximum level is specified. However, additives shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided that they do not mislead the consumer.

Article 3

▼M6

1. The presence of a food additive is permissible:

(a) in a compound foodstuff other than one mentioned in Article 2(3), to the extent to which the food additive is permitted in one of the ingredients of the compound foodstuff;

(b) in a foodstuff where a flavouring has been added, to the extent to which the food additive is permitted in the flavouring in compliance with this Directive and has been carried over to the foodstuff via the flavouring, provided the food additive has no technological function in the final foodstuff; or

(c) if the foodstuff is destined to be used solely in the preparation of a compound foodstuff and to an extent such that the compound foodstuff conforms to the provisions of this Directive.

▼B

2. Paragraph 1 does not apply to infant formulae, follow-on formulae and weaning foods, as referred to in Directive 89/398/EEC, except where specially provided for.

▼M6

3. The level of additives in flavourings shall be limited to the minimum necessary to guarantee the safety and quality of flavourings and to facilitate their storage. Furthermore, the presence of additives in flavourings must not mislead consumers or present a hazard to their health. If the presence of an additive in a foodstuff, as a consequence of adding flavourings, has a technological function in the foodstuff, it shall be considered as an additive of the foodstuff and not as an additive of the flavouring.

▼B

Article 4

This Directive shall apply without prejudice to specific Directives permitting additives listed in the Annexes to be used as sweeteners or colours.

▼B

Article 5

Where necessary, it may be decided by the procedure laid down in Article 6 of this Directive:

— whether a particular foodstuff not categorized at the moment this Directive was adopted belongs to a category of foodstuffs referred to in Article 2 or in one of the Annexes, or

— whether a food additive listed in the Annexes and authorized at '*quantum satis*' is used in accordance with the criteria referred to in Article 2, or

— whether a substance is a food additive within the meaning of Article 1.

▼M5

Article 6

1. The Commission shall be assisted by the Standing Committee on the Food Chain and Animal Health, set up by Article 58 of Regulation (EC) No 178/2002 ([1]), hereinafter referred to as 'the Committee'.

2. Where reference is made to this Article, Articles 5 and 7 of Decision 1999/468/EC ([2]) shall apply, having regard to the provisions of Article 8 thereof.

The period laid down in Article 5(6) of Decision 1999/468/EC shall be set at three months.

3. The Committee shall adopt its rules of procedure.

▼B

Article 7

Member States shall, within three years of the entry into force of this Directive, establish systems to monitor the consumption and use of food additives and report their findings to the Commission.

The Commission shall report to the European Parliament and the Council within five years of the entry into force of this Directive on the changes which have taken place in the food additives market, the levels of use and consumption.

In accordance with the general criteria in point 4 of Annex II to Directive 89/107/EEC, within five years of the entry into force of this Directive, the Commission shall review the conditions of use referred to in this Directive, and propose amendments where necessary.

Article 8

1. Directives 64/54/EEC, 70/357/EEC, 74/329/EEC and 83/463/EEC are hereby repealed.

2. References to these repealed Directives and to the purity criteria for certain food additives referred to in them shall henceforth be construed as references to this Directive.

Article 9

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive not later than 25 September 1996 in order to:

— allow, by 25 September 1996 at the latest, trade in and use of products conforming to this Directive,

— prohibit by 25 March 1997 at the latest, trade in and use of products not conforming to this Directive; products put on the market or

([1]) OJ L 31, 1.2.2002, p. 1.

([2]) Council Decision 1999/468/EC of 28 June 1999 laying down the procedures for the exercise of implementing powers conferred on the Commission (OJ L 184, 17.7.1999, p. 23).

▼B

labelled before that date which do not comply with this Directive may, however, be marketed until stocks are exhausted.

They shall forthwith inform the Commission thereof.

When Member States adopt these measures, they shall contain a reference to this Directive or shall be accompanied by such reference on the occasion of their official publication. The methods of making such reference shall be laid down by the Member States.

Article 10

This Directive shall enter into force on the seventh day following that of its publication in the *Official Journal of the European Communities*.

Article 11

This Directive is addressed to the Member States.

▼B

ANNEX I

FOOD ADDITIVES GENERALLY PERMITTED FOR USE IN FOODSTUFFS NOT REFERRED TO IN ARTICLE 2 (3)

Note

1. Substances on this list may be added to all foodstuffs with the exception of those referred to in Article 2 (3) following the *quantum satis* principle.

▼M6

2. The substances listed under numbers E 407, E 407a and E 440 may be standardised with sugars, on condition that this is stated in addition to the number and designation.

▼B

3. Explanation of symbols used:

   * The substances E 290, E 938, E 939, E 941, E 942, E 948 and ►M3 E 949 ◄ may also be used in the foodstuffs referred to in Article 2 (3).

   # The substances E 410, E 412, E 415 and E 417 may not be used to produce dehydrated foodstuffs intended to rehydrate on ingestion.

E No	Name
▼M6	
E 170	Calcium carbonate
▼B	
E 260	Acetic acid
E 261	Potassium acetate
E 262	Sodium acetates i) Sodium acetate ii) Sodium hydrogen acetate (sodium diacetate)
E 263	Calcium acetate
E 270	Lactic acid
E 290	Carbon dioxide*
E 296	Malic acid
E 300	Ascorbic acid
E 301	Sodium ascorbate
E 302	Calcium ascorbate
E 304	Fatty acid esters of ascorbic acid i) Ascorbyl palmitate ii) Ascorbyl stearate
E 306	Tocopherol-rich extract
E 307	Alpha-tocopherol
E 308	Gamma-tocopherol
E 309	Delta-tocopherol
E 322	Lecithins
E 325	Sodium lactate
E 326	Potassium lactate
E 327	Calcium lactate
E 330	Citric acid
E 331	Sodium citrates i) Monosodium citrate ii) Disodium citrate iii) Trisodium citrate
E 332	Potassium citrates i) Monopotassium citrate ii) Tripotassium citrate
E 333	Calcium citrates i) Monocalcium citrate

▼B

E No	Name	Foodstuff	Maximum level
▼M2		Spreadable fats as defined in Annexes B and C of Regulation (EC) No 2991/94	5 mg/kg
▼B		Meat products Fruit jellies Vegetable proteins	5 mg/kg (as flavour enhancer only)
E 999	Quillaia extract	Water-based flavoured non-alcoholic drinks	200 mg/l calculated as anhydrous extract
▼M2		Cider excluding *cidre bouché*	200 mg/l calculated as anhydrous extract
▼B E 1201	Polyvinylpyrrolidone	Dietary food supplements in tablet and coated tablet form	*quantum satis*
E 1202	Polyvinylpolypyrrolidone		
E 1505	Triethyl citrate	Dried egg white	*quantum satis*
▼M2 E 1518	Glyceryl triacetate (triacetin)	Chewing gum	*quantum satis*
E 459	Beta-cyclodextrine	Foodstuffs in tablet and coated tablet form	*quantum satis*
▼M6		Encapsulated flavourings in	
		— flavoured teas and flavoured powdered instant drinks	500 mg/l
		— flavoured snacks	1 g/kg in foodstuffs as consumed or as reconstituted according to the instructions of the manufacturer
▼M2 E 425	Konjac (7) (i) Konjac gum (ii) Konjac glucomannane	►M4 Foodstuffs in general (except those referred to in Article 2(3) and jelly confectionery including jelly-mini-cups) ◄	10 g/kg individually or in combination
▼M3 E 650	Zinc acetate	Chewing gum	1 000 mg/kg

I

(Acts adopted under the EC Treaty/Euratom Treaty whose publication is obligatory)

DIRECTIVES

COMMISSION DIRECTIVE 2008/84/EC

of 27 August 2008

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

(Text with EEA relevance)

(Codified version)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Commu nity,

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 authorised for use in foodstuffs intended for human consumption ([1]), and in particular Article 3 (3)(a) thereof,

Whereas:

(1) Commission Directive 96/77/EC of 2 December 1996 laying down specific purity criteria on food additives other than colours and sweeteners ([2]) has been substantially amended several times ([3]). In the interests of clarity and rationality the said Directive should be codified.

(2) 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 sweet eners ([4]).

(3) It is necessary to take into account 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).

(4) Food additives prepared by production methods or starting materials significantly different from those evaluated by the Scientific Committee for Food or different from those mentioned in this Directive should be submitted for safety evaluation by the European Food Safety Authority with emphasis on the purity criteria.

(5) The measures provided for in this Directive are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health.

(6) This Directive should be without prejudice to the obligations of the Member States relating to the time limits for transposition into national law of the Directives set out in Annex II, part B,

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 Annex I to this Directive.

Article 2

Directive 96/77/EC, as amended by the Directives listed in Annex II, part A, is repealed, without prejudice to the obligations of the Member States relating to the time limits for transposition into national law set out in Annex II, part B.

([1]) OJ L 40, 11.2.1989, p. 27.
([2]) OJ L 339, 30.12.1996, p. 1.
([3]) See Annex II, part A.
([4]) OJ L 61, 18.3.1995, p. 1.

References to the repealed Directive shall be construed as references to this Directive and shall be read in accordance with the correlation table in Annex III.

Article 3

This Directive shall enter into force on the 20th day following its publication in the *Official Journal of the European Union*.

Article 4

This Directive is addressed to the Member States.

Done at Brussels, 27 August 2008.

For the Commission

The President

José Manuel BARROSO

ANNEX I

Ethylene oxide may not be used for sterilising purposes in food additives.

E 170 (i) CALCIUM CARBONATE

Purity criteria for this additive are the same as set out for this additive in the Annex to Commission Directive 95/45/EC ([1]).

E 200 SORBIC ACID

Definition	
Chemical name	Sorbic acid
	Trans, trans 2,4 hexadienoic acid
Einecs	203 768 7
Chemical formula	$C_6H_8O_2$
Molecular weight	112,12
Assay	Content not less than 99 % on the anhydrous basis
Description	Colourless needles or white free flowing powder, having a slight characteristic odour and showing no change in colour after heating for 90 minutes at 105 °C
Identification	
A. Melting range	Between 133 °C and 135 °C, after vacuum drying for four hours in a sulphuric acid desiccator
B. Spectrometry	An isopropanol solution (1 in 4 000 000) shows absorbance maximum at 254 ± 2 nm
C. Positive test for double bonds	
D. Sublimation point	80 °C
Purity	
Water content	Not more than 0,5 % (Karl Fischer method)
Sulphated ash	Not more than 0,2 %
Aldehydes	Not more than 0,1 % (as formaldehyde)
Arsenic	Not more than 3 mg/kg
Lead	Not more than 5 mg/kg
Mercury	Not more than 1 mg/kg
Heavy metals (as Pb)	Not more than 10 mg/kg

E 202 POTASSIUM SORBATE

Definition	
Chemical name	Potassium sorbate
	Potassium (E, E) 2,4 hexadienoate
	Potassium salt of trans, trans 2,4 hexadienoic acid
Einecs	246 376 1
Chemical formula	$C_6H_7O_2K$
Molecular weight	150,22
Assay	Content not less than 99 % on the dried basis

([1]) OJ L 226, 22.9.1995, p. 1.

E 950 ACESULFAME K

Purity criteria for this additive are the same as set out for this additive in Annex I to Directive 2008/60/EC.

E 951 ASPARTAME

Purity criteria for this additive are the same as set out for this additive in Annex I to Directive 2008/60/EC.

E 953 ISOMALT

Purity criteria for this additive are the same as set out for this additive in Annex I to Directive 2008/60/EC.

E 957 THAUMATIN

Purity criteria for this additive are the same as set out for this additive in Annex I to Directive 2008/60/EC.

E 959 NEOHESPERIDINE DIHYDROCHALCONE

Purity criteria for this additive are the same as set out for this additive in Annex I to Directive 2008/60/EC.

E 965(i) MALTITOL

Purity criteria for this additive are the same as set out for this additive in Annex I to Directive 2008/60/EC.

E 965(ii) MALTITOL SYRUP

Purity criteria for this additive are the same as set out for this additive in Annex I to Directive 2008/60/EC.

E 966 LACTITOL

Purity criteria for this additive are the same as set out for this additive in Annex I to Directive 2008/60/EC.

E 967 XYLITOL

Purity criteria for this additive are the same as set out for this additive in Annex I to Directive 2008/60/EC.

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 *Quillaia saponaria* Molina, or other *Quillaia* species, trees of the family *Rosaceae*. It contains a number of triterpenoid saponins consisting of glycosides of quillaic acid. Some sugars including glucose, galactose, arabinose, xylose, and rhamnose are also present, along with tannin, calcium oxalate and other minor components
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
Coliforms	Not more than 30/g
E. coli	Absent by test in 25 g

E 1105 LYSOZYME

Synonyms	Lysozyme hydrochloride
	Muramidase
Definition	Lysozyme is a linear polypeptide obtained from hens' egg whites consisting of 129 amino acids. It possesses enzymatic activity in its ability to hydrolyse the β(1 4) linkages between N acetylmuramic acid and N acetylglucosamine in the outer membranes of bacterial species, in particular gram positive organisms. Is usually obtained as the hydro chloride
Chemical name	Enzyme Commission (EC) No: 3.2.1.17
Einecs	232 620 4
Molecular weight	About 14 000
Assay	Content not less than 950 mg/g on the anhydrous basis
Description	White, odourless powder having a slightly sweet taste
Identification	
A. Isoelectric point 10,7	
B. pH of a 2 % aqueous solution between 3,0 and 3,6	
C. Absorption maximum of an aqueous solution (25 mg/100 ml) at 281 nm, a minimum at 252 nm	
Purity	
Water content	Not more than 6,0 % (Karl Fischer method) (powder form only)
Residue on ignition	Not more than 1,5 %

[Code of Federal Regulations]
[Title 21, Volume 3]
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TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (CONTINUED)

PART 172_FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION--Table of Contents

Subpart F_Flavoring Agents and Related Substances

Sec. 172.510 Natural flavoring substances and natural substances used in conjunction with flavors.

Natural flavoring substances and natural adjuvants may be safely used in food in accordance with the following conditions.

(a) They are used in the minimum quantity required to produce their intended physical or technical effect and in accordance with all the principles of good manufacturing practice.

(b) In the appropriate forms (plant parts, fluid and solid extracts, concentrates, absolutes, oils, gums, balsams, resins, oleoresins, waxes, and distillates) they consist of one or more of the following, used alone or in combination with flavoring substances and adjuvants generally recognized as safe in food, previously sanctioned for such use, or regulated in any section of this part.

Common name	Scientific name	Limitations
Aloe	Aloe perryi Baker, A. barbadensis Mill., A. ferox Mill., and hybrids of this sp. with A. africana Mill. and A. spicata Baker.	
Althea root and flowers	Althea officinalis L.	
Amyris (West Indian sandalwood)	Amyris balsamifera L.	
Angola weed	Roccella fuciformis Ach.	In alcoholic beverages only
Arnica flowers	Arnica montana L., A. fulgens Pursh, A. sororia Greene, or A. cordifolia Hooker.	Do.
Artemisia (wormwood)	Artemisia spp.	Finished food thujone free \1\
Artichoke leaves	Cynara scolymus L.	In alcoholic beverages only
Benzoin resin	Styrax benzoin Dryander, S. paralleloneurus Perkins, S. tonkinensis (Pierre) Craib ex Hartwich, or other spp. of the Section Anthostyrax of the genus Styrax.	
Blackberry bark	Rubus, Section Eubatus	
Boldus (boldo) leaves	Peumus boldus Mol.	Do.
Boronia flowers	Boronia megastigma Nees	
Bryonia root	Bryonia alba L., or B. diocia Jacq.	Do.
Buchu leaves	Barosma betulina Bartl. et Wendl., B. crenulata (L.) Hook. or B. serratifolia Willd.	
Buckbean leaves	Menyanthes trifoliata L.	Do.
Cajeput	Melaleuca leucadendron L. and other Melaleuca spp.	
Calumba root	Jateorhiza palmata (Lam.) Miers.	Do.
Camphor tree	Cinnamomum camphora (L.) Nees et Eberm.	Safrole free
Cascara sagrada	Rhamnus purshiana DC.	
Cassie flowers	Acacia farnesiana (L.) Willd	
Castor oil	Ricinus communis L.	
Catechu, black	Acacia catechu Willd.	
Cedar, white (aborvitae), leaves and twigs	Thuja occidentalis L.	Finished food thujone free \1\
Centuary	Centaurium umbellatum Gilib.	In alcoholic beverages only
Cherry pits	Prunus avium L. or P. cerasus L.	Not to exceed 25 p.p.m. prussic acid
Cherry-laurel leaves	Prunus laurocerasus L.	Do.
Chestnut leaves	Castanea dentata (Marsh.) Borkh.	
Chirata	Swertia chirata Buch.-Ham.	In alcoholic beverages only
Cinchona, red, bark	Cinchona succirubra Pav. or its hybrids.	In beverages only; not more than 83 p.p.m. total cinchona alkaloids in finished beverage
Cinchona, yellow, bark	Cinchona ledgeriana Moens, C. calisaya Wedd., or	Do.

	hybrids of these with other spp. of Cinchona..	
Copaiba	South American spp. of Copaifera L.	
Cork, oak	Quercus suber L., or Q. occidentalis F. Gay.	In alcoholic beverages only
Costmary	Chrysanthemum balsamita L...	Do.

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Costus root	Saussurea lappa Clarke.....	
Cubeb	Piper cubeba L. f.....	
Currant, black, buds and leaves	Ribes nigrum L.....	
Damiana leaves	Turnera diffusa Willd.....	
Davana	Artemisia pallens Wall.....	
Dill, Indian	Anethum sowa Roxb. (Peucedanum graveolens Benth et Hook., Anethum graveolens L.).	
Dittany (fraxinella) roots	Dictamnus albus L.....	Do.
Dittany of Crete	Origanum dictamnus L.....	
Dragon's blood (dracorubin)	Daemonorops spp.....	
Elder tree leaves	Sambucus nigra L.....	In alcoholic beverages only; not to exceed 25 p.p.m. prussic acid in the flavor
Elecampane rhizome and roots	Inula helenium L.....	In alcoholic beverages only
Elemi	Canarium commune L. or C. luzonicum Miq.	
Erigeron	Erigeron canadensis L.....	
Eucalyptus globulus leaves	Eucalyptus globulus Labill..	
Fir (``pine'') needles and twigs	Abies sibirica Ledeb., A. alba Mill., A. sachalinesis Masters or A. mayriana Miyabe et Kudo.	
Fir, balsam, needles and twigs	Abies balsamea (L.) Mill....	
Galanga, greater	Alpinia galanga Willd.....	Do.
Galbanum	Ferula galbaniflua Boiss. et Buhse and other Ferula spp.	
Gambir (catechu, pale)	Uncaria gambir Roxb.....	
Genet flowers	Spartium junceum L.....	
Gentian rhizome and roots	Gentiana lutea L.....	
Gentian, stemless	Gentiana acaulis L.....	Do.
Germander, chamaedrys	Teucrium chamaedrys L.....	Do.
Germander, golden	Teucrium polium L.....	Do.
Guaiac	Guaiacum officinale L., G. santum L., Bulnesia sarmienti Lor.	
Guarana	Paullinia cupana HBK.....	
Haw, black, bark	Viburnum prunifolium L.....	
Hemlock needles and twigs	Tsuga canadensis (L.) Carr. or T. heterophylla (Raf.) Sarg.	
Hyacinth flowers	Hyacinthus orientalis L.....	
Iceland moss	Cetraria islandica Ach.....	Do.
Imperatoria	Peucedanum ostruthium (L.). Koch (Imperatoria ostruthium L.).	
Iva	Achillea moschata Jacq.....	Do.
Labdanum	Cistus spp.....	
Lemon-verbena	Lippia citriodora HBK.....	Do.
Linaloe wood	Bursera delpechiana Poiss. and other Bursera spp.	
Linden leaves	Tillia spp.....	Do.
Lovage	Levisticum officinale Koch..	
Lungmoss (lungwort)	Sticta pulmonacea Ach.....	
Maidenhair fern	Adiantum capillus-veneris L.	Do.
Maple, mountain	Acer spicatum Lam.....	
Mimosa (black wattle) flowers	Acacia decurrens Willd. var. dealbata.	
Mullein flowers	Verbascum phlomoides L. or V. thapsiforme Schrad.	Do.
Myrrh	Commiphora molmol Engl., C. abyssinica (Berg) Engl., or other Commiphora spp.	
Myrtle leaves	Myrtus communis L.....	Do.
Oak, English, wood	Quercus robur L.....	Do.
Oak, white, chips	Quercus alba L.....	
Oak moss	Evernia prunastri (L.) Ach., E. furfuracea (L.) Mann, and other lichens.	Finished food thujone free \1\
Olibanum	Boswellia carteri Birdw. and other Boswellia spp.	
Opopanax (bisabolmyrrh)	Opopanax chironium Koch (true opopanax) of Commiphora erythraea Engl. var. Llabrescens.	
Orris root	Iris germanica L. (including its variety florentina	

	Dykes) and I. pallida Lam.	
Pansy	Viola tricolor L.	In alcoholic beverages only
Passion flower	Passiflora incarnata L.	
Patchouly	Pogostemon cablin Benth. and P. heyneanus Benth.	
Peach leaves	Prunus persica (L.) Batsch.	In alcoholic beverages only; not to exceed 25 p.p.m. prussic acid in the flavor
Pennyroyal, American	Hedeoma pulegioides (L.) Pers.	
Pennyroyal, European	Mentha pulegium L.	

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Pine, dwarf, needles and twigs	Pinus mugo Turra var. pumilio (Haenke) Zenari.	
Pine, Scotch, needles and twigs	Pinus sylvestris L.	
Pine, white, bark	Pinus strobus L.	In alcoholic beverages only
Pine, white oil	Pinus palustris Mill., and other Pinus spp.	
Poplar buds	Populus balsamifera L. (P. tacamahacca Mill.), P. candicans Ait., or P. nigra L.	Do.
Quassia	Picrasma excelsa (Sw.) Planch, or Quassia amara L.	
Quebracho bark	Aspidosperma quebracho-blanco Schlecht, or (Quebrachia lorentzii (Griseb)).	Schinopsis lorentzii (Griseb.) Engl.
Quillaia (soapbark)	Quillaja saponaria Mol.	
Red saunders (red sandalwood)	Pterocarpus san alinus L.	In alcoholic beverages only
Rhatany root	Krameria triandra Ruiz et Pav. or K. argentea Mart.	
Rhubarb, garden root	Rheum rhaponticum L.	Do.
Rhubarb root	Rheum officinale Baill., R. palmatum L., or other spp. (excepting R. rhaponticum L.) or hybrids of Rheum grown in China.	
Roselle	Hibiscus sabdariffa L.	Do.
Rosin (colophony)	Pinus palustris Mill., and other Pinus spp.	Do.
St. Johnswort leaves, flowers, and caulis	Hypericum perforatum L.	Hypericin-free alcohol distillate form only; in alcoholic beverages only
Sandalwood, white (yellow, or East Indian)	Santalum album L.	
Sandarac	Tetraclinis articulata (Vahl.), Mast.	In alcoholic beverages only
Sarsaparilla	Smilax aristolochiaefolia Mill., (Mexican sarsaparilla), S. regelii Killip et Morton (Honduras sarsaparilla), S. febrifuga Kunth (Ecuadorean sarsaparilla), or undetermined Smilax spp. (Ecuadorean or Central American sarsaparilla).	
Sassafras leaves	Sassafras albidum (Nutt.) Nees.	Safrole free
Senna, Alexandria	Cassia acutifolia Delile.	
Serpentaria (Virginia snakeroot)	Aristolochia serpentaria L.	In alcoholic beverages only
Simaruba bark	Simaruba amara Aubl.	Do.
Snakeroot, Canadian (wild ginger)	Asarum canadense L.	
Spruce needles and twigs	Picea glauca (Moench) Voss or P. mariana (Mill.) BSP.	
Storax (styrax)	Liquidambar orientalis Mill. or L. styraciflua L.	
Tagetes (marigold)	Tagetes patula L., T. erecta L., or T. minuta L. (T. glandulifera Schrank).	As oil only
Tansy	Tanacetum vulgare L.	In alcoholic beverages only; finished alcoholic beverage thujone free \1\
Thistle, blessed (holy thistle)	Onicus benedictus L.	In alcoholic beverages only
Thymus capitatus (Spanish ``origanum'')	Thymus capitatus Hoffmg. et Link.	
Tolu	Myroxylon balsamum (L.) Harms.	
Turpentine	Pinus palustris Mill. and other Pinus spp. which yield terpene oils exclusively.	
Valerian rhizome and roots	Valeriana officinalis L.	
Veronica	Veronica officinalis L.	Do.
Vervain, European	Verbena officinalis L.	Do.
Vetiver	Vetiveria zizanioides Stapf.	Do.

Violet, Swiss	Viola calcarata L.	
Walnut husks (hulls), leaves, and green nuts	Juglans nigra L. or J. regia L.	
Woodruff, sweet	Asperula odorata L.	In alcoholic beverages only
Yarrow	Achillea millefolium L.	In beverages only; finished beverage thujone free \1\
Yerba santa	Eriodictyon californicum (Hook, et Arn.) Torr.	
Yucca, Joshua-tree	Yucca brevifolia Engelm.	
Yucca, Mohave	Yucca schidigera Roezl ex Ortgies (Y. mohavensis Sarg.).	

\1\ As determined by using the method (or, in other than alcoholic beverages, a suitable adaptation thereof) in section 9.129 of the ``Official Methods of Analysis of the Association of Official Analytical Chemists,'' 13th Ed. (1980), which is incorporated by reference. Copies may be obtained from the AOAC INTERNATIONAL, 481 North Frederick Ave., suite 500, Gaithersburg, MD 20877, or may be examined at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal--register/code--of--federal--regulations/ibr--locations.html.

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[42 FR 14491, Mar. 15, 1977, as amended at 43 FR 14644, Apr. 7, 1978; 49 FR 10104, Mar. 19, 1984; 54 FR 24897, June 12, 1989; 69 FR 24511, May 4, 2004; 72 FR 10357, Mar. 8, 2007]

U.S. Department of Health & Human Services

U.S. Food and Drug Administration

Food

Agency Response Letter GRAS Notice No. GRN 000165

CFSAN/Office of Food Additive Safety

November 9, 2005

Nancy Rachman, Ph.D.
Exponent, Inc.
1730 Rhode Island Ave., NW
Suite 1100
Washington, D.C. 20036

Re: GRAS Notice No. GRN 000165

Dear Dr. Rachman:

The Food and Drug Administration (FDA) is responding to the notice, dated March 4, 2005, that you submitted on behalf of the American Beverage Association (ABA) in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on March 8, 2005, filed it on March 10, 2005, and designated it as GRAS Notice No. GRN 000165.

The subject of the notice is Quillaia Extract Type 1 (Quillaia). The notice informs FDA of the view of ABA that Quillaia is GRAS, through scientific procedures, for use as a foaming agent in semi-frozen carbonated and non-carbonated beverages at levels not to exceed 500 milligrams/kilogram (dried basis) in beverage concentrate prior to the incorporation of water and carbon dioxide or air in retail establishments.

ABA describes the identity, composition, and method of manufacture of Quillaia. Quillaia is obtained by aqueous extraction of the milled inner bark or the wood of pruned stems and branches of Quillaia saponaria. ABA states that the extract is treated with a stabilizing agent such as polyvinylpyrrolidone (PVP) and then filtered through diatomaceous earth. Following filtration, the liquid is concentrated. The resulting concentrate may be sold as such or spray-dried and sold as a powder containing carriers such as lactose and maltodextrin. ABA notes that the liquid form of the product is reddish brown and the powdered form is light brown with a pinkish tinge. Quillaia is composed of triterpenoid saponins consisting of glycosides of quillaic acid. Other components include polyphenols, tannins, calcium oxalate, and sugars such as glucose, galactose, arabinose, xylose, and rhamnose.

As part of its notice, ABA provides specifications for Quillaia established by Joint Expert Committee on Food Additives (JECFA) and the European Commission. These specifications include limits for arsenic, lead, and mercury that may be present in Quillaia.

ABA notes that the safety of Quillaia has been evaluated by JECFA and provides a copy of JECFA's safety evaluation as part of the GRAS notice. JECFA evaluated the publicly available scientific literature and determined an acceptable daily intake of 0-5 milligrams/kilogram body weight/day (mg/kg bw/d). FDA has approved Quillaia extract as a food additive for use as a flavor in multiple food categories (21 CFR 172.510). In addition, ABA states that recent search of the publicly available scientific literature revealed no new information indicative of any adverse effects associated with the consumption of Quillaia.

ABA estimates the total daily intake of Quillaia from its proposed use as 1.4 mg/kg bw/d at the mean and 2.2 mg/kg bw/d at the 90th percentile level. As part of its evaluation of GRN 000165, FDA asked ABA to include the estimated daily intake of Quillaia for its current use as a flavoring agent. In an amendment dated May 31, 2005 and a revision to the May amendment dated June 17, 2005, ABA reported that the daily per capita intake of Quillaia from its uses in all food applications as 0.18 mg/kg bw/d as determined by the total poundage of Quillaia extract reported to have been used in food in 1995 per consumer.

Based on the information provided by ABA, as well as other information available to FDA, the agency has no questions at this time regarding ABA's conclusion that Quillaia is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of Quillaia. As always, it is the continuing responsibility of ABA to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the information in your notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and

copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Sincerely,

Laura Tarantino, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

Links on this page:

Summary of Data Supporting the Expanded Use of Quillaja Extracts, Q-Naturale® 100, Q-Naturale® 200 and Q-Naturale® 300 Emulsifiers, in Foods As Generally Recognized As Safe (GRAS) by Scientific Principles

- Final -

Prepared for: National Starch Food Innovation
10 Finderne Avenue
Bridgewater, NJ
08807

Prepared by: Cantox U.S. Inc., an Intertek company
1011 US Highway 22, Suite 200
Bridgewater, New Jersey, USA
08807-2950

July 6, 2011

EXPERT PANEL CONSENSUS STATEMENT REGARDING THE EXPANDED USE OF QUILLAJA EXTRACTS, Q-NATURALE® 100, Q-NATURALE® 200 AND Q-NATURALE® 300 EMULSIFIERS, IN FOODS AS GENERALLY RECOGNIZED AS SAFE (GRAS) BY SCIENTIFIC PRINCIPLES

EXPERT PANEL CONSENSUS STATEMENT REGARDING THE EXPANDED USE OF QUILLAJA EXTACTS, Q-NATURALE® 100, Q-NATURALE® 200 and Q-NATURALE® 300 EMULSIFIERS, IN FOODS AS GENERALLY RECOGNIZED AS SAFE (GRAS)

The undersigned, an independent Panel of recognized Experts (hereinafter referred to as the Expert Panel), qualified by scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was commissioned by National Starch Food Innovation (NSFI) to determine whether the expanded use of quillaja extracts, Q-Naturale® 100, Q-Naturale® 200 and Q-Naturale® 300 emulsifiers, in foods would entail a reasonable certainty of no harm and could be considered Generally Recognized as Safe (GRAS) by scientific procedures.

Quillaja extract is currently approved for use as a natural substance used in conjunction with flavors and as a foaming agent in semi-frozen carbonated and non-carbonated beverages and beverage bases. NSFI intends to market Q-Naturale® 100, Q-Naturale® 200 and Q-Naturale® 300 (refer to Table 1 for overview of product compositions), as an emulsifier and encapsulating agent for vitamins, nutrients, colorants, and clouding agents, for expanded food-uses including, alcoholic beverages, additional beverage and beverage bases, tea, processed fruits and fruit juices, and processed vegetables and vegetable juices. Q-Naturale® 100, Q-Naturale® 200 and Q-Naturale® 300 would be used in food at levels resulting in an estimated 90th percentile all user intake of 1.0 mg/kg body weight (bw)/day of saponins (6.7 mg/kg bw/day quillaja extract) from all current and proposed food-uses. In addition to use in conventional foods, NSFI will market Q-Naturale® 100, Q-Naturale® 200 and Q-Naturale® 300 for use in dietary supplement applications. In this capacity, the quillaja extracts, Q-Naturale® 100, Q-Naturale® 200 and Q-Naturale® 300, can be used for their food additive properties of emulsification and encapsulation in the preparation of dietary supplements at levels not to exceed Good Manufacturing Practice (GMP).

Table 1 Overview of the composition of Q-Naturale® products

Q-Naturale® 100	Q-Naturale® 200	Q-Naturale® 300
quillaja (containing not less than 14.94% saponins)	quillaja (containing not less than 14.94% saponins)	quillaja (containing not less than 14.94% saponins)
Preservative [sodium benzoate (0.1%)]	No preservative	No preservative
Does not meet organic standards	Does not meet organic standards	Meets organic standards

To assist the Panel in its determination, a dossier summarizing technical and safety information was provided.

The Panel noted a prior GRAS notice (GRAS Notice No. GRN 000165) for use of quillaja extract as a foaming agent in semi-frozen carbonated and non-carbonated beverages. As part of this notice, and subsequent amendments dated May 31, 2005 and June 17, 2005, the American Beverage Association (ABA) reported the daily per capita intake of quillaja from its uses in all

food applications as 0.18 mg/kg/body weight (bw)/day. FDA's response letter to GRAS Notice No. GRN 000165, dated November 9, 2005, stated that the FDA had no questions regarding ABA's conclusion that quillaja is GRAS under the intended conditions of use.

In addition, the Panel considered that quillaja extract is cited in 21 CFR Part 172.510 as a food additive permitted for direct addition to food for human consumption. It is listed under the common name of Quillaia (Soapbark) among multiple natural flavoring substances and natural substances used in conjunction with flavors, which may be safely used in food in accordance with the outlined conditions.

With respect to the safety of quillaja extract for human consumption the Panel assigned primary importance to GRAS Notice No. GRN 000165, which showed no unresolved safety issues and generated no questions from the FDA. The Panel also reviewed subchronic and chronic toxicity studies in rats and mice, and found no evidence of adverse effects.

Information describing the identity, composition, and method of manufacture of Q-Naturale® 100, Q-Naturale® 200 and Q-Naturale® 300 were carefully reviewed by the Expert Panel to ensure that the quality of the products meets standards for food production. Analytical data verified that the final Q-Naturale® products meet the specifications for quillaia extract (type I) established by Joint Expert Committee on Food Additives (JECFA) and conformed to applicable standards regarding residue limits.

Finally, the Panel acknowledged that the estimated 90th percentile all user intake of 1.0 mg/kg body weight (bw)/day of saponins (6.7 mg/kg bw/day quillaja extract) from all current and proposed food-uses is consistent with levels calculated by applying a conservative 100-fold safety factor to the NOAELs of 700 and 1500 mg/kg bw/day reported for the mouse (Phillips *et al.*, 1979) and rat (Drake *et al.*, 1982), respectively.

EXPERT PANEL CONSENSUS STATEMENT REGARDING THE EXPANDED USE OF QUILLAJA EXTACTS, Q-NATURALE® 100, Q-NATURALE® 200 and Q-NATURALE® 300 EMULSIFIERS, IN FOODS AS GENERALLY RECOGNIZED AS SAFE (GRAS)

After a critical independent evaluation of the available technical and safety information for quillaja extracts, Q-Naturale® 100, Q-Naturale® 200 and Q-Naturale® 300 emulsifiers, the undersigned members of the Expert Panel conferred and unanimously determined that Q-Naturale® 100, Q-Naturale® 200 and Q-Naturale® 300, meeting food-grade specifications, and produced according to current GMP, when used in newly proposed food-uses of alcoholic beverages, additional beverage and beverage bases, tea, processed fruits and fruit juices, and processed vegetables and vegetable juices at levels resulting in an estimated 90th percentile all user intake of 1.0 mg/kg body weight (bw)/day of saponins (6.7 mg/kg bw/day quillaja extract) from all current and proposed food-uses per day as well as in dietary supplements is considered Generally Recognized as Safe (GRAS).

[redacted] 7/20/11
Date

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Date

Adjunct Professor
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[redacted] 7/22/11
Date

Vice President & Senior Scientific Consultant
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CANADA

CONSOLIDATION

CODIFICATION

Food and Drug Regulations

Règlement sur les aliments et drogues

C.R.C., c. 870

C.R.C., ch. 870

Current to May 18, 2010

À jour au 18 mai 2010

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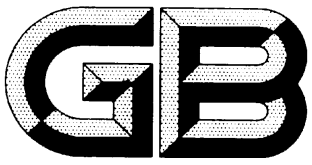
http://laws-lois.justice.gc.ca

Item No.	Column I Additive		Column II Permitted in or Upon		Column III Purpose of Use		Column IV Maximum Level of Use
P.4.1	Potassium Ferrocyanide		Wine		Fining agent		Good Manufacturing Practice
P.5	Potassium Stearate	(1)	Chewing gum	(1)	Plasticizing agent	(1)	Good Manufacturing Practice
		(2)	Emulsifying preparations containing propylene glycol monoesters	(2)	Stabilizing agent	(2)	2%
P.6	Propane		Unstandardized foods		Pressure dispensing and aerating agent		Good Manufacturing Practice
P.7	Propylene Glycol	(1)	Oil-soluble annatto	(1)	Solvent	(1)	Good Manufacturing Practice
		(2)	Unstandardized foods	(2)	Humectant	(2)	Good Manufacturing Practice
Q.1	Quillaia Extract		Beverage bases; Beverage mixes; Soft drinks		Foaming Agent		Good Manufacturing Practice
S.1	Saponin		Beverage bases; Beverage mixes; Soft drinks		Foaming agent		Good Manufacturing Practice
S.1.01	Silicon Dioxide		Edible vegetable oil-based cookware coating emulsions		Suspending agent		2.0% of preparation
S.1.1	Sodium Acid Pyrophosphate		Frozen fish fillets; frozen minced fish; frozen lobster; frozen crab; frozen clams; frozen shrimp		To reduce processing losses and to reduce thaw drip		Used in combination with sodium tripolyphosphate and sodium pyrophosphate tetrabasic, total added pyrophosphate not to exceed 0.5% calculated as sodium phosphate, dibasic
S.2	Sodium Aluminum Sulphate		Flour; Whole wheat flour		Carrier of benzoyl peroxide		900 p.p.m. in accordance with subparagraphs B.13.001(*e*)(vi) and B.13.005(*d*)(vi)
S.3	Sodium	(1)	Confectionery	(1)	Aerating agent	(1)	Good Manufacturing Practice
	Bicarbonate	(2)	Salt	(2)	To stabilize potassium iodide in salt	(2)	Good Manufacturing Practice
S.3A	Sodium Carbonate		In combination with sodium hexametophosphate for use on frozen fish fillets, frozen lobster, frozen crabs, frozen clams and frozen shrimp		To reduce thaw drip		15% of the combination of sodium carbonate and sodium hexametaphosphate



中华人民共和国国家标准

GB 2760—2011

食品安全国家标准

食品添加剂使用标准

2011-04-20 发布　　2011-06-20 实施

中华人民共和国卫生部　发布

表 B.2(续)

序号	编码	香料中文名称	香料英文名称	FEMA编号
157	N159	奶油发酵起子蒸馏物(黄油蒸馏物)	Butter starters distillate	2173
158	N160	卡南伽油	Cananga oil (*Cananga odorata* Hook. F. and Thoms)	2232
159	N161	月桂叶提起物/油树脂	Laurel leaves extract/oleoresin (*Laurus nobilis* L.)	2613
160	N162	生姜提取物(生姜浸膏)	Ginger extract (Ginger concrete.) (*Zingiber officinale*)	2521
161	N163	白栎木屑提取物	Oak chips extract (*Quercus alba* L.)	2794
162	N164	龙蒿油	Estragon oil (*Artemisia dracunculus* L.)	2412
163	N165	白樟油	Camphor oil, white (*Cinnamomum camphora* (L.) Presl)	2231
164	N166	肉豆蔻衣油	Mace oil (*Myristica fragrans* Houtt.)	2653
165	N167	众香叶油	Pimento leaf oil (*Pimenta officinalis* Lindl.)	2901
166	N168	西班牙鼠尾草油	Sage oil, Spanish (*Salvia lavandulaefolia* VahL.)	3003
167	N169	红桔油	Tangerine oil (*Citrus reticulata* Blanco)	3041
168	N170	杂薰衣草油	Lavandin oil (*Lavandula hydrida*)	2618
169	N171	杏仁油	Apricot Kernel oil (*Prunus armeniaca* L.)	2105
170	N172	苏合香油	Styrax oil (*Liquidambar* spp.)	—
171	N173	苏合香提取物	Styrax extract (*Liquidambar* spp.)	3037
172	N174	长角豆油	Locust bean oil (*Ceratonia siliqua* L.)	—
173	N175	角豆提取物	Carob bean extract (*Ceratonia siliqua* L.)	2243
174	N176	皂树皮提取物	Quillaia (*Quillaja saponaria* Molina)	2973
175	N177	乳香油	Olibanum oil (*Boswellia* spp.)	2816
176	N178	没药油	Myrrh oil (*Commiphora* spp.)	2766
177	N179	良姜根提取物	Galangal root extract (*Alpinia* spp.)	2499
178	N180	苏格兰松油	Pine oil, scotch (*Pinus sylvestris* L.)	2906
179	N181	小茴香油(普通小茴香油)	Fennel oil, (common) (*Foeniculum vulgare* Mill)	2481
180	N182	苦杏仁油	Almond oil, bitter (*Prunus amygdalus*)	2046
181	N183	阿魏油	Asafoetida oil (*Ferula asafoetida* L.)	2108
182	N184	金合欢净油	Cassie absolute (*Acacia farnesiana* (L.) Willd.)	2260

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The Japan Food Chemical Research Foundation

List of Existing Food Additives

Last update : 2011/05/09

List of Existing Food Additives

This list of food additives from natural origin is complied and published by the Ministry of Health and Welfare on April 16, 1996.

These additives are listed here in alphabetic order. The number preceding the name of each additive is the sequence number given to the corresponding additive in the original Japanese list.

Effective from May 6, 2011

No.	Name	Note
236	Absinth extract	A substance composed mainly of sesquiterpenes obtained from the whole absinth grass.
10	α-Acetolactate decarboxylase	–
146	Acid clay	–
147	Acid phosphatase	–
3	Actinidine	–
56	Activated acid clay	–
55	Active carbon	A substance obtained by carbonizing and activating carbon-containing substances.
5	Acylase	–
11	5'-Adenylic acid	–
2	Agarase	–
4	Agrobacterium succinoglycan	A substance composed mainly of succinoglycan obtained from the cultured solution of bacteria belonging to Agrobacteriurn.
17	L-Alanine	–
23	Alginate lyase	–
22	Alginic acid	–
24	Aluminium	–
196	Amino acid-sugar reaction product	A substance obtained by heating the mixture of amino acids and monosaccharides.
14	Aminopeptidase	–
15	alpha-Amylase	–
16	beta-Amylase	–
12	Annatto extract	A substance composed mainly of norbixin and bixin obtained from the seed coats of annatto.
25	Anthocyanase	–
19	Arabino galactan	–
20	L-Arabinose	–
21	L-Arginine	–
145	Artemisia sphaerocephala seed gum	A substance composed mainly of polysaccharides obtained from the seed coats of SABAKU-YOMOGI (*Artemisia sphaerocephala* KRASCH).
6	Ascorbate oxidase	–
7	L-Asparagine	–

305	Phospholipase	–
260	Phytase	–
261	Phytic acid	A substance composed mainly of inositol hexaphosphate obtained from rice bran or corn seeds.
262	Phytin (extract)	A substance ccomposed mainly of magnesium inositol hexaphosphate obtained from rice bran or corn seeds.
242	Platinum	–
309	ε-Polylysine	–
308	Polyphenol oxidase	–
195	Powdered bile	A substance composed mainly of cholic acid and desoxycholic acid obtained from bile.
280	Powdered cellulose	A substance composed mainly of cellulose obtained by decomposing pulp, excluding "Microcrystalline cellulose".
281	Powdered rice hulls	A substance composed mainly of cellulose obtained from rice hulls.
170	Powdered stevia	A substance composed mainly of steviol glycosides obtained by grinding stevia leaves.
278	L-Proline	–
275	Propane	–
276	Propoils extract	A substance composed mainly of flavonoids obtained from honeycomb.
274	Protease	–
143	Psyllium seed gum	A substance composed mainly of polysaccharides obtained from the seed coats of blond psyllium.
273	Pullulan	–
272	Pullulanase	–
323	Purple corn colour	A substance composed mainly of cyanidine-3-glucoside obtained from corn seeds.
322	Purple sweet potato colour	A substance composed mainly of cyanidine acylglucosides and peonidin acylglucosides obtained from the tuberous roots of sweet potatos.
324	Purple yam colour	A substance composed mainly of cyanidine acylglucosides obtained from yam tuberous roots.
95	Quercetin	–
173	Quicklime	–
88	Quillaia extract (Quillaja extract)	A substance composed mainly of saponins obtained from the bark of quillaia trees.
339	Rakanka extract	A substance composed mainly of mogulosides obtained from rakanka fruits (*Momordica grosvenori* SWINGLE).
85	Redbark cinchona extract	A substance composed mainly of quinidine, quinine and cinchonine obtained from the bark of redbark cinchona trees.
360	Rennet	–
139	Resin of depolymerized natural rubber	A substance composed mainly of diterpenes, triterpenes and tetraterpenes obtained from "rubber".
345	L-Rhamnose	–
344	Rhamsan gum	A substance composed mainly of polysaccharides obtained from the cultured solution of bacteria belonging to Alcaligenes.
351	D-Ribose	–
140	Rice bran oil extract	A substance composed mainly of ferulic acid obtained from rice bran oil.

PREVENTION OF FOOD ADULTERATION ACT, 1954

with

PREVENTION OF FOOD ADULTERATION RULES, 1955

with

FOOD SAFETY AND STANDARDS ACT, 2005

(BILL NO. 123 OF 2005)

and

Commodity Index

with

Exhaustive Short Notes

(Amended Uptodate)

22nd Edition, 2006

2006

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Price: Rs. 300.00

(1)	(2)	(3)	(4)
384.	252	Potassium nitrate	Preservative, colour fixative
385.	249	Potassium nitrite	Preservative, colour fixative
386.	922	Potassium persulphate	flour treatment agent
387.	340	Potassium phosphates	acidity regulator, Sequestrant, emulsifier, Texturizer, Stabilizer, water retention agent
388.	452 (ii)	Potassium polyphosphate	Emulsifier, Stabilizer, acidity regulator, raising agent, Sequestrant, water retention agent
389.	283	Potassium propionate	preservative
390.	560	Potassium silicate	anticaking agent
391.	337	Potassium sodium tartrate	Stabilizer, sequestrant
392.	202	Potassium sorbate	preservative
393.	515	Potassium sulphates	acidity regulator
394.	225	Potassium sulphite	Preservative, antioxidant
395.	336	Potassium tartrates	Stabilizer, sequestrant
396.	460 (ii)	Powdered cellulose	Emulsifier, anticaking agent, texturizer, dispersing agent
397.	407 a	Processed Euchema seaweed	Thickener, stabilizer
398.	944	Propane	Propellant
399.	280	Propionic acid	preservative
400.	310	Propyl gallate	antioxidant
401.	216	Propyl p-hydroxybenzoate	preservative
402.	1520	Propylene glycol	Humectant, wetting agent, dispersing agent
403.	405	Propylene glycol alginate	Thickener, emulsifier
404.	477	Propylene glycol esters of fatty acids	emulsifier
405.	1101 (i)	Protease	flour treatment agent, Stabilizer, tenderizer, flavour enhancer
406.	1101	Proteases	flour treatment agent, Stabilizer, tenderizer, flavour enhancer
407.	999	Quillaia extracts	foaming agent
408.	104	Quinoline yellow	colour
409.	128	Red 2G	colour

DISCLAIMER

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Director-General,
Agri-Food and Veterinary Services

Front Page [Jump to: Front Page / Arrangement of Provisions / Actual Provisions]

SALE OF FOOD ACT

(CHAPTER 283, SECTION 56 (1))

FOOD REGULATIONS

History	G.N. No. S 264/88	->	1990 REVISEDEDITION	->	RG1 2005 REVISED EDITION

[1st October 1988]

Arrangement of Provisions [Jump to: Front Page / Arrangement of Provisions / Actual Provisions]

"expiry date" , in relation to a prepacked food, means the date after which the food, when kept in accordance with any storage conditions set out on the label of that food, may not retain its normal wholesomeness, nature, substance and quality;

"food additive" includes —

(a) all substances, which are components of food, the intended use of which results or may reasonably be expected to result, directly or indirectly, in their affecting the characteristics of food but does not include any foreign substance mixed with food as a result of contamination, or improper handling of the food during the preparation, processing, packing or storage of the food; and

(b) anti-caking agents, anti-foaming agents, anti-oxidants, artificial sweetening agents, chemical preservatives, colouring matters, emulsifiers or stabilizers, flavouring agents, flavour enhancers, humectants, nutrient supplements, sequestrants and other general purpose food additives;

"infant" means a person not more than 12 months of age;

"package" includes every means by which food may be cased, enclosed, contained or packed;

"prepacked" means packed or made up in advance ready for sale in a wrapper or container, and where any food packed or made up in a wrapper or container is found on any premises where such food is packed, kept or stored for sale, the food shall be deemed to be prepacked unless the contrary is proved, and it shall not be sufficient proof of the contrary to show that the food had not been labelled in accordance with the provisions of these Regulations;

"premises" means a building or part thereof and any forecourt, yard or place of storage used in connection with a building or part thereof and includes, in relation to dairies and farms, any land other than building.

(2) In these Regulations, the symbols specified in the first column of the following table shall have the meanings specified in relation to those symbols in the second column of the table:

First column	*Second column*
Symbol	*Meaning*
C	degrees in Celsius scale of temperature
cm	centimetres
g	grams
i.u	international units
kcal	kilocalories
kg	kilograms
kJ	kilojoules
mcg	micrograms
mg	milligrams
ml	millilitres
mm	millimetres
ppm	parts per million
%	per cent
sq dm	square decimetres

and solely for the external colouring of dragees and the decoration of sugar-coated flour confectionery, silver or aluminium in leaf or powder form.

4. The aluminium or calcium salts (lakes) of any of the scheduled water-soluble colours.

SIXTH SCHEDULE

Regulation 21 (2)

PERMITTED EMULSIFIERS AND PERMITTED STABILISERS

Acetylated mono-glycerides; lactated mono-diglycerides; tartaric acid glycerides; diacetyl tartaric acid glycerides; citric acid glycerides;

Agar;

Alginic acid; ammonium alginate; calcium alginate; potassium alginate; sodium alginate;

Carrageenan;

Caseinate, sodium and calcium;

Cellulose, methyl, ethyl, methyl ethyl, hydroxy propyl and hydroxy propyl methyl derivatives of; carboxy methyl cellulose; croscarmellose sodium;

Dioctyl sodium sulphosuccinate;

Furcelleran;

Gums, acacia, carob, gellan, ghatti, guar, karaya, tragacanth, and xanthan;

Konjac flour;

Lecithin;

Mono and diglycerides of fatty acids;

Pectin, calcium pectate; sodium pectate;

Polyglycerol esters of fatty acids;

Polyoxyethylene (20) sorbitan monolaurate (polysorbate 20);

Polyoxyethylene (20) sorbitan mono-palmitate (polysorbate 40);

Polyoxyethylene (20) sorbitan monostearate (polysorbate 60);

Polyoxyethylene (20) sorbitan mono-oleate (polysorbate 80);

Polyoxyethylene (20) sorbitan tristearate (polysorbate 65);

Propylene glycol esters of fatty acids; propylene glycol alginate;

Quillaia (only in soft drinks, not exceeding 200 parts per million);

Starches, bleached (with chlorite, hypochlorite, hydrogen peroxide, or peracetic acid) and hypochlorite-oxidised; di-starch phosphate prepared using sodium triphosphate, di-starch phosphate prepared using phosphorus oxychloride; phosphated di-starch phosphate; starch acetates; acetylated di-starch glycerol; acetylated di-starch adipate; acetylated di-starch phosphate, starches octenyl succinic anhydride modified; hydroxypropyl distarch phosphate;

Stearoyl-2-lactylic acid and its sodium and calcium salts; Stearyl tartrate;

Sorbitan monostearate; sorbitan tristearate; sorbitan mono-palmitate; sorbitan monolaurate; sorbitan mono-oleate.

SEVENTH SCHEDULE

Regulation 25 (2)

PERMITTED NUTRIENT SUPPLEMENT

Ascorbic acid;

Biotin;

Calcium carbonate;

Calcium citrate;

Calcium glycerophosphate;

Calcium oxide;

Calcium pantothenate;

Calcium phosphate (mono-, di- and tri-basic);

Calcium pyrophosphate;

Calcium sulphate;

Beta-carotene;

Choline bitartrate;

Choline chloride;

Ferric ammonium citrate;

Ferric phosphate;

Ferric pyrophosphate;

Ferrous gluconate;

Scope and Application Standards of Food Additives

〔1. Preservatives / 2. Sanitizing agents / 3. Antioxidants〕〔4. Bleaching agents / 5. Color fasting agents / 6. Leavening agents〕〔7. Food quality improvement, fermentation and food processing agents〕〔8. Nutritional additives〕〔9. Colors / 10. Flavoring agents〕〔11. Seasoning agents / 12. Pasting agents〕〔13. Coagulating agents / 14. Chemicals for Food Industry / 15. Solvents〕〔16. Emulsifiers / 17. Others〕

16. Emulsifiers

Promulgation Date	Code	Food Additive Items	Scope and Application Standards	Limitations
1999.10.21	16001	Glycerin Fatty Acid Ester（Mono- and Diglycerides）	All foods: as practically needed.	
1987.07.22.	16002	Sucrose Fatty Acid Ester	All foods: as practically needed.	
1999.10.21	16003	Sorbitan Fatty Acid Ester	All foods: as practically needed.	
1987.07.22.	16005	Propylene Glycol Fatty Acid Ester	All foods: as practically needed.	
1999.10.21	16006	Diacetyl Tartaric Acid Esters of Mono- and Diglycerides（DATEM）	All foods: as practically needed.	
1987.07.22.	16007	Sodium Aluminum Phosphate, Basic	All foods: as practically needed.	
1999.10.21	16008	Polysorbate 20	All foods: as practically needed.	
1999.10.21	16009	Polysorbate 60	All foods: as practically needed.	
1999.10.21	16010	Polysorbate 65	All foods: as practically needed.	
1999.10.21	16011	Polysorbate 80	All foods: as practically needed.	

1987.07.22	16012 Hydroxypropyl Cellulose	All foods: as practically needed.
1987.07.22	16013 Hydroxypropyl Methylcellulose (Propylene Glycol Ether of Methylcellulose)	All foods: as practically needed.
1999.10.21	16014 Mono- and Diglycerides, Citrated	All foods: as practically needed.
1999.10.21	16015 Mono- and Diglycerides, Tartrated	All foods: as practically needed.
1999.10.21	16016 Mono- and Diglycerides, Lactated	All foods: as practically needed.
1999.10.21	16017 Mono- and Diglycerides, Ethoxylated	All foods: as practically needed.
1999.10.21	16018 Mono- and Diglycerides, Monosodium Phosphate Derivatives	All foods: as practically needed.
1999.10.21	16019 Succinylated Monoglycerides （SMG）	All foods: as practically needed.
1999.10.21	16020 Polyglycerol Esters of Fatty Acids	All foods: as practically needed.
1999.10.21	16021 Polyglycerol Esters of Interesterified Ricinoleic Acids	All foods: as practically needed.
1999.10.21	16022 Sodium Stearyl-2-Lactylate （SSL）	All foods: as practically needed.
1999.10.21	16023 Calcium Stearyl-2-Lactylate （CSL）	All foods: as practically needed.
1999.10.21	16024 Salts of Fatty Acids	All foods: as practically needed.
1999.10.21	16025 Polyoxyethylene （20） Sorbitan Monopalmitate; Polysorbate 40	All foods: as practically needed.
1999.10.21	16026 Polyoxyethylene （20） Sorbitan Monostearate	All foods: as practically needed.
1999.10.21	16027 Polyoxyethylene （20） Sorbitan Tristearate	All foods: as practically needed.

17. Others

Promulgation Date	Code	Food Additive Items	Scope and Application Standards	Limitations
1987.07.22.	17001	Piperonyl Butoxide	Grains, beans: not more than 0.024 g/kg.	For insects control purpose.
1987.08.13.	17002	Polyvinyl Acetate	Peel coating for vegetable width="100%"s and fruits: as practically needed.	
1987.07.22.	17003	Silicon Resin	All foods: not more than 0.05 g/kg.	For defoaming purpose.
1987.07.22.	17005	Diatomaceous Earth	All foods: not more than 5 g/kg.	For adsorption or filtration purpose in food processing.
1987.07.22.	17006	Enzyme Product	All foods: as practically needed.	For manufacturing or processing purpose.
1987.08.13.	17007	Sodium Oleate	Peel coating for vegetable width="100%"s and fruits: as practically needed.	
1987.08.13.	17008	Oxyethylene Higher Aliphatic Alcohol	Peel coating for vegetable width="100%"s and fruits: as practically needed.	
1987.07.22.	17009	Shellac	All foods: as practically needed.	For manufacturing or processing purpose.
1987.07.22.	17010	Petroleum Wax	Chewing gums, bubble gums, fruits, vegetable width="100%"s, cheeses, egg shells: as practically needed.	For protecting film purpose.
1987.07.22.	17011	Petroleum Wax, Synthetic	Chewing gums, bubble gums, fruits, vegetable width="100%"s, cheeses, egg shells: as practically needed.	For protecting film purpose.
1997.05.26	17012	Liquid Paraffin（Mineral Oil）	All foods: not more than 0.15% .	For manufacturing or processing purpose.

1997.11.18	17013	Polyethylene Glycol 200-9500	table width="100%"t and capsule formed foods: as practically needed.	For manufacturing or processing purpose.
2007.01.08	17014	(Polygalloyl glucose, Tannic Acid)	Non-alcoholic beverages: not more than 0.005%.	For filtering aid purpose.
2002.05.06.	17015	Quillaia Extracts	Water-based flavoured drinks: no more than 0.2 g/kg or 0.2 g/l.	
2005.07.07	17016	Polyvinyl alcohol	Film coating for table width="100%"t formed foods: not more than 1.8 %.	
2008.03.12	17017	Magnesium silicate (synthetic)	Fats and oils filtering: not more than 2 %.	For filtering aid and anticaking agent purpose.

BỘ Y TẾ	CỘNG HOÀ XÃ HỘI CHỦ NGHĨA VIỆT NAM
Sè: **3742** /2001/Q§-BYT	**Độc lập - Tự do - Hạnh phúc**

Hà Nội, ngày 31 tháng 8 năm 2001

QUYẾT ĐỊNH CỦA BỘ TRƯỞNG BỘ Y TẾ
Về việc ban hành " Quy định danh mục các chất phụ gia được phép sử dụng trong thực phẩm"

BỘ TRƯỞNG BỘ Y TẾ

- Căn cứ Luật Bảo vệ sức khỏe nhân dân ngày 30/6/1989 và Điều lệ Vệ sinh ban hành kèm theo Quyết định số 23-HĐBT ngày 24/01/1991 của Hội đồng Bộ trưởng (nay là Chính phủ);
- Căn cứ Nghị định số 68/ CP ngày 11/10/1993 của Chính phủ quy định chức năng, nhiệm vụ, quyền hạn và tổ chức bộ máy của Bộ Y tế;
- Căn cứ Nghị định số 86/CP ngày 08/12/1995 của Chính phủ về việc phân công trách nhiệm quản lý nhà nước đối với chất lượng hàng hóa;
- Theo đề nghị của Cục trưởng Cục Quản lý chất lượng vệ sinh an toàn thực phẩm và Vụ trưởng Vụ Khoa học và Đào tạo - Bộ Y tế,

QUYẾT ĐỊNH

Điều 1. Ban hành kèm theo Quyết định này **"Quy định Danh mục các chất phụ gia được phép sử dụng trong thực phẩm"**.

Điều 2. Quyết định này có hiệu lực sau 15 ngày kể từ ngày ký ban hành và thay thế Mục I phần phụ gia thực phẩm của "Danh mục tiêu chuẩn vệ sinh đối với lương thực, thực phẩm" ban hành kèm theo Quyết định số 867/1998/QĐ-BYT ngày 04/4/1998 của Bộ trưởng Bộ Y tế.

Điều 3. Cục trưởng Cục Quản lý chất lượng vệ sinh an toàn thực phẩm có trách nhiệm tổ chức, chỉ đạo, hướng dẫn triển khai và kiểm tra việc thực hiện Quyết định này.

Điều 4. Các Ông, Bà: Chánh Văn phòng, Chánh Thanh tra, Vụ trưởng các Vụ: Pháp chế, Khoa học và Đào tạo; Cục trưởng Cục Quản lý chất lượng vệ sinh an toàn thực phẩm - Bộ Y tế, Giám đốc Sở Y tế tỉnh, thành phố trực thuộc Trung ương, Thủ trưởng các đơn vị trực thuộc Bộ Y tế, Thủ trưởng y tế ngành chịu trách nhiệm thi hành Quyết định này.

KT. BỘ TRƯỞNG BỘ Y TẾ
THỨ TRƯỞNG

Lê Văn Truyền

Số thứ tự phụ gia **70**

Tên tiếng Việt	:	**Chất chiết xuất từ Annatto** (*)	**INS:**	**160b**
Tên tiếng Anh	:	*Annatto Extracts*	**ADI:**	**0-0,065**
Chức năng	:	Phẩm màu		

STT	Nhóm thực phẩm	ML	Ghi chú
1.	Đồ uống có sữa, có hương liệu hoặc lên men (VD: sữa sô cô la, sữa cacao, bia trứng, sữa chua uống, sữa đặc)	50	8
2.	Sữa lên men (nguyên kem), không xử lý nhiệt sau lên men	50	
3.	Các sản phẩm tương tự phomát	70	74
4.	Thức ăn tráng miệng có sữa (VD: kem, sữa lạnh, bánh putđing, sữa chua hoa quả hoặc có hương liệu...)	100	
5.	Mứt, mứt cô đặc, mứt hoa quả	200	
6.	Cacao, sô cô la và các sản phẩm tương tự	25	9
7.	Kẹo cứng, kẹo mềm, kẹo nuga...	25	9
8.	Sản phẩm dùng để trang trí thực phẩm	30	9
9.	Bánh có sữa, trứng	15	9
10.	Sản phẩm thịt, thịt gia cầm và thịt thú lên men, xay nhỏ chưa xử lý nhiệt	50	9
11.	Thủy sản, sản phẩm thủy sản xay nhỏ đông lạnh, kể cả nhuyễn thể, giáp xác, da gai	30	9
12.	Thủy sản, sản phẩm thủy sản hun khói, sấy khô, lên men hoặc ướp muối, kể cả nhuyễn thể, giáp xác, da gai	15	9,22
13.	Nước chấm dạng sữa (VD: nước sốt mayonne, nước sốt salát)	100	8
14.	Nước chấm không có sữa (VD: tương cà chua, tương ớt, nước chấm có kem)	100	8
15.	Nước quả ép thanh trùng pasteur đóng hộp hoặc đóng chai	GMP	
16.	Necta quả thanh trùng pasteur đóng hộp hoặc đóng chai	GMP	
17.	Nước giải khát có hương liệu, bao gồm cả nước uống dành cho thể thao, nước uống có hàm lượng khoáng cao và các loại nước uống khác	50	
18.	Bia và nước giải khát chế biến từ mạch nha	GMP	96
19.	Rượu vang	GMP	

Chú thích (*)

Loại chất màu : Phẩm màu tự nhiên **Chỉ số màu** : C.I. (1975) No. 75120

Nhóm chất màu : Họ Carotene **Tên khác** :

Số thứ tự phụ gia **71**

Tên tiếng Việt	:	**Chất chiết xuất từ Quillaia**	**INS:**	**999**
Tên tiếng Anh	:	*Quillaia Extracts*	**ADI:**	**0-5**
Chức năng	:	Tạo bọt		

STT	Nhóm thực phẩm	ML	Ghi chú
1.	Nước giải khát có hương liệu, bao gồm cả nước uống dành cho thể thao, nước uống có hàm lượng khoáng cao và các loại nước uống khác	1500	

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)". A group ADI of 0-1 mg quillaia saponins /kg bw for Quillaia ExtractsTypes 1 & 2 was established at 65th JECFA (2005)

SYNONYMS Quillaja extract, Soapbark extract, Quillay bark extract, Bois de Panama, Panama bark extract, Quillai extract; INS No. 999(i)

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 (Vol. 4) 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 (Vol. 4)	Powder form: not more than 6% (Karl Fischer Method)
Loss on drying (Vol. 4)	Liquid form: 50 to 80% (2 g, 105°, 5 h)
pH (Vol. 4)	3.7 -5.5 (4 % solution)
Ash (Vol. 4)	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)
Tannins	Not more than 8% on a dried basis See description under TESTS
Lead (Vol. 4)	Not more 2 mg/kg. Determine using an atomic absorption technique appropriate to the specified level. The selection of the sample size and method of sample preparation may be based on the principles of the method described in FNP 5, "Instrumental Methods".

TESTS

PURITY TESTS

Tannins

Weigh either 3.0 g of the powder form or an equivalent amount of liquid sample, accounting for solids content determined from loss on drying. Dissolve in 250 ml of water. Adjust the pH to 3.5 with acetic acid. Dry 25 ml of this solution at 105° for 5 h and determine the weight of the dried solid, in g (W_i). Mix 50 ml of the solution with 360 mg of polyvinyl polypyrrolidone. Stir the solution for 30 min at room temperature; then centrifuge at 800 × g. Recover the supernatant and dry this solution at 105° (5 h).Weigh the recovered solid (W_f, in g). The percentage of tannins in the sample is:

$$\% \text{ tannins (dried basis)} = 100 \times (W_i - W_f/2) / W_i$$

METHOD OF ASSAY

Principle:

The saponins QS-7, QS-17, QS-18 and QS-21 are separated by reversed phase HPLC and their quantitation is used as an indicator for total saponins levels in Quillaia extract (Type 1).

Sample preparation:

Powders: Weigh 0.5 g of sample and dissolve in 9.5 g of water. Filter through a 0.2 µm filter.

Aqueous extracts (~ 550 mg solids/ml): Weigh 1 g of sample and dilute with 9 g of water. Filter through a 0.2 µm filter.

In either case, the sample volume is ca. 10 ml.

Standard preparation:

Weigh 1.5 g of purified saponins (SuperSap, Natural Response, Chile; Quil-A, Superfos, Denmark or similar, containing a known saponin content) and dissolve in 100 ml of water. Filter through a 0.2 µm filter.

High performance liquid chromatography (HPLC):

HPLC conditions:

Column: Vydac 214TP54 (4.6 x 250 mm length, 5 µm pore) or equivalent
Column temperature: room temperature
Pump: gradient
Solvent A: 0.15% trifluoroacetic acid in HPLC-grade water.
Solvent B: 0.15% trifluoroacetic acid in HPLC-grade acetonitrile.
Gradient:

Time(min)	% solvent A	% solvent B
0	70	30
40	55	45
45	70	30

Flow rate: 1 ml/min
Detection wavelength: 220 nm
Injection volume: 20 µl

Calculation:

The concentration of saponins, C_{sap}, in mg/ml, in the solution prepared as directed under sample preparation is:

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

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.)

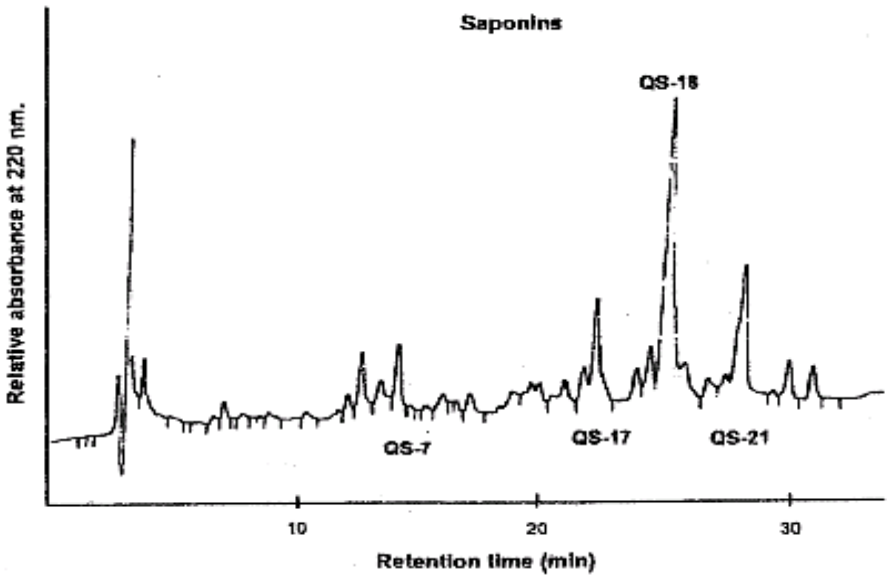
The percentage of saponins in the test sample is:

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

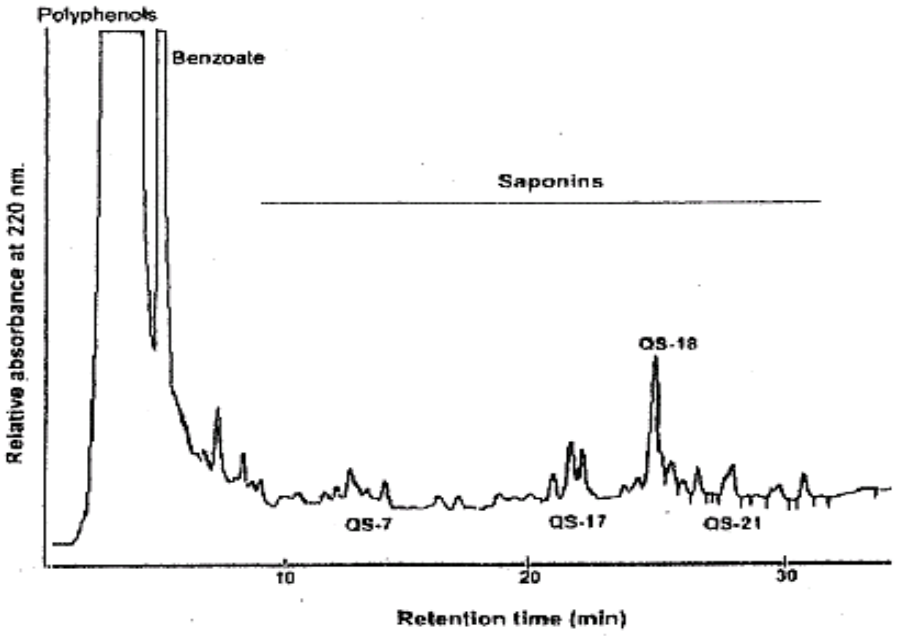
where W_{sample} is the weight of the sample (mg) taken for the sample preparation and 0.1 is the inverse of the sample volume, 10 ml.

Appendix

Chromatogram of Standard (15 mg solids/ml equivalent to 13.5 mg saponins/ml).



Chromatogram of Quillaia extract (Type 1) (55 mg solids/ml)



QUILLAIA EXTRACT (TYPE 2)

Revised specifications prepared at the 65th JECFA and published in FNP 52 Add 13 (2005), superseding specification prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). A group ADI of 0-1 mg quillaia saponins /kg bw for Quillaia ExtractsTypes 1 & 2 was established at 65th JECFA (2005)

SYNONYMS Quillaja extract, Soapbark extract, Quillay bark extract, Bois de Panama, Panama bark extract, Quillai extract; INS No. 999(ii)

DEFINITION Quillaia extract (Type 2) is obtained either by chromatographic separation or ultrafiltration of the aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaja saponaria* Molina (family *Rosaceae*). It contains triterpenoid saponins (quillaia saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are minor components. Some sugars and calcium oxalate will also be present.

Quillaia extract (Type 2) is available commercially as a liquid product or as a spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol.

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 65 % and not more than 90 % on the dried basis

DESCRIPTION Light red-brownish liquid or powder

FUNCTIONAL USES Emulsifier, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water, insoluble in ethanol, acetone, methanol, and butanol

Foam Dissolve 0.5 g of the powder form in 9.5 ml of water or 1 ml of the liquid form in 9 ml of water. Add 1 ml of this solution to 350 ml of water in a 1000-ml graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam volume (ml) after 30 min. Typical volumes are about 260 ml.

Chromatography Determine as in METHOD OF ASSAY. The retention time of major sample peak corresponds to the major saponin peak (QS-18) of the standard.

Colour and turbidity Powder form only: Dissolve 0.5 g in 9.5 ml of water. The solution shall not turbid Determine the absorbance of the solution against water at 520 nm. The absorbance shall be less than 0.7.

PURITY

Water (Vol. 4) Powder form: not more than 6% (Karl Fischer Method)

Loss on drying (Vol. 4)	Liquid form: 50 to 80% (2 g, 105°, 5 h)
pH (Vol. 4)	3.7 -5.5 (4 % solution)
Ash (Vol. 4)	Not more than 5% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from Loss on drying)
Tannins	Not more than 8% on a dried basis See description under TESTS
Lead (Vol. 4)	Not more 2 mg/kg. Determine using an atomic absorption technique appropriate to the specified level. The selection of the sample size and method of sample preparation may be based on the principles of the method described in FNP 5, "Instrumental Methods".

TESTS

PURITY TESTS

Tannins

Weigh either 3.0 g of the powder form or an equivalent amount of liquid sample, accounting for solids content determined from loss on drying. Dissolve in 250 ml of water. Adjust the pH to 3.5 with acetic acid. Dry 25 ml of this solution at 105° for 5 h and determine the weight of the dried solid, in g (W_i). Mix 50 ml of the solution with 360 mg of polyvinyl polypyrrolidone. Stir the solution for 30 min at room temperature; then centrifuge at 800 × g. Recover the supernatant and dry this solution at 105° (5 h).Weigh the recovered solid (W_f, in g). The percentage of tannins in the sample is:

$$\% \text{ tannins (dried basis)} = 100 \times (W_i - W_f/2) / W_i$$

METHOD OF ASSAY

Principle:
The saponins QS-7, QS-17, QS-18 and QS-21 are separated by reversed phase HPLC and their quantitation is used as an indicator for total saponins levels in Quillaia extract (Type 2).

Sample preparation:
Powders: Weigh 0.5 g of sample and dissolve in 9.5 ml of water. Filter through a 0.2 µm filter.
Aqueous extracts (~ 550 mg solids/ml): Weigh 1 g of sample and dilute with 9 ml of water. Filter through a 0.2 µm filter.
In each case, the sample volume is ca. 10 ml.

Standard preparation:
Weigh 1.5 g of purified saponins (SuperSap, Natural Response, Chile; Quil-A, Superfos, Denmark or similar, containing a known saponin content) and dissolve in 100 ml of water. Filter through a 0.2 µm filter.

High performance liquid chromatography (HPLC):
HPLC conditions:
Column: Vydac 214TP54 (4.6 x 250 mm length, 5 µm particle size) or equivalent
Column temperature: Room temperature
Pump: Gradient
Solvent A: 0.15% trifluoroacetic acid in HPLC-grade water.
Solvent B: 0.15% trifluoroacetic acid in HPLC-grade acetonitrile.

Gradient:

Time(min)	% solvent A	% solvent B
0	70	30
40	55	45
45	70	30

Flow rate: 1 ml/min
Detection wavelength: 220 nm
Injection volume: 20 µl

Calculation:
The concentration of saponins, C_{sap}, in mg/ml, in the solution prepared as directed under sample preparation is:

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

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 corresponding to the saponins will appear after the major peak corresponding to the polyphenols)

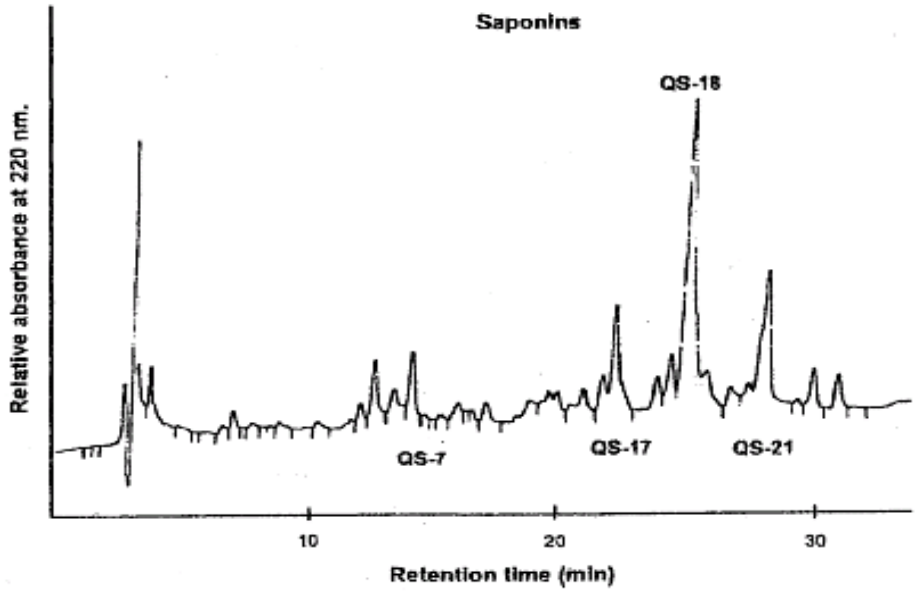
The percentage of saponins in the test sample is:

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

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.

Appendix

Chromatogram of Standard (15 mg solids/ml equivalent to 13.5 mg saponins/ml).



This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to prepare specifications for the identity and purity of food additives.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents), assessments of intake, and the establishment and revision of specifications for food additives.

A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (Beeswax, Candelilla wax, Calcium L-5-Methyltetrahydrofolic acid (L-5-MTHF), Phospholipase A1 from *Fusarium venenatum* expressed in *Aspergillus oryzae*, Pullulan, Quillaia extract Type 1, Quillaia extract Type 2) and seven groups of flavouring agents. Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, changes in the status of specifications, and further information requested or desired.

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EVALUATION OF CERTAIN FOOD ADDITIVES

WHO Technical Report Series – 934

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934

EVALUATION OF CERTAIN FOOD ADDITIVES

Sixty-fifth report of the joint FAO/WHO Expert Committee on food additives

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The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

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Joint FAO/WHO Expert Committee on Food Additives

Geneva, 7–16 June 2005

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Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:
Safety evaluation of certain food additives. **WHO Food Additive Series, No. 56, in preparation.**

Specifications are issued separately by FAO under the title:
Compendium of food additive specifications, Addendum 13. **FAO Food and Nutrition Paper, No. 52, Add. 13.**

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the Member States that contribute to the work of the International Programme on Chemical Safety (IPCS).

The IPCS is a joint venture of the United Nations Environment Programme, the International Labour Organization and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

Assessment of dietary exposure

Pullulan is used as a substitute for gelatin in the production of capsule shells, as an ingredient of coated tablets and in edible, flavoured films (breath fresheners). The amount of pullulan ingested from one unit of each of these products is 135 mg per capsule, 30 mg per tablet and 29 mg per film.

Data on consumption of food supplements were available from France and the United Kingdom. For consumers at the 97.5th percentile, intake of seven capsules per day was reported to correspond to a dietary exposure to pullulan of 950 mg/day. As dietary supplements for children are usually formulated as tablets, the consumption of pullulan by children was estimated to be lower than that of adults and typically not to exceed 90 mg/day on the basis of intake of three tablets per day, as reported in the United Kingdom. If regular maximum daily consumption of seven capsules (950 mg/day of pullulan) and of one standard packet of breath-freshening films (700 mg/day of pullulan) is assumed, the maximum daily exposure would be 1.65 g.

Pullulan is used in Japan in various foodstuffs, at levels ranging from 2 g/kg in ham and sausages to 30 g/kg in various processed products; use of 50 g/kg was reported in hard sweets. A conservative estimate of dietary exposure from various foods by the budget method, assuming the presence of pullulan at the maximum reported level in a limited fraction of the diet (30 g/kg in 1/16 of the diet, corresponding to 187 g/day), resulted in a value of about 6 g/day. Consumption of sweets by children was considered separately, with consumption figures for France and the USA, resulting in an estimate of about 2.5 g/day.

The Committee recognized that the conservative estimates should not be summed.

Evaluation

The Committee concluded that the current uses of pullulan as a food additive and the studies on its safety provided sufficient information to allocate an ADI ‘not specified’.

A toxicological monograph, new specifications and a chemical and technical assessment were prepared.

3.1.6 ***Quillaia extract type 2***

Explanation

Quillaia extracts (synonyms: quillaja extracts, bois de Panama, Panama bark extracts, quillaia extracts, Quillay bark extracts, soapbark extracts; CAS

No. 68990-67-0, INS No. 999) are obtained by aqueous extraction of the milled inner bark or whole wood of *Quillaja saponaria* Molina (family Rosaceae), which is a large evergreen tree with shiny, leathery leaves and a thick bark, native to China and several South American countries, particularly Bolivia, Chile and Peru. Quillaia extracts (types 1 and 2) are used in foods, primarily for their foaming and emulsifying properties, which are attributed to their saponin content.

Quillaia extracts were considered previously by the Committee, at its twenty-sixth, twenty-ninth, fifty-seventh and sixty-first meetings (Annex 1, references *59*, *70*, *154* and *166*) At its fifty-seventh meeting, the Committee assessed all relevant information on toxicity and dietary exposure, as requested by the Codex Committee on Food Additives and Contaminants at its Thirty-second Session (*14*). At that time, the Committee gave temporary status to the ADI of 0–5 mg/kg bw allocated previously to the unpurified extract, pending further clarification of the specifications. The existing specifications were revised and designated 'tentative'.

At its sixty-first meeting, the Committee reviewed new information relating to the chemical characterization of quillaia extracts and further information for specifications. The Committee agreed that separate specifications were needed for the two forms of quillaia extract, type 1 ('unpurified') and type 2 ('semi-purified'). Specifications for the total saponin content of type-1 extract were set at 20–26% (dried weight basis), and the Committee agreed that, while there was some variation in saponin content, the material tested toxicologically was representative of the material specified as type-1 extract. The 'temporary' assignment to the ADI of 0–5 mg/kg bw was therefore removed. Specifications for the total saponin content of type-2 extract after a concentration step of ultra-filtration or chromatography were set at 75–90% (dried weight basis). The saponin profile of the type-2 extract when prepared by membrane ultrafiltration was similar to that of the extract assayed according to the specifications. No information was available on the saponin profile of the extract prepared by chromatography. Limited information was available on the composition of the non-saponin fraction. Although the Committee established separate specifications for type-1 and type-2 quillaia extracts, it was unable to establish an ADI for the type-2 extract owing to the limited information on the qualitative and quantitative composition of this extract. A decision about which additional toxicological studies were needed on the type-2 extract remained suspended in the absence of further data on composition.

Further evaluation of quillaia extract type 2 was requested by the Codex Committee on Food Additives and Contaminants at its Thirty-sixth Session (*14*). At its present meeting, the Committee considered additional information

on the production and composition of type-2 extract prepared by membrane ultrafiltration in Chile and by chromatography in Japan. The Committee also considered data on the acute toxicity in rats of preparations of quillaia type-1 and type-2 extracts.

Chemical and technical considerations

Quillaia extracts (types 1 and 2) can contain over 100 tri-terpenoid saponins, the glycosides of the aglycone quillaic acid. Other constituents are polyphenols, tannins, oxalate salts, simple sugars and trace amounts of fats and nitrogen compounds. Quillaia extract type 2 is derived from type 1 extract subjected to chromatography or ultrafiltration to reduce the amount of non-saponin soluble solids, such as polyphenols and tannins. Type 2 extract is used in food, primarily for its foaming and emulsifying properties, which are attributed to the saponin content. The chemical compositon and manufacture of type-1 extract were discussed by the Committee at its sixty-first meeting.

Chromatographic analysis indicated that the saponin profile of type-2 extract prepared by membrane ultrafiltration or chromatography is similar to that of type-1 extract. The new information indicated that the range of saponin contents is broader than established in the specifications at the sixty-first meeting; therefore, the specification for total saponin content of type-2 extract was revised to 65–90% on a dried basis at the present meeting.

Toxicological data

The Committee considered data on the acute oral toxicity in rats of two preparations of quillaia extract from Chile: a type-2 extract prepared by ultrafiltration and a type-1 extract. Groups of five Sprague-Dawley rats of each sex were given single oral doses ranging from 3000 to 20 000 mg/kg bw and were observed for clinical signs of toxicity for 14 days. The LD_{50} for the type-2 extract was 6600 mg/kg bw; when expressed in relation to the 14% saponin content of the standardized test material (72% on dry matter basis), the LD_{50} was 900 mg/kg bw. The LD_{50} for the type-1 extract was 11 400 mg/kg bw; when expressed in relation to the 8.8% saponin content of the standardized test material (20% w/w on dry matter basis), the LD_{50} was 1000 mg/kg bw. On the basis of the saponin content, the LD_{50}s for the two extracts were the same: about 900 mg/kg bw. No other new studies of toxicity were available.

Assessment of dietary exposure

Quillaia extracts are used as foaming agents in soft drinks and cocktail mixes and as emulsifiers in foods such as baked goods, sweets, frozen dairy products, gelatine and puddings. Their major food use is in soft drinks. If a

similar technological function based on saponin content and the interchangeability of type-1 and type-2 extracts are assumed, the estimated dietary exposure to saponins from type-1 and type-2 extracts would be the same.

Evaluation

The previous requirement of the Committee for information on the qualitative and quantitative composition of the type-2 extract was considered to have been met, and the existing specifications for type-2 extract were revised. On the basis of the new information on composition, the Committee assessed the need for additional toxicological studies on this extract, as it had decided at its previous meeting, and concluded that no additional studies were necessary.

The Committee noted that there was no difference between type-1 and type-2 extracts with respect to acute toxicity when expressed in relation to the quillaia saponin content of the extracts.

On the basis of the conclusion of the Committee at its previous meeting that the material tested toxicologically was representative of the material specified as type-1 extract, and assuming that the toxicity is due to the saponin content, the Committee agreed that the ADI for quillaia type-1 extract could be changed to an ADI based on the saponin content and incorporated into a group ADI for type-1 and type-2 extracts. On the basis of the lower end of the range of the saponin content (20%), the ADI for type-1 extract would be 0–1 mg/kg bw expressed as quillaia saponins.

The Committee established a group ADI of 0–1 mg/kg bw for type-1 and type-2 extract expressed as quillaia saponins. The previously established ADI of 0–5 mg/kg bw for type-1 extract was withdrawn.

No toxicological monograph was prepared. The existing specifications and the chemical and technical assessment were revised.

3.1.7 *Quillaia extract type 1: Assessment of dietary exposure*

Explanation

Quillaia extracts were considered previously by the Committee, at its twenty-sixth, twenty-ninth, fifty-seventh and sixty-first meetings (Annex 1, references *59*, *70*, *154* and *166*). At its fifty-seventh meeting, the Committee assessed all relevant information on toxicity and dietary exposure and allocated a temporary ADI of 0–5 mg/kg bw to the unpurified extract, pending clarification of the specifications. At its sixty-first meeting, the

Committee reviewed new information relating to the chemical characterization of quillaia extracts and further information for specifications. The Committee agreed that separate specifications were needed for the two forms of quillaia extract, type 1 ('unpurified') and type 2 ('semi-purified') and also concluded that the data submitted for toxicological and dietary exposure assessment were specific to the material described as type-1 extract. The 'temporary' assignment to the ADI of 0–5 mg/kg bw for quillaia extract type 1 was therefore removed.

At its present meeting, the Committee decided to express the ADI on the basis of the saponin content. Taking the lower end of the specified range, a group ADI of 0–1 mg/kg bw, expressed as quillaia saponins, was established (see section 3.1.6).

Quillaia extracts can be used as foaming agents in soft drinks and cocktail mixes and as emulsifiers in foods such as baked goods, sweets, frozen dairy products, gelatine and puddings. Their main food use is in soft drinks. The Codex Alimentarius Commission adopted a provision for the use of quillaia extract as a foaming agent at a level of 100 mg/kg in food category 14.1.4, 'water-based flavoured drinks', of the Codex General Standard for Food Additives.

At its fifty-seventh meeting, the Committee estimated dietary exposure to quillaia extracts by a stepwise procedure, assuming a quillaia concentration of 500 mg/kg in all water-based flavoured drinks. On the basis of this use level, the Committee concluded that consumption at the 95th percentile of the distribution of consumption of soft drinks, particularly by children, could exceed the ADI. Estimates of exposure based on consumption of soft drinks in the USA that are likely to contain quillaia at a level of 100 mg/kg were below the ADI.

The previous exposure estimates considered by JECFA did not explicitly include use of quillaia extract in semi-frozen carbonated and non-carbonated beverages. The Codex Committee on Food Additives and Contaminants at its Thirty-sixth Session (*14*) therefore requested further information on dietary exposure to quillaia extract type 1 at levels up to 500 mg/kg in these products.

The composition of semi-frozen carbonated and non-carbonated beverages is similar to that of the corresponding unfrozen beverages. They differ in the content of foaming agents and carbonation or addition of air to expand their volume up to 180% of the original. Therefore, a use level of 500 mg/kg in unexpanded semi-frozen carbonated and non-carbonated beverages corresponds to 295 mg/l as consumed, expressed as quillaia extract.

Assessment of dietary exposure based on model diets

Dietary exposure to quillaia extracts was estimated after assuming the presence of the additive at 295 mg/l in all water-based flavoured drinks. High-percentile consumption of soft drinks is 446–1600 ml/day, resulting in a level of exposure to quillaia extracts of 130–470 mg per person per day or 44–160% of the group ADI of 0–1 mg/kg bw expressed as quillaia saponins (see section 3.1.6).

The Committee noted that this assessment assumes that semi-frozen carbonated and non-carbonated beverages are the only source of quillaia extracts. This assumption is based on the fact that no data were submitted about levels of quillaia extracts in solid foods and that it is unlikely that a person who consumes semi-frozen carbonated and non-carbonated beverages would also consume other beverages potentially containing the same additive.

Assessment of dietary exposure based on a probabilistic approach

A probabilistic exposure assessment was submitted, combining the frequency of consumption of semi-frozen carbonated and non-carbonated beverages with the amounts consumed per eating occasion. The amounts were estimated from the size of the containers available and on the consumption of 'frozen novelties' in the USA, used as a surrogate for semi-frozen carbonated and non-carbonated beverages. Assuming that the frequency and the amount per eating occasion are independent variables, dietary exposure to quillaia extracts is below the group ADI of 0–1 mg/kg bw, expressed as quillaia saponins, at the 90th percentile.

The hypothesis of independence between the amount consumed and the frequency of consumption could not be verified from the available information. Therefore, the possibility that a consumer of large amounts of semi-frozen carbonated and non-carbonated beverages is also a frequent consumer cannot be excluded. Assuming 100% dependence between the frequency and the amount of consumption, it is possible to estimate the number of consumers who potentially exceed the group ADI of 0–1 mg/kg bw, as follows: semi-frozen carbonated and non-carbonated beverages are consumed in the USA by 1–7% of the total population, which corresponds to 10 000–70 000 consumers per million. Of those consumers, 15% consume semi-frozen carbonated and non-carbonated beverages at least once a day, corresponding to 1500–10 500 individuals per million, and 1% drink > 1 l/day. Thus, the consumption of 15–100 individuals per million in the whole population could exceed the group ADI under these conditions.

An addendum to the monograph was prepared.

Annex 2

Acceptable daily intakes, other toxicological information and information on specifications

1. Food additives and ingredients evaluated toxicologically or assessed for dietary exposure

Food additive	Specifi cations[a]	Acceptable daily intake (ADI) and other toxicological recommendations
Beeswax	R	No safety concern at predicted dietary intake (< 650 mg per person per day), based on long history of use and lack of toxicity observed with major components
Candelilla wax	R	No safety concern at predicted dietary intake (< 650 mg per person per day)
Calcium L 5 methyltetrahydrofolate	N	No safety concern for proposed use in dry crystalline or microencapsulated form as alternative to folic acid used in dietary supplements, foods for special dietary uses and other foods. Safety of folate fortification and supplementation as such not evaluated.
Phospholipase A1 from *Fusarium venenatum* expressed in *Aspergillus oryzae*	N	Information provided too limited to assess safety
Pullulan	N	ADI 'not specified'[b]
Quillaia extract type 1	S	Previous ADI converted to an ADI based on saponin content from the lower end of speci fied saponin range and established as group ADI for quillaia extract type 1 and quillaia extract type 2. Assessment of dietary exposure included additional use of quillaia extract type 1 in semi frozen carbonated and non carbonated beverages (≤ 500 mg/kg product). In a model diet approach, high per centile consumption estimated to lead to intake of 44 157% of ADI, assuming pre sence of quillaia extract type 1 at 295 mg/l in all water based flavoured drinks. In a proba bilistic exposure assessment and assuming that the frequency and amount per eating occasion are independent variables, the estima ted dietary exposure was below the ADI at the 90th percentile. Assuming 100% dependence between frequency and amount consumed, estimated that 100 700 individuals per million in the entire population could exceed the ADI .

Food additive	Specifications[a]	Acceptable daily intake (ADI) and other toxicological recommendations
Quillaia extract type 2	R	Previous ADI established for quillaia extract type 1 converted to an ADI based on saponin content from the lower end of the specified saponin range and established as a group ADI for quillaia extract type 1 and type 2.

[a] N: new specifications prepared; R: existing specifications revised; S: existing specifications maintained.

[b] ADI 'not specified' is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

2. Food additives considered for specifications only

Food additive	Specifications[a]
Aspartame acesulfame salt	R
Hexanes	See below
Laccase from *Myceliophthora thermophila* expressed in *Aspergillus oryzae*	R
Monomagnesium phosphate and trisodium diphosphate	Wb
Sucrose esters of fatty acids	R, T

[a] R: existing specifications revised; T: tentative specifications; W: existing specifications withdrawn.

[b] As no information was received on these substances, the existing tentative specifications were withdrawn.

Hexanes

As used in the food industry, 'hexane' is a mixture of hydrocarbons. Recent changes in environmental regulations have led to a change in composition of hexanes since the original specifications were established. In addition, the composition of hexanes depends on the region of production, the source of the raw material and the site of production. Therefore, the Committee concluded that the present articles of commerce differ from those previously evaluated by the Committee and that the composition of the residues and their levels in foods may not be the same as those evaluated in the original safety assessment. The Committee also concluded that there was insufficient information available to change the current specifications, and therefore recommended a re-evaluation of hexanes.

JECFA - Monographs & Evaluations

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Your Query "**quillaia extract**" matched 12 documents out of 8835.
10 documents displayed.

1.0000 QUILLAIA EXTRACTS (JECFA Food Additives Series 48)

09-21-10, http://www.inchem.org/documents/jecfa/jecmono/v48je03.htm
Summary: 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). 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...

0.9864 544. Quillaia extracts (WHO Food Additives Series 17)

09-21-10, http://www.inchem.org/documents/jecfa/jecmono/v17je24.htm
Summary: Quillaia (synonyns; soapbark, soap tree bark, murillo bark, quillaia, Panama bark, Panama wood, and China bark) is the dried inner bark derived of cork of Quillaia saponaria Molina and other species of Quillaia (Rosaceae). Quillaia extract is the aqueous extract of the above bark and contains 3 or possibly 4 triterpenoid saponins (2 major, 1 minor, 1 trace) constituting about 10% of the extract. In lifetime studies in the mouse and rat, at dietary levels up to 1.5%, there were minor changes ...

0.9474 JECFA Evaluations-QUILLAIA EXTRACTS-

09-21-10, http://www.inchem.org/documents/jecfa/jeceval/jec_2074.htm
Summary: QUILLAIA EXTRACTS See: QUILLAIA EXTRACT (TYPE 1) QUILLAIA EXTRACT (TYPE 2) INS: 999 Synonyms: SOAPBARK EXTRACT; QUILLAY BARK EXTRACT; PANAMA BARK EXTRACT; QUILLAI EXTRACT; BOIS DE PANAMA Functional class: EMULSIFIER; FOAMING AGENT Latest evaluation: 2003 Comments: Quillaia extracts were divided into quillaia extract (type 1) and quillaia extract (type 2) Report: TRS 922-JECFA 61/37 Tox monograph: FAS 48-JECFA 57/17 (2001) Previous status: 2001, TRS 909-JECFA 57/14, COMPENDIUM ADDENDUM 9/FNP ...

0.9416 JECFA Evaluations-QUILLAIA EXTRACT (TYPE 1)-

09-21-10, http://www.inchem.org/documents/jecfa/jeceval/jec_2075.htm
Summary: QUILLAIA EXTRACT (TYPE 1) See: QUILLAIA EXTRACTS QUILLAIA EXTRACT (TYPE 2) INS: 999 Synonyms: QUILLAJA EXTRACT; SOAPBARK EXTRACT; QUILLAY BARK EXTRACT; BOIS DE PANAMA; PANAMA BARK EXTRACT; QUILLAI EXTRACT Functional class: EMULSIFIER; FOAMING AGENT Latest evaluation: 2005 ADI: 0-0.1 mg quillaia saponins/kg bw Comments: Group ADI with quillaia extract (type 2) Report: TRS 934-JECFA 65/31 Specifications: COMPENDIUM ADDENDUM 13/FNP 52 Add.

0.9282 JECFA Evaluations-QUILLAIA EXTRACT (TYPE 2)-

09-21-10, http://www.inchem.org/documents/jecfa/jeceval/jec_2076.htm
Summary: QUILLAIA EXTRACT (TYPE 2) See: QUILLAIA EXTRACTS QUILLAIA EXTRACT (TYPE 1) Synonyms: QUILLAJA EXTRACT; SOAPBARK EXTRACT; QUILLAY BARK EXTRACT; BOIS DE PANAMA; PANAMA BARK EXTRACT; QUILLAI EXTRACT Functional class: EMULSIFIER; FOAMING AGENT Latest evaluation: 2005 ADI: 0-1 mg quillaia saponins/kg bw Comments: Group ADI with quillaia extract (type 1) Report: TRS 934-JECFA 65/28 Specifications: COMPENDIUM ADDENDUM 13/FNP 52 Add.

0.8351 ANNEX 4 (JECFA 52, 2004)

09-21-10, http://www.inchem.org/documents/jecfa/jecmono/v52je27.htm
Summary: ADI "not specified" is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. The ADI was maintained and the use of the chemical as a flavouring agent subsumed ...

0.7967 521. Introduction (WHO Food Additives Series 17)

09-21-10, http://www.inchem.org/documents/jecfa/jecmono/v17je01.htm
Summary: INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY WORLD HEALTH ORGANIZATION TOXICOLOGICAL EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD WHO FOOD ADDITIVES SERIES 17 Prepared by: The 26th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) World Health Organization, Geneva 1982 The International Programme on Chemical Safety (IPCS), established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and ...

0.7742 606. Tragacanth Gum (WHO Food Additives Series 20)

09-21-10, http://www.inchem.org/documents/jecfa/jecmono/v20je16.htm
Summary: 19. Specifications for the identity and purity of food additives and their toxicological evaluation; some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). 38. Evaluation of certain food additives: some food colours, thickening agents, smoke codensates, and certain other substances (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives).

0.7742 ANNEX 4 (JECFA Food Additives Series 48)

09-21-10, http://www.inchem.org/documents/jecfa/jecmono/v48je24.htm
Summary: N, new specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or required; T, the existing, new, or revised specifications are tentative and new information is needed; W, existing specifications withdrawn. Invertase from Saccharomyces cerevisiae that meets the specifications developed at the present meeting was considered to be acceptable because S. cerevisiae is commonly used in the preparation of food. The new specifications for ...

0.7742 Introduction (JECFA Food Additives Series 48)

09-21-10, http://www.inchem.org/documents/jecfa/jecmono/v48je01.htm
Summary: Safety evaluation of certain food additives and contaminants / prepared by the fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives. JECFA serves as a scientific advisory body to FAO, WHO, their Member States, and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives and Contaminants and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally ...

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JECFA - Monographs & Evaluations

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0.7742 Annexes (WHO Food Additives Series 56)

02-19-08, http://www.inchem.org/documents/jecfa/jecmono/v56je14.pdf
Summary: This volume contains monograph's prepared at the sixty-fifth meeting of the joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Geneva, Switzerland, from 7 to 16 June 2005. This volume and others in the WHO Food Additives series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminats in food, government and food regulatory officers, industrial testing laboratories, toxicological ...

0.7742 Quillaia extract type 1 (addendum) (WHO Food Additives Series 56)

02-19-08, http://www.inchem.org/documents/jecfa/jecmono/v56je06.pdf

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The International Programme on Chemical Safety (IPCS) WHO ILO UNEP

in partnership with CCOHS Canadian Centre for Occupational Health and Safety

IOMC INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among UNEP, ILO, FAO, WHO, UNIDO, UNITAR and OECD

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives

QUILLAIA EXTRACTS

See:	QUILLAIA EXTRACT (TYPE 1) QUILLAIA EXTRACT (TYPE 2)
INS:	999
Synonyms:	SOAPBARK EXTRACT; QUILLAY BARK EXTRACT; PANAMA BARK EXTRACT; QUILLAI EXTRACT; BOIS DE PANAMA
Functional class:	EMULSIFIER; FOAMING AGENT
Latest evaluation:	2003
Comments:	Quillaia extracts were divided into quillaia extract (type 1) and quillaia extract (type 2)
Report:	TRS 922-JECFA 61/37
Tox monograph:	FAS 48-JECFA 57/17 (2001)
Previous status:	2001, TRS 909-JECFA 57/14, COMPENDIUM ADDENDUM 9/FNP 52 Add.9/89, FAS 48-JECFA 57/17. 0-5 mg/kg bw (TEMPORARY). TE. R,T 2000, COMPENDIUM ADDENDUM 8/FNP 52 Add.8/93. R 1985, TRS 733-JECFA 29/41, FNP 34-JECFA 29/183; COMPENDIUM/1239, FAS 17-JECFA 26/180 (1982). 0-5 mg/kg bw. N,T 1982, TRS 683-JECFA 26/30, NOT PREPARED, FAS 17-JECFA 26/180. NO ADI ALLOCATED. NO. O

27 Jan 06

See Also:
- Toxicological Abbreviations
- Quillaia extracts (JECFA Food Additives Series 48)
- Quillaia extracts (WHO Food Additives Series 17)

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives

QUILLAIA EXTRACT (TYPE 1)

See:	QUILLAIA EXTRACTS QUILLAIA EXTRACT (TYPE 2)
INS:	999
Synonyms:	QUILLAJA EXTRACT; SOAPBARK EXTRACT; QUILLAY BARK EXTRACT; BOIS DE PANAMA; PANAMA BARK EXTRACT; QUILLAI EXTRACT
Functional class:	EMULSIFIER; FOAMING AGENT
Latest evaluation:	2005
ADI:	0-0.1 mg quillaia saponins/kg bw
Comments:	Group ADI with quillaia extract (type 2)
Report:	TRS 934-JECFA 65/31
Specifications:	COMPENDIUM ADDENDUM 13/FNP 52 Add. 13/39 (republished)
Previous status:	2005, TRS 922-JECFA 61/37, COMPENDIUM ADDENDUM 11/FNP 52 Add.11/61, FAS 48-JECFA 57/17 (2001). 0-5 (SAPONIN CONTENT OF 20-26%). FU. R. SEE "QUILLAIA EXTRACTS". THE PREVIOUS SPECIFICATIONS FOR QUILLAIA EXTRACTS PREPARED AT JECFA 57 (2001) HAVE BEEN REPLACED BY THESE AND SEPARATE SPECIFICATIONS FOR "QUILLAIA EXTRACT (TYPE 2)."
Intake:	An assessment of dietary exposure considered the additional use of quillaia extract type 1 in semi-frozen carbonated and non-carbonated beverages (up to 500 mg/kg product). Using a model diet approach, high-percentile consumption was estimated to lead to an exposure of 44 to 157% of the ADI, assuming the presence of quillaia extract type 1 at 295 mg/l in all water-based flavoured drinks. Using a probabilistic exposure assessment and assuming that the frequency and amount per eating occasion are independent variables, the estimated dietary exposure was below the ADI at the 90th percentile. Assuming 100% dependency between frequency and amount consumed, it was estimated that 100-700 individuals per million over the whole population could exceed the ADI under these conditions.

31 Jan 06

See Also:
Toxicological Abbreviations

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives

QUILLAIA EXTRACT (TYPE 2)

See:	QUILLAIA EXTRACTS QUILLAIA EXTRACT (TYPE 1)
Synonyms:	QUILLAJA EXTRACT; SOAPBARK EXTRACT; QUILLAY BARK EXTRACT; BOIS DE PANAMA; PANAMA BARK EXTRACT; QUILLAI EXTRACT
Functional class:	EMULSIFIER; FOAMING AGENT
Latest evaluation:	2005
ADI:	0-1 mg quillaia saponins/kg bw
Comments:	Group ADI with quillaia extract (type 1)
Report:	TRS 934-JECFA 65/28
Specifications:	COMPENDIUM ADDENDUM 13/FNP 52 Add. 13/43
Tox monograph:	FAS for JECFA 65 in press
Previous status:	2005, TRS 922-JECFA 61/37, COMPENDIUM ADDENDUM 11/FNP 52 Add.11/65, FAS 48-JECFA 57/17 (2001). NO ADI ALLOCATED (SAPONIN CONTENT OF 75-90%. NO ADI WAS ALLOCATED DUE TO LIMITED INFORMATION ON THE QUALITATIVE AND QUANTITATIVE COMPOSITION OF THE PRODUCT.) NO. N. SEE "QUILLAIA EXTRACTS". THE PREVIOUS SPECIFICATIONS FOR QUILLAIA EXTRACTS PREPARED AT JECFA 57 (2001) HAVE BEEN REPLACED BY THESE AND SEPARATE SPECIFICATIONS FOR "QUILLAIA EXTRACT (TYPE 1)."

31 Jan 06

See Also:
Toxicological Abbreviations

WHO FOOD ADDITIVES SERIES: 48

SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

QUILLAIA EXTRACTS

First draft prepared by Jennifer Eastwood[1], Elizabeth Vavasour[1] and Janis Baines[2]
[1]Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada
[2]Australian New Zealand Food Authority, Canberra, Australia

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 ^{o}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 $\leq$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 $\leq$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 $\leq$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 of 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	1986–87
	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 drinks[c]; consumers 87%	National survey; 7-day records; children 1 5–4 5 years; sample, 1675; assumed body weight, 14 5 kg	1992
	Child respondents Child consumers	Mean, 260 97th percentile, 800	Mean, 1 7 97th percentile, 5 2	34 100	Maximum manufacturers' use level, 95 mg/kg; extract in all water-based drinks		

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	1989–92 (combined surveys)

[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.

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

QUILLAIA EXTRACTS

Explanation

This compound has not previously been reviewed by the Joint FAO/WHO Expert Committee on Food Additives.

Quillaia (synonyns; soapbark, soap tree bark, murillo bark, quillaia, Panama bark, Panama wood, and China bark) is the dried inner bark derived of cork of Quillaia saponaria Molina and other species of Quillaia (Rosaceae). This plant is a large evergreen tree with shiny, leathery leaves and thick bark, which is native to China and Peru and cultivated in southern California (Leung, 1980).

Quillaia extract is the aqueous extract of the above bark and contains 3 or possibly 4 triterpenoid saponins (2 major, 1 minor, 1 trace) constituting about 10% of the extract. The sugars, glucose, galactose, arabinose, xylose, rhamnose and 2 further unidentified sugars, are also present. The extract also contains tannin and about 11% calcium oxalate. The 2 major saponins are quillaia sapogenin and quillaic acid (Commission of the European Communities, 1978).

BIOLOGICAL DATA

TOXICOLOGICAL STUDIES

General biological activity

Saponins vary widely in the kind and intensity of biological activity. Some of the more important reported activities include haemolytic, local irritant, inflammatory (especially on intestine), cytotoxic and antimicrobial.

Powdered quillaia bark or saponin concentrate has highly local irritant and stimulatory properties. It also possesses expectorant properties as well as depressant activity on the heart and respiration. Severe toxic effects due to large oral doses have been reported to be liver damage, respiratory failure, gastric pain, diarrhoea, haemolysis of red blood corpuscles, convulsions and coma (Leung, 1980).

Special studies on pharmacological effects

Saponins extracted with alcohol from soapbark trees, administered orally to rabbits with experimental atherosclerosis, resulted in increased plasma lecithin to cholesterol ratio, normalized blood cholesterol levels, and decreased elevated blood pressure. Subcutaneous injection of the saponin extract did not affect atherosclerotic symptoms (Efimova et al., 1966).

Groups of 12 Swiss mice (strain and sex undefined) were injected in the footpad with aliquots of 7 different 5% extracts of Quillaia saponica bark. Animals surviving after 24 hours and control animals were sacrificed. Degree of oedema was measured in the mice. Haemolysis of rabbit erythrocytes was also measured using the same quillaia extracts. The adjuvant strength of the various quillaia extracts in stimulating immunity to staphylococcal toxin was also measured. The samples of quillaia showed no relationship between their inflammatory effect on mice, their haemolytic effect for rabbit erythrocytes, their

toxicity for Swiss mice and their antistaphylococcal immunity-stimulation effect for rabbits (Richou et al., 1965).

Acute toxicity (Saponins extracted from the soapbark tree)

Species	Route	LD_{50} (mg/kg bw)	Reference
Mouse	Oral	1 625	Efimova et al., 1966
	s.c.	650	Efimova et al., 1966
	i.p.	275	Efimova et al., 1966
	i.v.	275	Efimova et al., 1966

Short-term studies

Rat

Groups of 15 male and 15 female weanling rats of the CFE strain were housed 5 per cage and fed on diets containing 0 (control), 0.6, 2.0 or 4.0% quillaia extract for 13 weeks. Groups of 5 male and 5 female rats from the same lot of animals were fed on diets containing 0 (control), 2.0% or 4.0% quillaia extract for 2 or 6 weeks.

Animal weights and food intake were measured at the beginning of the experiment and weekly throughout the study. Urine analysis was carried out during the final week of the study. At sacrifice, absolute and relative organ weights were determined, and a histological examination was made of tissue and organs from the 4% group. Haematological studies and serum chemistry were carried out for all groups.

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

saponin did not affect the results of haematological examinations, serum and 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 both sexes at the same levels. No histopathological effects attributable to treatment were found. The no-effect level in this study was 0.6% of the diet equivalent to an intake of approximately 400 mg/kg bw per day (Gaunt, Grasso & Gangolli, 1974).

Long-term studies

Mouse

Groups of 48 male and 48 female TO strain mice were fed diets containing 0, 0.1, 0.5 and 1.5% quillaia extract for 84 weeks. The mice were observed regularly for abnormalities of condition or behaviour, and some males were weighed at intervals. Haematological studies were made at weeks 24, 56 and 84. No compound-related effects were reported. At the highest dose level, there was a decreased weight gain in the male mice. Quillaia at the levels fed had no adverse effect on condition, behaviour or death rate. At the termination of the study a detailed autopsy and histopathological examination of

tissues and organs showed no compound-related effects.

Quillaia extract fed at levels up to 1.5% in the diet (2.2 g/kg bw per day) was not carcinogenic; the slightly lower body weight gain in the mice on the highest dietary level and some organ weight changes, albeit of doubtful significance, indicate a no-effect level for quillaia extract of 0.5% in the diet (or 700 mg/kg bw per day) (Phillips et al., 1979).

Rat

Groups of 48 male and 48 female rats were housed in groups of 4 and fed on diets containing 0 (control), 0.3, 1.0 or 3.0% quillaia extract for 108 weeks. Rats used in the study were a Wistar-derived strain from a specified pathogen-free breeding colony. Haematological studies were made at weeks 15, 25, 52 and 108 of the study and urinalysis at weeks 13, 24 and 78. At the termination of the study a complete autopsy was carried out, including histological examination of the tissues and organs.

Male rats fed the highest dietary level had lower body weights throughout the experiment than control animals, the differences being statistically significant between 10 and 22 months. Females on the lowest dietary level had significantly higher body weights than the control animals during the first 6 months of the study. The lower body weights and reduced incidence of glomerulonephrosis in the male rats fed 3% quillaia are considered to be due to reduced food consumption. Preference tests run before the start of the 2-year study showed that

the rats avoided diet containing quillaia extract. Haematological parameters and urinalysis showed no compound-related effects and were within normal range.

In general, the incidence of histological findings was similar in treated and control animals. The only lesions with incidences greater than those of control were fibrosis of the heart and dilatation of the glands of the gastric mucosa in females, at the lowest dietary level of quallaia. These effects are considered to be fortuitous since there was no dose relationship and no similar occurrence in males.

A variety of benign and malignant tumours were found. The incidence of haemangiomas and haemangiosarcomas in the lymh nodes were similar in both control and treated animals. The only tumour showing a statistical difference from the control incidence was thyroid adenoma, which occurred more frequently in females fed 1% quillaia extract in the diet. This finding was not considered treatment related, since incidence did not increase with level of quillaia extract fed and the total incidence of thyroid adenoma in both sexes fed 1% quillaia extract was not statistically different from the total control incidence (Drake et al., 1982).

Comments

A short-term study in the rat showed that even at the highest test level (up to 4% of the diet) the only effects observed were some decrease in body weight gain, and relative liver weight. In lifetime studies in the mouse and rat, at dietary levels up to 1.5%, there were minor changes in body weight gain, and some relative organ weights. No compound-related histological changes were reported. The no-effect level for quillaia extract in the diet of mice was 0.5% and in the rat 1.0%. No evaluation is possible at this time because specifications for quillaia extract are not available.

EVALUATION

Estimate of acceptable daily intake for man

Not allocated.

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See Also:
Toxicological Abbreviations
Quillaia extracts (JECFA Food Additives Series 48)
QUILLAIA EXTRACTS (JECFA Evaluation)

National Starch
FOOD INNOVATION

APPENDIX F

STATUTORY DECLARATION

Date: 22 May 2012

I, [redacted] (Technical Service Manager), make the following declaration under the *Statutory Declarations Act 1959*:

1. the information provided in this application fully sets out the matters required
2. the information provided in this application is true to the best of my knowledge and belief
3. no information has been withheld that might prejudice this application, to the best of my knowledge and belief

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the *Statutory Declarations Act 1959*, and I believe that the statements in this declaration are true in every particular.

[redacted]
Technical Service Manager
National Starch Pty Ltd
[redacted]

National Starch
FOOD INNOVATION

APPENDIX G
LITERATURE

LITERATURE

1. Peter R. Cheeke. Dietary saponins are not absorbed.
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Dietary Saponins Are Not Absorbed

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There are several lines of evidence suggesting that dietary saponins are not absorbed. Saponins have very low oral toxicity whereas if injected they may be highly toxic. Saponins are 10 to 1000 times less toxic when given orally than when given by intravenous injection (Oakenfull and Sidhu, 1989). Gestetner et al. (1968) studied the digestion and absorption of saponins in chicks, rats, and mice. There were no detectable saponins in the blood of these animals. Oakenfull (1981) reviewed the significance of saponins in food, and concluded that saponins are not toxic to humans because they are not absorbed from the digestive tract. Price et al. (1987) reached a similar conclusion. Price et al. (1987) also concluded that the lack of toxicity of orally ingested saponins to humans is due in part to the large surface area of the gastrointestinal tract in relation to the concentration of saponins to which it is exposed.

Saponins are non-dialyzable so their absorption would be expected to be essentially nil. Alfalfa saponin was found to be about 50 times more toxic to sheep when administered intravenously than when given orally (Cheeke, 1971).

Saponins are potent hemolytic agents, causing red blood cell hemolysis when administered intravenously, even at very low concentrations. Hemolysis is not seen when saponins are given orally, implying that they are not absorbed.

In conclusion, several lines of evidence indicate that saponins are not absorbed. Furthermore, there is no evidence that orally-administered saponins cause red blood cell hemolysis, which is a very sensitive indicator of saponin toxicity.

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Chapter 4

SAPONINS

David Oakenfull and Gurcharn S. Sidhu

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rich in medicagenic acid are more toxic to the flour beetle (*Tribolium castraneum*) than soy saponins.[178] While hydrolytic removal of one sugar group from medicagenic acid increased its toxicity, the same process made soy saponins nontoxic.

Not all insects are equally susceptible to saponins. Some have evolved strategies for overcoming the saponin defenses of the plants on which they prey. Several alfalfa pests, such as alfalfa weevil (*Hypera postica*), spotted aphid (*Thereoaphid maculata*), clover root curculio (*Stona hispidulus*), and seed chalcid (*Bruchophagus roddi*) are hardly affected by the saponin content of their diet.[180] It has even been suggested that the alfalfa weevil may have taken positive advantage of the saponins in its diet for its development. However, an apparent correlation between the total saponin content of a plant and resistance to insect predation must be considered with caution.[181] Aphids feed on sieve tubes and may remain unaffected by saponins present in other parts of the leaves or in the roots of alfalfa.[181] It has recently been shown that alfalfa saponins in the diet neither inhibit feed intake nor have any repellent effect on the green pea aphid *Acyrthosiphon pisum*; yet, ingested alfalfa saponins are definitely toxic (radioactive tracer techniques were used).[181,182]

Thus, it seems there is no direct link between the presence in a plant of saponins and the plant's resistance to insect predation. Some saponins are vastly more toxic than others; some insects have developed strategies for dealing with the toxic properties of saponins.

2. Fish, Tadpoles, and Snails

Toxicity to fish is a characteristic property of saponins which has been known and exploited for centuries.[1] Saponins added to the water rapidly cause paralysis and death. Small fish, such as minnows or guppies, and tadpoles and freshwater snails have been used to bioassay saponin toxicity. Saponins extracted from the African medicinal plant *Phytolacca dodecandra* have been suggested as a means of controlling the water snail *Biomphalaria glabrata* which transmits the endemic disease schistosomiasis.[183,184] Saponin-containing tubers of another African plant, *Talinum tenuissimum*, have also been reported to be effective in this way.[185]

Attempts have been made to relate the toxicity of saponins to fish to their ability to lower surface tension, their ability to increase the permeability of cell membranes, or to their hemolytic power.[186] None of these correlations, though, have been particularly convincing. The effect seems to be directly related to damage caused by the saponins to the delicate membranes of the gills. Scanning electron microscopy has revealed the extensive damage that occurs in the gills of the climbing perch (*Anabas testidineus*) exposed to the saponins from *Mollugo pentaphylla* in solution at 50 ppm.[187] The saponin caused swelling of the lamellar and interlamellar epithelium with some dissociation of the epithelium and development of microridges in the minute water spaces between the lamellae. These changes caused asphyxiation and death of the fish.

E. Effects on Birds and Mammals

There has been a tendency in the past to treat saponins exclusively as antinutritional or toxic constituents of plants when consumed by animals or humans. Recent work has brought to light more positive, beneficial effects from dietary saponins, and in this section we shall discuss both these aspects.

1. Digestion and Absorption of Saponins

Saponins are 10 to 1000 times less toxic orally than when given by intravenous injection. They are absorbed very slowly, if at all, from the gastrointestinal tract.[143] Gestetner et al.[188] have studied the digestion and absorption of saponins in chicks, rats, and mice. The researchers fed the animals diets containing soy saponins and failed to detect any saponins in the blood. The enzymes of the gastrointestinal tract had no effect on the saponins, but they were hydrolyzed to their respective sapogenins by the cecal microflora.

Fd Cosmet. Toxicol. Vol. 12 pp. 641–650. Pergamon Press 1974. Printed in Great Britain

SHORT-TERM TOXICITY OF QUILLAIA EXTRACT IN RATS

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(*Received 8 March 1974*)

Abstract—Quillaia extract was fed to groups of 15 male and 15 female rats for 13 wk at dietary concentrations of 0 (control), 0·6, 2·0 or 4·0%. There was a transitory reduction in the rate of body-weight gain, associated with a reduced intake of food and water, but by the end of the study the weights of the treated rats did not differ significantly from those of the controls. The feeding of quillaia saponin did not affect the results of haematological examinations (including erythrocyte fragility tests in hypotonic saline), serum and urine analyses, renal concentrating ability or the urinary cell excretion. The relative liver weight was reduced in males given 2·0 or 4·0% quillaia and the relative stomach weight was increased in both sexes at the same levels. No histopathological effects attributable to treatment were found. The no-untoward-effect level in this study was 0·6% of the diet, equivalent to an intake of approximately 400 mg/kg/day.

INTRODUCTION

Saponins are glycosides, usually nitrogen-free, consisting of a sapogenin and a sugar. The sapogenin may be either a steroid or triterpene. The commonest sugar moiety is glucose, although Coulson (1958) identified, in addition, arabinose, xylose, rhamnose and galactose when examining the saponins from lucerne, white clover and Quillaja. Jackson & Shaw (1959) identified a similar range of sugars in alfalfa saponins. The material chosen for the present studies (quillaia extract) was a triterpenoid saponin (Cheeke, 1971), in which two major sapogenins have been identified as well as one minor one and possibly a fourth, present only in trace amounts (Coulson, 1958).

This group of chemicals is found in over 400 species of plants including some, such as spinach, beetroot, sugar beet, asparagus, alfalfa, clover and soya, of importance as human or animal foods. They have been widely studied, particularly in relation to their growth-promoting effects and in relation to bloat production in ruminants. However, relatively little is known about the particular material used in the present work and it is questionable whether general conclusions can be drawn from studies with other saponins in view of their chemical diversity.

Saponins have amphipathic properties because of the water solubility of the carbohydrate moiety and lipid solubility of the sapogenin and are therefore used as emulsifiers and foaming agents, particularly in soft drinks, where the usage levels may be up to 300 ppm. They are also present in some flavours, such as sarsaparilla and liquorice (Merck Index, 1960), and are used in confectionery, toiletry preparations and pharmaceuticals.

As far as the use of quillaia extract in food in concerned, it is permitted at present in the UK under the Emulsifiers and Stabilisers in Food Regulations (Statutory Instrument 1962, no. 72) although specifically excluded from the definition of emulsifiers and stabilisers. However, a report of the Food Additives and Contaminants Committee (1970 & 1972) recommended its future inclusion in the permitted list, with the provisions that it

should be restricted to material of British Pharmacopoeia standard and used in soft drinks at not more than 200 ppm. It was classified as provisionally acceptable for use in food but a need for further information, including data from long-term studies and studies on possible intestinal irritation, was indicated.

Quillaia saponin has been shown to be degraded in the intestine. After oral doses in dogs two sapogenins are excreted in the faeces (Bäck, 1917), and Ewart (1931) has stated that the liberation of the sapogenins could be brought about by trypsin, diastase and pancreatic juice. However, Gestetner, Birk & Tencer (1968) were not able to confirm this in mice given soya saponin as they found no release of sapogenin in the small intestine. The probability is that micro-organisms in the gastro-intestinal tract are important in the degradation of saponins. This has been investigated in cattle in relation to bloat formation, and Gutierez & Davis (1962) were able to isolate saponin-digesting bacteria from the rumen of cattle fed on pastures carrying plants of high saponin content. Their work suggests that the bacteria utilize the carbohydrate moiety, leaving the insoluble sapogenin. In rats, mice and hens, Gestetner *et al.* (1968) found that soya-bean saponin was degraded only in the caecum and colon. They demonstrated that this required the presence of the micro-organisms and they were able to extract from these organisms an enzyme preparation capable of releasing the sapogenin. They considered that this was a broad-spectrum system and not specific to one saponin.

Ewart (1931) was of the opinion that saponins were detoxified in the liver, as animals with extensive liver damage were more readily affected by oral doses than were normal animals. He also suggested that prolonged treatment with some saponins could lead to liver damage. The work of Fieger (1918) showed that the sapogenin was excreted in the urine, indicating some absorption from the intestine after oral administration. In the case of quillaia, however, the degree of absorption appeared to be low. A lack of absorption from the gastro-intestinal tract was confirmed by Erbring & Vogel (1963), working with horse-chestnut saponin, and by Newman, Kummerow & Scott (1958), who showed that daily oral doses of 0·2 g digitoxin caused no haemolysis in cats whereas a marked haemolysis occurred within 10 min of iv injection. Vogel & Marek (1962) reached a similar conclusion with a range of saponins, which they found were considerably less toxic after oral administration than after iv injection. A lack of absorption in the case of soya-bean saponin was confirmed by blood analysis in rats, mice and chickens following oral doses (Gestetner *et al.* 1968).

It has been reported that quillaia saponin causes an irritation of the gastro-intestinal tract in hens and dogs (Bäck, 1917), and Brune & Günther (1961) reported a marked diarrhoea in rats given diets containing pure saponins. The *Extra Pharmacopoeia* (1972) quotes the toxic effect of quillaia extract as a violent local irritation of the gastro-intestinal mucosa. This can be severe enough to allow absorption and the development of systemic signs such as haemolysis, liver damage, respiratory failure, bladder irritation, convulsions and coma. Vogel & Marek (1962) confirmed the irritancy of saponins using the mucosa of the rabbit eye. They found that the lowest concentration causing visible damage ranged with various saponins from 1 in 2300 to 1 in 10,000.

Leroy & Marbarger (1969) exposed hamsters to an atmosphere containing droplets of 0·5 or 1·0% solutions of quillaia saponin. After 60–90 days with 1 hr exposure daily there was hyperplasia of the bronchial epithelium and focal lesions consisting of giant cells and histiocytes containing haemosiderin and fat. It seems probable that this effect may be related to the irritant properties of the saponin.

In *in vitro* systems, saponins cause haemolysis, although the degree of activity varies considerably (Ewart, 1931). Oser (1966) showed that a concentration of 1·78 g quillaia extract/100 ml caused haemolysis of 50% of a sample of washed rat erythrocytes. In general this activity, most easily detected with washed erythrocytes, is reduced by the presence of serum and even more reduced by cholesterol, with which saponins form complexes (Solé, 1954). Because of this protective action of cholesterol, Ewart (1931) considers that haemolysis only occurs *in vivo* with relatively large doses of saponins, even after iv treatment. It has been suggested (Glauert, Dingle & Lucy, 1962) that haemolysis results from the complexing of saponin with the cholesterol of the erythrocyte membrane, causing a rearrangement of the cholesterol so as to leave pores in the membrane.

Diets containing quillaia saponin caused a reduced rate of body-weight gain in chickens and this was reversed by the addition of cholesterol to the diet (Peterson, 1950). This reversal was thought to be due to combination in the intestine rather than to high serum cholesterol levels. The ability of cholesterol to reverse the impairment of body-weight gain caused by quillaia saponin was disputed by Newman *et al.* (1958) who considered that the differences were due to changes in food intake. A dose-related reduction in the rate of body-weight gain was seen in rats fed on diets containing 0·5–2·0% quillaia saponin while a level of 3·0% was lethal (Coulson & Evans, 1960). There was no consistent evidence from their studies that this effect was prevented by cholesterol. Oser (1966) fed a diet containing 0·05% quillaia extract to rats for 12 wk with no effect on weight gain, food intake, haematology (including erythrocyte fragility), urine analysis, organ weights, serum chemistry or the incidence of histopathological findings.

It has been suggested that saponins have an anti-vitamin D property (Brune & Günther, 1961), as their simultaneous administration prevented the sparing effect of vitamin D in rats fed on a rachitogenic diet. However, the work of Coulson & Evans (1960) with quillaia saponin failed to demonstrate this effect. It has been reported that there is a reduction of the activity of the enzymes of the Krebs cycle in tissues exposed to saponins *in vitro* (Cheeke, 1971), and Shaw & Jackson (1957) found that clover extracts reduced the *in vitro* respiration of the rat diaphragm. They also found a reduction in spontaneous intestine motility and in erythrocyte cholinesterase activity. In view of this latter observation the suggestion of Ewart (1931) that saponins may lead to central nervous system damage is of interest.

The present paper gives the results of a short-term study of quillaia extract in rats, carried out as part of the BIBRA Safety Evaluation Programme. It is part of a series of studies including long-term work in rats and mice, which will be reported later.

EXPERIMENTAL

Materials. The sample of quillaia extract used in these studies was supplied by Food Industries Ltd., Birkenhead, Cheshire. It was a spray-dried aqueous extract of quillaia bark, prepared in such a manner that 100 parts by weight of bark yielded approximately 15 parts of extract. In addition, the sample contained 5% lactose added to the extract before drying. The extract was stated to contain less than 10% moisture and less than 10% ash (at 550°C).

Animals. Weanling rats of the CFE strain, obtained from an SPF breeding colony, were housed in an animal room maintained at 20 ± 1°C and a relative humidity of 40–50%. Spillers' Laboratory Small Animal Diet and water were available *ad lib.*

Experimental design and conduct. Groups of 15 male rats (130–175 g body weight) and 15 females (105–135 g body weight) were housed five in a cage and fed on diets containing 0 (control), 0·6, 2·0 or 4·0% quillaia extract for 13 wk. Groups of five male and five female rats from the same batch were fed on diets containing 0 (control), 2·0 or 4·0% quillaia extract for 2 or 6 wk.

The animals were weighed and the food intake was measured before the experimental diets were fed and then weekly throughout the study. At the end of the appropriate feeding period, the rats were killed by exsanguination under barbiturate anaesthesia and an autopsy was conducted, during which brain, pituitary, thyroid, heart, liver, spleen, stomach, small intestine, caecum, kidneys, adrenals and gonads were weighed. Samples of these organs and of oesophagus, colon, rectum, lung, lymph nodes, skeletal muscle, trachea, uterus, urinary bladder and pancreas were preserved in 10% buffered formalin. Paraffin-wax sections of the tissues from the animals fed for 13 wk on the diet containing 4·0% quillaia extract and from half the control animals were stained with haematoxylin and eosin for microscopic examination.

Blood collected at autopsy was examined for haemoglobin content, packed cell volume and counts of erythrocytes, reticulocytes, leucocytes and the different types of leucocytes. *In vitro* haemolysis was assessed at wk 6 using the blood of five animals of each sex from the control group and that fed 4% quillaia extract. At wk 13, five females from these two groups were examined. The blood was diluted 1 : 100 in 0·85, 0·60 or 0·40% buffered saline (Dacie & Lewis, 1968) or in distilled water. The haemoglobin liberated was estimated after 30 min at room temperature and the values in saline were expressed as a percentage of the values in distilled water (total haemolysis).

Serum collected at autopsy was examined for contents of urea, glucose, total protein, and albumin and for the activities of the glutamic–oxalacetic transaminase, glutamic–pyruvic transaminase and lactic dehydrogenase.

During the last week of the feeding period, urine was collected from all rats and examined for microscopic constituents and content of blood, bile and ketones. A concentration test was carried out involving measuring the specific gravity and volume of urine produced in a 6-hr period without water. At wk 6 and 13 this was extended to include similar measurements on urine produced in a 2-hr period immediately after a water load of 25 ml/kg and 16–20 hr after the water load. A count of the number of cells in the urine was made using the 6-hr collection.

RESULTS

No abnormalities of behaviour or condition were seen in the rats fed on the diets containing quillaia extract. Particularly it was noted that there was no diarrhoea or other sign of gastro-intestinal irritation. The rate of body-weight gain was reduced at all levels of feeding, although the weight gain of the treated rats over the whole experimental period did not differ significantly from that of the controls (Table 1). The greatest difference in weight gain occurred in the first few days of feeding, and at the highest level (4·0%) there was a weight loss over the first 24 hr. The differences between the body weights of control rats and those given 4·0% quillaia saponin were statistically significant up to day 78 in males but only for the first 2 wk in females. Food consumption was reduced throughout the study in both sexes at all dietary levels (Table 1) and the mean intakes over the whole experimental period were significantly less than controls in females given 2·0 or 4·0% quillaia saponin

Table 1. *Body weight and food and water intake of rats fed on diets containing 0–4·0% quillaia extract for 13 wk*

Dietary level (%)	Body weight (g) at day				Weight gain (g) at day 92	Food intake (g/rat/day) at day				Mean intake (g/rat/day)	Water intake (ml/rat/day) at day				Mean water intake (ml/rat/day)
	0†	29	57	92		1	29	57	92		2	31	59	94	
Males															
0	150	333	428	496	346	19·4	24·0	22·0	21·3	21·9	25·3	29·5	28·5	25·0	27·9
0·6	150	319	407	466	316	18·2	26·4	19·4	21·5	20·5	25·0	20·9	26·3	23·6	25·9
2·0	150	321	419	478	328	14·7	22·9	20·4	20·2	20·3	23·4	26·9	24·9	25·8	25·1*
4·0	150	306***	409*	457	307	9·4	22·3	20·4	20·2	20·0	24·9	26·5	25·2	23·8	23·0***
Females															
0	123	214	263	272	149	15·6	22·7	17·5	17·2	17·8	21·3	22·2	21·6	25·0	23·1
0·6	121	215	264	293	172	15·6	19·2	17·2	17·6	17·4	22·6	22·0	23·9	25·6	23·9
2·0	123	207	251	277	154	13·3	16·8	11·3	12·4	14·0**	20·3	22·4	24·6	28·0	23·4
4·0	122	206	254	281	159	6·4	15·5	15·1	16·6	15·3**	19·0	21·0	22·2	24·4	21·4

†First day of feeding.

Values are the means for groups of 15 rats for body weight, and for three cages of five rats for food intake and water consumption. The values marked with asterisks differ significantly (Student's *t* test for body weight and ranking method of White, 1952, for food intake and water consumption) from those of controls: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$.

in the diet. The largest differences from the control values were seen in the early part of the study, particularly during the first 24 hr.

The water consumption (Table 1) was reduced in males at all levels of treatment, and the mean intake over the experimental period in the case of the two highest quillaia levels (2·0 and 4·0%) was less than the controls to a statistically significant degree. In the females, this effect was seen only at the highest level of feeding. Calculation of the levels of intake of saponin over the experimental period established a mean daily intake of 0·36, 1·18 and 2·47 g/kg in males and 0·44, 1·37 and 3·03 g/kg in females given dietary levels of 0·6, 2·0 and 4·0% respectively.

No adverse differences between treated and control rats were seen in the haematological examinations, the results of which at wk 13 are given in Table 2, and the erythrocytes from treated rats were no more susceptible to haemolysis in hypotonic salt solutions than were those of controls. There were no differences between treated and control rats in the results of the serum analyses or in the urinary cell counts and renal concentration tests (Table 3) and no abnormal constituents were found in the urine.

No abnormalities were seen at autopsy and the significant differences in organ weights were confined almost entirely to males and consisted of decreases in the weights of the liver, spleen, kidneys, adrenals and pituitary, in some cases only at wk 2 or 6. These changes were found in groups that had low average body weights, and when the organ weights were expressed relative to body weight, there were no differences from controls in the adrenal and pituitary weights. The relative liver weights of males given 2·0 or 4·0% quillaia saponin were significantly lower than those of the controls, although no similar effect was seen in females. The relative spleen weight was reduced in females given 4·0% quillaia saponin in the diet for 2 wk and relative kidney weight was reduced with the highest dietary level in males at wk 6 and in females at wk 13. The organ weights at wk 13 are given in Table 4. Some increases in relative organ weight were seen. The relative stomach weights of rats fed on the diet containing 4·0% quillaia saponin were increased

Table 2. *Haematological findings in rats fed on diets containing 0–4% quillaia extract for 13 wk*

Dietary level (%)	Hb (g/100 ml)	PCV (%)	RBC (10^6/mm^3)	Retics (% of RBC)	Leucocytes Total (10^3/mm^3)	Differential (%) N	E	L	M
				Males					
0	14·8	46	7·29	1·0	6·54	15	1	81	3
0·6	14·7	46	7·15	1·0	5·39	14	1	82	3
2·0	14·5	46	7·27	1·3	5·64	18	0	79	3
4·0	14·6	44	7·15	1·2	5·94	17	1	79	3
				Females					
0	14·0	46	6·67	0·9	5·64	18	1	79	2
0·6	13·8	45	6·26	1·0	4·41	15	1	81	3
2·0	13·8	45	6·52	1·0	4·87	15	1	81	3
4·0	13·7	45	6·65	0·7	4·37	14	1	83	2

Hb = Haemoglobin PCV = Packed cell volume RBC = Red blood cells
Retics = Reticulocytes N = Neutrophils E = Eosinophils
L = Lymphocytes M = Monocytes

Values are means for groups of 15 rats. Basophils were not present at more than 0·5% in any group and no inclusions were seen in the erythrocytes. No adverse effects of the treatment were found at wk 2 and 6.

Table 3. *Results of urinary cell counts and concentration tests in rats fed on diets containing 0–4% quillaia extract for 13 wk*

Dietary level (%)	Concentration test				Dilution test (2 hr)		Cell count (10^3/hr)
	Specific gravity		Volume (ml)				
	0–6 hr	16–20 hr	0–6 hr	16–20 hr	Specific gravity	Volume (ml)	
				Males			
0	1·054	1·067	2·0	1·1	1·009	5·6	3·7
0·6	1·053	1·070	1·6	0·7	1·009	7·9	3·5
2·0	1·047	1·070	1·8	0·7	1·009	6·6	3·0
4·0	1·055	1·063	1·2	0·5	1·013	5·2	3·3
				Females			
0	1·062	1·067	1·2	0·6	1·007	5·6	2·8
0·6	1·065	1·060	0·8	0·7	1·011	3·6	2·9
2·0	1·060	1·059	0·9	0·7	1·007	4·7	3·6
4·0	1·067	1·061	0·9	0·6	1·009	4·4	4·7

Values are means for groups of 12 rats. No differences between treated and control animals were found at wk 2 and 6.

throughout the study and at wk 13 the same effect was seen in males fed on the 2·0% diet. An increase in relative testis weight was seen at wk 2 at the highest dietary level.

The only histopathological changes seen were slight renal tubular dilatation and lymphocyte cuffing of the bronchi. The incidence of these findings was greater in the control rats than in those fed for 13 wk on the diet containing 4·0% quillaia saponin.

DISCUSSION

The reduced body-weight gain seen in this study is in keeping with results previously reported for chickens (Newman *et al.* 1958; Peterson 1950) and rats (Coulson & Evans, 1960). In the present study, the reduced rate of body-weight gain was transitory; it was most marked in the first few days of feeding and its persistence was dose related. There were parallel changes in the intakes of food and water. Such a reduction in food intake and body-weight gain occurring in the early stages of a study with subsequent recovery suggests the intake of an unpalatable diet rather than a toxic effect. It is likely that the reduction in water intake is also related to the reduced food consumption since such reductions have been seen in starved rats (Cizek & Nocenti, 1965) and in rats fed reduced quantities of food (Strominger, 1947).

The lack of any effect on the haematological parameters and the normal osmotic fragility of the erythrocytes extends the work of Oser (1966) to cover higher dosage levels.

The decreases in organ weight are difficult to interpret as they were seen in only one sex and were not associated with any histopathological change. However, particularly in the case of the liver, these reductions were seen consistently throughout the study. They cannot be accounted for solely in terms of differences of body weight, as similar changes were evident in the relative organ weights. Thus, until evidence to the contrary is produced, the dietary levels producing changes in both absolute and relative organ weights must be regarded as having a toxic effect.

Table 4. *Mean organ weights of rats fed on diets containing 0–4% quillaia extract for 13 wk*

Sex and dietary level (%)	Organ weights												Body weight (g)
	Brain	Heart	Liver	Spleen	Stomach	Small intestine	Caecum	Kidneys	Adrenals†	Gonads†	Pituitary†	Thyroid†	
						Weight (g)							
Male													
0	1·90	1·37	12·39	0·79	1·65	8·57	0·99	2·86	65	3·87	10·3	19	468
0·6	1·94	1·32	11·21*	0·83	1·65	8·09	1·08	2·67	58	3·74	11·7	23	443
2·0	1·93	1·32	10·86**	0·82	1·76	8·00	1·10	2·28*	64	3·70	9·8	15	453
4·0	1·88	1·29	10·57***	0·82	1·75	7·97	1·07	2·55*	56*	3·66	10·8	20	438
Female													
0	1·81	0·92	6·51	0·60	1·29	6·25	0·82	1·69	69	101	12·6	16	281
0·6	1·80	0·90	6·32	0·61	1·27	6·32	0·82	1·65	66	99	13·4	19	282
2·0	1·83	0·86	6·29	0·58	1·30	6·21	0·84	1·59	67	99	11·7	19	270
4·0	1·77	0·87	6·68	0·58	1·39	6·17	0·89	1·55	58	104	11·9	14	271
						Relative weight (g/100 g body weight)							
Male													
0	0·41	0·29	2·64	0·17	0·35	1·83	0·21	0·61	14	0·82	2·2	4·1	
0·6	0·44	0·30	2·53	0·19	0·37	1·83	0·24	0·60	13	0·85	2·6	5·1*	
2·0	0·43	0·29	2·40***	0·18	0·39**	1·77	0·24	0·57*	14	0·82	2·2	3·2	
4·0	0·43*	0·30	2·41***	0·19	0·40**	1·82	0·24	0·58	13	0·84	2·5	4·5	
Female													
0	0·65	0·33	2·32	0·21	0·46	2·24	0·29	0·60	25	36	4·5	5·8	
0·6	0·64	0·32	2·24	0·22	0·45	2·24	0·29	0·59	23	35	4·7	6·9	
2·0	0·67	0·32	2·32	0·21	0·48	2·30	0·31	0·58	25	37	4·3	7·0*	
4·0	0·65	0·32	2·45	0·21	0·51**	2·28	0·33*	0·57*	20	39	4·4	5·0	

†Values for these organs are expressed in mg and mg/100 g body weight.
‡Values for female gonads are expressed in mg and mg/100 g body weight.
Values are means for groups of 15 rats. Those marked with asterisks differ significantly (Student's t test) from those of controls: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$.

Similarly the increased stomach weight seen at the two higher dosage levels must be regarded as an effect of the saponin. These increases may be due to a local irritant effect, and as such, are likely to depend on the concentration present in the diet rather than on the total amount consumed. As the maximum concentration used in human food is likely to be 200 ppm (Food Additives and Contaminants Committee, 1970) and this enlargement of the stomach was seen only with concentrations of 20,000 ppm or above, this is unlikely to represent a hazard for man. Although this increase in stomach weight may be associated with an irritant effect, no signs, such as diarrhoea, were seen during the study and irritation was not confirmed by the findings at autopsy or in the histopathological examination.

On the basis of the results of this study, the no-untoward-effect level for quillaia extract is 0·6% of the diet of rats, a level equivalent to an intake of approximately 400 mg/kg/day. The lowest level at which any effects were seen was 2·0%, equivalent to approximately 1200 mg/kg/day, so the true no-effect level lies somewhere between these two figures. This point may be settled when the results of the long-term studies are available.

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LONG-TERM TOXICITY STUDY OF QUILLAIA EXTRACT IN MICE

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Abstract—Groups of 48 male and 48 female mice were fed quillaia extract in the diet at levels of 0 (control), 0·1, 0·5 or 1·5% for 84 wk. The material had no adverse effect on the death rate or the incidence of histopathological findings, including tumours. However, there was a lower rate of body-weight gain at the 1·5% dietary level, and there were isolated statistically significant differences between the treated and control animals, mainly at the 1·5% dietary level, in the haematological examinations and in some absolute and relative organ weights of both sexes. It is concluded that, in mice, quillaia extract fed at levels up to 1·5% in the diet (approximately 2·2 g/kg/day) did not exert a carcinogenic effect. The no-untoward-effect level from this study is considered to be 0·5% in the diet, giving an intake of approximately 0·7 g quillaia extract/kg/day.

INTRODUCTION

Saponins are the glycosides of a number of related steroids or triterpenes. The most commonly found carbohydrate moeity is glucose, although galactose, arabinose, xylose and rhamnose have been identified in the wide range of saponins examined (Jackson & Shaw, 1959; Marker, Wagner, Ulshafer, Wittbecker, Goldsmith & Ruof, 1947). The saponins from quillaia species are of the triterpene glycoside type and in this material two major and two minor components have been identified (Cheeke, 1971; Coulson, 1958).

The amphipathic properties of saponins have led to the use of quillaia extract as an emulsifier and foaming agent, particularly in soft drinks. The material is also widely used in toiletry preparations and pharmaceuticals. Quillaia extract is permitted in the UK in soft drinks, at not more than 200 ppm (w/w) under The Emulsifiers and Stabilisers in Food Regulations 1975 (Statutory Instrument 1975, no. 1486). This followed recommendations from the Food Additives and Contaminants Committee (1970 & 1972) that the future inclusion of extracts prepared from quillaia of British Pharmacopoeia standard was provisionally acceptable for restricted food use subject to a requirement for further data from long-term studies.

The toxicological data on quillaia extract were reviewed by Gaunt, Grasso & Gangolli (1974), who found a no-untoward-effect level for quillaia of 0·6% of the diet (equivalent to an intake of approximately 400 mg/kg/day) in a short-term study in rats. The findings at the 2 and 4% dietary levels were confined to lower liver weights and higher stomach weights.

The present paper gives the results of a long-term study of the effects of quillaia extract in mice, carried out as part of a safety evaluation programme, which included short-term (Gaunt *et al.* 1974) and long-term work on this material in the rat.

EXPERIMENTAL

Materials. The quillaia extract used in this study was supplied by Food Industries Ltd., Bromborough Port, Wirral, Merseyside. It was a spray-dried aqueous extract of quillaia bark, prepared in such a manner that 100 parts by weight of bark yielded approximately 15 parts of extract before drying. The extract was stated to contain less than 10% moisture and less than 10% ash (at 550°C). It conformed to the requirements of The Emulsifiers and Stabilizers in Food Regulations 1975 (Statutory Instrument 1975, no. 1486).

Animals and diet. Mice of the TO strain obtained from a specified-pathogen-free breeding colony (A. Tuck & Son, Rayleigh, Essex) were used. They were housed at 21 ± 1°C in a relative humidity of 50–60%, and were given water and ground Oxoid pasteurized breeding diet supplemented with vitamin K, *ad lib.*

Experimental design and conduct. Groups of 48 male and 48 female mice were fed diets containing 0, 0·1, 0·5 or 1·5% quillaia extract for 84 wk. The males were caged singly and the females in groups of four. The condition and behaviour of the animals were observed frequently and sixteen of the individually caged mice were weighed at intervals. Any mouse showing signs of ill-health was closely observed and was killed if its condition deteriorated. An autopsy was conducted on all animals unless this was precluded by advanced autolysis.

At the end of the study the surviving animals were killed by exsanguination from the aorta under barbiturate anaesthesia. At autopsy, all macroscopic abnormalities were noted and the brain, heart, liver, kidneys, spleen, stomach, small intestine, caecum and testes were weighed. Samples of these organs, together with salivary gland, thyroid, adrenal glands, lymph nodes, aorta, pancreas, pituitary, prostate, seminal vesicles, ovaries, uterus, urinary bladder, lungs, colon, rectum, spinal cord, skeletal muscle, eye, Harderian gland and other tissues that appeared abnormal were preserved in 10% buffered formalin. Paraffin-wax sections of these tissues were stained with haematoxylin and eosin for microscopic examination.

Blood samples were collected from a caudal vein of each of ten male and ten female mice from the

Table 1. *Cumulative mortality record of mice fed diets containing 0–1·5% quillaia extract for 84 wk*

	Cumulative mortality in							
	Males given dietary levels (%) of				Females given dietary levels (%) of			
Wk	0	0·1	0·5	1·5	0	0·1	0·5	1·5
26	0	1	0	0	0	0	1	0
52	4	3	3	1	0	4	3	2
64	10	6	6	4	2	8	5	4
72	12	10	9	5	4	10	6	9
80	16	18	12	9	9	11	10	12

Figures represent the number of mice dead or killed *in extremis* from groups of 48. There was no significant positive trend in cumulative mortality due to treatment ($P > 0·05$) using the logrank conditionally expected numbers method (Peto & Pike, 1973).

control group and those on the two higher dietary levels at wk 26 and 54 and from all surviving mice at wk 84. The blood samples were examined for haemoglobin concentration and packed cell volume, and counts were made of reticulocytes and total erythrocytes and leucocytes.

RESULTS

The ingestion of quillaia extract had no effect on the condition or behaviour of the animals. There were deaths in all groups during the course of this study, but there was no relationship between the number of deaths at any time and the dietary level of quillaia extract (Table 1). The body weights of all treated males (Table 2) were similar to those of the controls at each weighing, but overall there was a slightly lower gain in weight in the males given 1·5% quillaia extract. The difference between this group and the controls was statistically significant at autopsy.

The weights of the testes were lower ($p < 0·05$) in the mice fed 0·5 or 1·5% quillaia extract, but the differences from the control value were not statistically significant when the weights were expressed relative to body weight (Table 3). Other isolated organ weights differed significantly from those of the controls, but in each case the differences occurred in one sex only and were not apparent in both the absolute and relative figures. However, they all occurred in the mice that received the highest dietary level. The significant absolute values were reduced liver and kidney weights in males and a high small-intestine weight in females.

The erythrocyte counts were lower in some of the animals given 0·5 or 1·5% quillaia extract in the diet than in their controls, but these findings were not dose-related and were not accompanied by higher reticulocyte counts (Table 4). Also at wk 84 there was a higher packed cell volume in males fed the 1·5% dietary level, while this value was lower in the females given the 0·5% level. There were no significant differences from the corresponding control values in the other haematological measurements.

The histopathological findings are summarized in Table 5. There were no significant differences between treated and control mice in the incidence of the lesions and there was a tendency for fewer lesions to be seen in the group on the highest level of quillaia. The tumours found are listed in Table 6. A number of malignant tumours were detected, but these were mainly in the control groups. No malignant tumours were found in any of the treated female mice and none occurred more frequently in the treated than in the control males. The total incidence of malignant tumours in the three groups of treated mice was less than that in the controls. The most frequently found benign tumours were those of the lung and Harderian gland, but in no instance was there any evidence of a dose-related effect on tumour incidence or of a statistically significant incidence in the treated animals compared to the controls. In addition the total incidence of these benign tumours was similar in all groups.

Table 2. *Mean body weight of male mice fed diets containing 0–1·5% quillaia extract for 84 wk*

	Body weight (g) at wk								
Dietary level (%)	0†	1	4	10	14	28	40	57	84
0	30	32	37	41	45	52	56	59	58
0.1	31	32	36	40	44	52	58	60	58
0·5	30	31	35	41	45	52	59	61	59
1·5	32	32	35	38	42	50	56	58	56

† Value on first day of feeding.
The figures are means for 16 mice and those for treated animals did not differ significantly ($P > 0·05$ by Student's t test) from those of the controls.

Table 3. *Relative organ weights of mice fed diets containing 0–1·5% quillaia extract for 84 wk*

Organ	Relative organ weight (g/100 g body weight)			
Dietary level (%)...	0	0·1	0·5	1·5
Males				
Brain	0·85	0·88	0·91	0·96*
Heart	0·48	0·52	0·50	0·51
Liver	3·67	3·75	3·85	3·75
Spleen	0·26	0·32	0·38	0·25
Kidney	1·27	1·31	1·31	1·28
Stomach	0·71	0·75	0·77	0·82*
Small intestine	4·12	4·31	4·19	4·37
Caecum	0·38	0·40	0·42	0·41
Testes	0·57	0·55	0·53	0·57
Terminal body weight (g)...	53	51	51	47*
Females				
Brain	1·00	1·04	1·01	1·03
Heart	0·39	0·39	0·40	0·42
Liver	4·07	3·95	4·14	4·14
Spleen	0·41	0·31	0·46	0·41
Kidney	1·03	1·03	1·04	1·04
Stomach	0·93	0·91	0·85	0·97
Small intestine	3·80	3·95	3·92	4·56
Caecum	0·46	0·49	0·44	0·47
Terminal body weight (g)...	46	43	45	43

Figures are means for groups of 31 males or 37 females and those marked with an asterisk differ significantly (Student's *t* test) from those of the controls: $*P < 0·05$.

DISCUSSION

It is notoriously difficult to detect marginal differences in rates of body-weight gain in mice in view of their small size and relatively small weight gain. Nevertheless the weight gain of the small sample of mice fed 1·5% quillaia extract was slightly less (15%) than that of the controls during the study. When all the surviving mice were weighed during the post-mortem examination, the same group showed a significantly lower body weight than the controls. On this evidence, some slight effect on the body weight

Table 4. *Results of haematological examinations in mice fed diets containing 0–1·5% quillaia extract for 26 or 84 wk*

Sex and dietary level (%)	No. of mice examined	Hb (g/100 ml)	PCV (%)	RBC (10^6/mm^3)	Retics (% of REC)	WBC (10^3/mm^3)
			Wk 26			
Male						
0	10	17·2	53	9·98	1·7	15·1
0·5	10	15·4	49	8·30*	1·9	14·5
1·5	10	16·9	54	8·82**	1·8	16·5
Female						
0	10	16·4	53	9·53	1·0	12·2
0·5	10	16·6	53	9·06	1·4	14·7
1·5	10	16·0	51	8·55**	1·4	14·8
			Wk 84			
Male						
0	31	14·6	47	7·68	2·1	11·4
0·1	28	14·5	45	7·07	3·1	12·1
0·5	33	14·3	45	6·71**	2·2	10·5
1·5	37	15·6	50*	7·78	1·4	12·6
Female						
0	37	15·1	49	8·50	2·1	10·2
0·1	35	14·8	48	8·01	2·1	9·4
0·5	33	14·3	46*	8·09	1·8	11·5
1·5	34	14·6	47	8·07	2·6	10·2

Hb = Haemoglobin, PCV = Packed cell volume, RBC = Red blood cells, Retics = Reticulocytes, WBC = White blood cells

Figures are means for the numbers of mice shown and those marked with asterisks differ significantly (Student's *t* test) from those of the controls: $*P < 0·05$; $**P < 0·01$.

Table 5. *Incidence of histopathological findings (excluding tumours) found in mice fed diets containing 0–1·5% quillaia extract for 84 wk*

Tissue and histological finding	No. of mice affected							
	Males fed dietary level (%) of				Females fed dietary level (%) of			
	0	0·1	0·5	1·5	0	0·1	0·5	1·5
No. of mice examined...	45	42	41	43	44	43	43	46
Lung								
Chronic inflammatory infiltration	1	0	0	1	0	0	0	0
Liver								
Focal areas of vacuolation	12	12	5	14	23	16	22	14
Severe fatty change	5	3	9	3	7	9	5	1
Necrotic foci	11	5	5	1	3	2	0	0
Amyloid	2	3	0	0	0	0	0	0
Kidney								
Degenerative changes	11	13	9	9	13	5	7	3
Bladder								
Foci of chronic inflammation	4	3	1	2	2	0	1	0
Heart								
Chronic degenerative changes	2	1	2	0	0	0	0	0
Gastro-intestinal tract								
Chronic inflamation and degenerative changes	0	2	1	3	1	0	0	0
Testes								
Atrophy	3	1	1	1	—	—	—	—
Foci of chronic inflammation in seminal vesicles	3	3	1	0	—	—	—	—
Ovary								
Follicular cyst	—	—	—	—	5	6	10	8
Non-specific								
Chronic subcutaneous abscess	1	0	1	4	0	0	0	1
Calcified areas in tissues	5	3	2	1	3	1	0	0

The figures for the treated groups were not significantly greater ($P > 0.05$ by the chi-square test) than those of the controls.

at this highest treatment level cannot be excluded. This material has been reported to reduce the rate of body-weight gain when fed to rats and chickens (Coulson & Evans, 1960; Newman, Kummerow & Scott, 1958; Peterson, 1950). In the previous study in rats (Gaunt *et al.* 1974), a transitory effect on the rate of body-weight gain was observed but was attributed to the unpalatability of the diet rather than to a toxic effect. It was further suggested that the effect might have been due to local irritation of the gastro-intestinal tract by the high concentrations (2 or 4%). Food intake was not measured in the present study, but the similarity of the dietary levels in this and the rat study suggests the possibility of a similar cause in both species, although, as in the rats, there was no evidence in the mice of any direct effect on the gastro-intestinal tract. Gaunt *et al.* (1974) suggested that any direct effects on the alimentary tract are likely to be concentration- rather than dose-related and hence of little relevance to the assessment of risk in man.

Following treatment at the highest level, there were isolated statistically significant changes in the weights of the liver and kidneys in males and in the small intestine in females, and there were also increased relative brain and stomach weights in the male mice. Lower liver and higher gastro-intestinal weights were reported in the short-term rat study (Gaunt *et al.* 1974) and therefore it is possible that they represent a toxic effect although, in the present study, no pathological changes were seen on histological examination in the gastro-intestinal tract.

There were slightly lower testicular weights in the mice given 0·5 or 1·5% quillaia extract in the diet compared with the controls. However, this effect was not apparent when the weights were expressed relative to body weight and there was no evidence of pathological changes on histological examination. Thus these observations are unlikely to be of toxicological significance.

The absence of any malignant tumours in the treated female mice and the low incidence in the males given quillaia extract indicates a lack of any carcinogenic effect. The similarity of the incidences of benign tumours in all the groups is also indicative of a lack of carcinogenic effect. Consideration of the individual tumours does not suggest any specific effect on any organ. The tumours found in treated mice without comparable findings in the controls were of low incidence generally, only one tumour being found. This is suggestive of a spontaneous occurrence. This is supported by the general similarity of the tumour incidence in the present experiment to that expected in old mice (Cloudman, 1956; Tucker & Baker, 1967). The occasional sebaceous adenoma, for example, may represent a spontaneous occurrence, since this tumour has been reported by Murphy (1966) to occur spontaneously in mice.

Table 6. *Incidence of tumours in mice fed diets containing 0–1·5% quillaia extract for 84 wk*

Tissue and tumour	No. of mice affected							
	Males fed dietary levels (%) of				Females fed dietary levels (%) of			
	0	0·1	0·5	1·5	0	0·1	0·5	1·5
No. of mice examined...	45	42	41	43	44	43	43	46
Liver								
Malignant hepatoma	5	1	2	1	0	0	0	0
Haemangioma	1	0	0	0	0	0	0	0
Haemangiosarcoma	1	0	0	0	0	0	0	0
Hyperplastic nodules	8	4	3	6	1	2	1	0
Lung								
Papillary adenoma	7	3	7	12	9	5	4	5
Pituitary								
Adenoma	0	0	0	0	1	1	0	0
Harderian gland								
Adenoma	2	1	1	0	3	0	0	1
Stomach								
Fibrosarcoma	0	0	0	0	1	0	0	0
Uterus								
Fibromyoma	—	—	—	—	1	1	1	0
Adenocarcinoma	—	—	—	—	1	0	0	0
Ovary								
Granulosa cell tumour	—	—	—	—	0	1	0	0
Testis								
Interstitial cell tumour	0	0	1	0	—	—	—	—
Breast								
Adenocarcinoma	—	—	—	—	1	0	0	0
Reticulo-endothelial system								
Sarcoma in lymph node	1	0	0	0	0	0	0	0
Skin and subcutaneous tissue								
Squamous papilloma	0	0	1	0	0	0	0	0
Subcutaneous fibroma	1	0	0	0	0	0	0	0
Subcutaneous fibrosarcoma	0	0	0	0	1	0	0	0
Sebaceous gland adenoma	0	0	0	0	0	0	0	1
Sebaceous gland adenocarcinoma	0	0	0	0	1	0	0	0

The figures represent the incidence of tumours in the numbers of mice shown; those for the treated groups were not significantly greater ($P > 0.05$ by the chi-square test) than those of the controls.

On the basis of the results of this study in mice, there is no evidence that quillaia extract has a carcinogenic effect when given at dietary levels up to 1·5%, a level providing an intake of approximately 2·2 g/kg/day. However, the slightly lower rate of body-weight gain in the mice on the highest dietary level and some organ-weight changes, albeit of doubtful significance, indicate a no-untoward-effect level for quillaia extract of 0·5% of the diet in this study, providing an intake of approximately 700 mg/kg/day.

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LONG-TERM TOXICITY STUDY OF QUILLAIA EXTRACT IN RATS*

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Abstract—Groups of 48 male and 48 female rats were fed quillaia extract in the diet at levels of 0 (control), 0·3, 1·0 and 3·0% for 2 yr. The material had no adverse effects on death rate, water consumption, serum chemistry or haematological parameters or on the incidence of histopathological findings, including tumours. In the males given the 3% dietary level, the death rate, total leucocyte count at wk 108, incidence of kidney lesions and weights of the kidneys, heart and thyroid were all below the control values. These differences were explicable, however, in terms of a lowered body weight consequent on a decreased food intake. It is concluded that, in rats, quillaia extract fed at levels up to 3·0% in the diet did not have any carcinogenic effect. The no-untoward-effect level established in this study was 3·0% in the diet, approximately equivalent to an intake of 1·5 g/kg/day.

INTRODUCTION

Quillaia extract, an aqueous extract of the dried inner part of the bark of *Quillaia saponaria* Molina and other species of Quillaia (Rosaceae), contains three or possibly four saponins (two major, one minor and one trace constituent) together accounting for about 10% of the extract. Glucose, galactose, arabinose, xylose, rhamnose and two further unidentified sugars are also present. The two major saponins are quillaia sapogenin, which has a triterpenoid structure, and quillaic acid (EEC Scientific Committeee for Food, 1978). The amphipathic properties of saponins, a reflection of the water solubility of the carbohydrate moiety and the lipid solubility of the sapogenin, account for their use as foaming agents and emulsifiers.

In the UK, the Emulsifiers and Stabilisers in Food Regulations 1980 (Statutory Instrument 1980, no. 1833) restrict the use of quillaia extracts to soft drinks, at not more than 200 ppm by weight. This measure endorses the recommendation of the Food Additives and Contaminants Committee (1970 & 1972) that quillaia of British Pharmacopoeial standard should be provisionally acceptable for restricted use in food. The Committee stressed the need for further toxicological data, including long-term feeding studies and studies on possible intestinal irritation. The EEC Scientific Committee for Food (1978) established an acceptable daily intake of 5 mg/kg body weight for the spray-dried extract. Quillaia no longer comes within the scope of the EEC directive on food emulsifiers, stabilizers, thickeners and gelling agents (*Off. J. Europ. Commun.* 1980, **23** (L155), 23); instead it is subject to national regulations.

Gaunt, Grasso & Gangolli (1974) reviewed the toxicological status of quillaia and presented the results of a short-term study in rats. Relative liver weight was reduced in males given 2·0 or 4·0% quillaia in that study, the relative stomach weight was increased in both sexes at the same levels and a no-untoward-effect level of 0·6% in the diet was demonstrated. A long-term study in mice showed no carcinogenic response with dietary levels of up to 1·5% quillaia extract and established a no-untoward-effect level of 0·5% (Phillips, Butterworth, Gaunt *et al.* 1979). This paper describes the final part of this safety evaluation programme, a study involving the long-term dietary administration of quillaia extract to rats.

EXPERIMENTAL

Materials. The sample of quillaia extract used in these studies was supplied by Food Industries Ltd, Bromborough Port, Merseyside. It was a spray-dried aqueous extract of quillaia bark, prepared in such a way that 100 parts by weight of bark yielded approximately 15–18 parts of powdered extract. The extract was stated to contain less that 10% moisture and less than 10% ash (at 550 C). The specification conformed to that given in the UK Emulsifiers and Stabilisers in Food Regulations 1975 (Statutory Instrument 1975, no. 1486) and to that in the British Pharmacopoeia (1973).

Animals. Weanling rats of a Wistar-derived strain from a specified-pathogen-free breeding colony (A. Tuck and Son, Rayleigh, Essex) were housed in an air-conditioned room maintained at 20 ± 2°C and were given Spratt's Laboratory Animal Diet No. 1 and water *ad lib.*

Experimental design and conduct

Acceptability of test diets. Two short diet-acceptability studies were conducted. In the first, male rats (255–275 g body weight) were housed in pairs in individual plastics cages with access to both control diet and a second diet containing 0·3, 1·0 or 3·0% quillaia

*Further details of the data from this study are tabulated in BIBRA Research Report No. 1/1978, which can be obtained on request.

extract. The amount of each diet consumed was recorded daily for 21 days. In the second experiment, pairs of male rats (358–395 g body weight) were fed on diet containing either 0 (control), 0·3, 1·0 or 3·0% quillaia extract for 7 days, with daily measurement of body weight and food intake.

Long-term feeding study. Groups of 48 male and 48 female rats were housed four in a cage and fed on diets containing 0 (control), 0·3, 1·0 or 3·0% quillaia extract for 2 yr. They were observed regularly and any rats showing signs of ill health were isolated. These were returned to their cage if their condition improved; otherwise they were killed and autopsied. The rats were weighed at approximately 2-monthly intervals up to 2 yr and the consumption of food and water was measured for the 24-hr period prior to body-weight determinations. At wk 15, 25 and 52, blood was collected from the tail veins of ten male and ten female rats from each of the groups fed diets containing 0, 1·0 or 3·0% quillaia extract. After 108 wk, blood was collected from the aorta of all remaining animals during the post-mortem examination. On all samples, measurements were made of haemoglobin concentration and packed cell volume, together with total erythrocyte and leucocyte counts. Slides for differential leucocyte counts were prepared for all samples, but only those from the control and 3·0% dietary groups were examined.

At wk 13, 24 and 78, urine was collected from ten rats from the controls and the highest level of treatment. It was examined for appearance and microscopic constituents, and semi-quantitative tests for the content of protein, glucose, ketones, bile salts and blood were carried out. Concentration and dilution tests were carried out at these times, involving measurement of the specific gravity and volume of urine produced in a 6-hr period without water, over a 4-hr period commencing after 16 hr without water and over a 2-hr period following a water load of 25 ml/kg body weight. The number of cells present was counted in the 2-hr sample.

Serum separated from blood collected at autopsy was examined for contents of urea, glucose, total protein and albumin, and the activities of glutamic–oxalacetic transaminase, glutamic–pyruvic transaminase and lactate dehydrogenase were determined.

Animals that died during the study were autopsied unless this was precluded by advanced autolysis or cannibalism. Those found *in extremis* during the study or surviving beyond wk 106 were killed by exsanguination from the aorta under barbiturate anaesthesia and examined for macroscopic abnormalities. Where possible the animals were deprived of food overnight before killing and the brain, heart, liver, spleen, kidney, stomach, small intestine, caecum (with and without its contents), adrenal glands, gonads, pituitary and thyroid were weighed. Samples of these tissues, together with salivary glands, thymus, various lymph nodes, pancreas, aorta, nasal bones, lungs, trachea, oesophagus when present, colon, rectum, skeletal muscle, spinal cord, sciatic nerve, uterus or prostate and seminal vesicles, urinary bladder, mammary tissue, eye and Harderian gland, and other tissues if they appeared abnormal, were preserved in buffered formalin. Paraffin-wax sections of all available tissues except nasal bones were stained with haematoxylin and eosin for histological examination. Sections of the nasal bones were prepared from the animals on the highest dietary level. Samples of femoral bone marrows were stained, but were not examined in view of the absence of other haematological findings.

RESULTS

Acceptability tests

Rats offered the choice between the control and the 0·3% quillaia diet consumed on average 23 g of the control diet and only 1·3 g of the test diet daily. The corresponding average daily intakes were 23·6 g (control) and 2·0 g (test) when the 1·0% quillaia diet was the alternative and 27·4 g (control) and 0·8 g (test) with 3·0% quillaia. When there was no choice of diet, the average intakes over 7 days were 25, 29, 26 and 21 g/day for rats given diet containing 0, 0·3, 1·0 and 3·0% quillaia, respectively. The corresponding weight gains were 26, 29, 30 and 11 g.

Long-term feeding study

Rats given up to 3% quillaia extract in the diet appeared normal. In the male rats fed 1% of the extract, the number of deaths exceeded those among control animals from wk 73 onwards. However, the values were statistically significant only between wk 85 and 91. There was a trend towards a lower number of deaths in male rats fed 3% quillaia extract (Table 1), the difference being statistically significant at wk 97 and 99.

Male rats fed the highest dietary level had lower body weights throughout the experiment than did control animals (Table 2), the differences being statistically significant in the examinations covering the period 10–22 months. Females on the lowest dietary level had significantly higher body weights during the first 6 months of the study.

In the group of male rats fed 3% quillaia extract in the diet, food consumption was consistently lower than that of the controls (Table 3) and water consumption was lower than in the controls from wk 71 (Table 3). During this latter period, the water intake of the males given 3% quillaia extract remained approximately constant whereas that of the controls increased markedly. The calculated daily intakes of quillaia extract are also shown in Table 3.

At wk 13 and 24 the results of the renal concentration and dilution tests showed no statistically significant differences between the control rats and those given 3% quillaia extract. At wk 78, the urine produced in the 4-hr period following water deprivation for 16 hr by male rats fed 3% quillaia extract was of a higher specific gravity than that of the controls (Table 4). This difference was not observed in females nor in male rats at any other time. The cell excretion rates were similar in the treated and control rats.

There were no statistically significant differences between treated and control animals in the results of serum analyses. The only statistically significant findings ($P < 0·05$) in the red-cell parameters were an increase in haemoglobin level at wk 15 in males fed 1% quillaia (17·5 g/100 ml *v.* 16·5 g/100 ml in the controls) and an increased erythrocyte count at wk 108 in females on the same diet (7·60 *v.* 7·18 million/mm^3). There was no trend in other determinations to sup-

Table 1. *Cumulative totals of deaths in rats fed diets containing 0–3% quillaia extract for up to 2 yr*

Duration of test (wk)	Dietary level (%)...	Total no. of deaths							
		Males				Females			
		0	0·3	1·0	3·0	0	0·3	1·0	3·0
79		2	3	4	1	6	4	4	2
83		2	3	6	1	8	6	5	3
87		3	3	13**	2	9	7	6	7
91		6	5	15*	3	9	9	9	9
95		9	7	17	3	10	10	9	9
99		14	7	19	7*	12	10	9	10
103		16	13	20	9	13	13	10	14

The figures are the total numbers of animals dead or killed *in extremis* from groups of 48. Those marked with asterisks differ significantly from the corresponding control value (chi-square test): $*P < 0.05$; $**P < 0.01$.

port either of these isolated findings. Total leucocyte counts were higher in all treated male rats examined at wk 15 and in those given 3% quillaia at wk 25 (Table 5). In contrast, these counts were lower at wk 108 in both sexes given the highest dietary level. There were no significant alterations in the differential counts except at wk 15, when the neutrophils showed an increase and the lymphocytes a decrease at the 3% level.

The weights of heart, kidneys and thyroid were lower than the control values in male rats fed 3% and thyroid weights were also lower among males fed 1% quillaia extract (Table 6). These differences were statistically significant, but no such differences were observed in the females. When the weights were expressed in relation to body weight (Table 6) the same organs showed low values compared with the controls although the differences did not reach statistical significance. In the females, weights of the small intestine and caecum were higher than in controls at both the highest and 0·3% dietary level. These groups also showed higher stomach weights. When expressed in relation to body weight, the weights of the stomach, intestine and caecum in these two groups of females remained higher than the control values, but the values for the stomach and small intestine were not statistically significant in the group on 0·3% quillaia. The only other significant changes in relative organ weights were a higher liver weight in females given the 3% level and a lower liver weight in males given 1%.

In general the incidence of histological findings was similar in treated and control animals. The only lesions with incidences greater than those of the controls were fibrosis of the heart and dilatation of the glands of the gastric mucosa in the females fed 0·3% extract (Table 7). A variety of benign and malignant tumours was found (Table 8). Some of the tumours occurred only in the treated groups, but the numbers were small, most were not found in the animals given the highest treatment level and no relation to dose was observed. Thus, for example, the incidences of

Table 2. *Mean body weights of rats fed diets containing 0–3% quillaia extract for up to 2 yr*

Duration of test (wk)	Body weight (g)							
	Males given dietary levels (%) of:				Females given dietary levels (%) of:			
	0	0·3	1·0	3·0	0	0·3	1·0	3·0
4	256	258	256	249*	172	179*	173	175
8	356	365	358	344	219	226*	220	219
16	452	458	454	440	259	269*	263	263
25	503	508	504	493	282	296*	289	288
34	550	554	547	528	302	317	310	305
42	598	604	591	568*	329	337	331	326
48	632	636	621	596*	350	362	356	345
54	655	663	649	618*	379	384	378	366
63	684	689	677	641**	404	407	403	395
71	703	700	697	657**	421	426	422	410
80	712	707	697	660**	435	445	445	417
89	683	670	689	637*	433	451	457	420
106	662	683	662	623	431	445	462	417

Results are means for all survivors in each group. Those marked with asterisks differ significantly (Student's t test) from their corresponding controls: $*P < 0.05$; $**P < 0.01$.

Table 3. *Mean food and water consumption and calculated intake of emulsifier by rats fed diets containing 0–3% quillaia extract for up to 2 yr*

Dietary level (%)	Mean intakes at wk: 4	25	54	71	89	106
Food consumption (g/rat/day)						
Males						
0	23·0	21·5	21·1	20·2	18·7	16·8
0·3	22·7	20·2	19·1	19·1	18·8	16·3
1·0	22·0	18·3	18·8	18·8	16·8	18·8
3·0	21·1	19·7	18·7	18·4	18·8	14·5
Females						
0	17·5	17·1	16·8	14·6	14·8	14·7
0·3	16·4	17·1	15·8	15·0	14·1	13·3
1·0	16·1	15·6	15·5	13·3	13·7	17·0
3·0	16·6	14·8	16·0	14·8	13·8	13·6
Water consumption (ml/rat/day)						
Males						
0	31·1	28·1	24·9	31·3	37·3	37·2
0·3	32·2	27·9	28·4	29·6	33·0	30·9
1·0	29·6	25·2	29·1	31·0	33·1	34·5
3·0	31·1	29·5	29·4	27·1	29·4	30·9
Females						
0	27·2	27·7	30·8	30·9	32·9	37·3
0·3	32·5	28·9	30·4	30·8	38·1	34·6
1·0	29·8	29·3	31·4	30·4	33·0	36·6
3·0	29·6	29·6	31·0	34·0	34·3	36·0
Quillaia intake (mg/kg/day)						
Males						
0·3	260	120	90	80	100	70
1·0	780	360	310	290	320	280
3·0	2540	1200	960	810	838	700
Females						
0·3	280	170	130	110	100	90
1·0	930	540	460	350	330	370
3·0	2850	1540	1380	1140	1110	980

haemangiomas and haemangiosarcomas in the lymph nodes were similar in both control and treated animals. The one tumour showing a statistical difference from the control incidence was thyroid adenoma which occurred more frequently in females given 1% quillaia extract in the diet.

DISCUSSION

Many of the differences between the male rats given 3% quillaia extract and the control males may be accounted for in terms of the lower body weights recorded in the former group from wk 42. These include lower mortality, a higher concentrating ability in the renal function test at wk 78, together with a lower incidence of the more severe renal lesions, and decreased kidney, heart and thyroid weights.

It is known that animals on a reduced food intake show a prolonged lifespan (Gaunt, Hardy, Grasso *et al.* 1976; Ross & Bras, 1971; Simms, 1967). Moreover it has been established by other workers (Simms, 1967) and seen in this laboratory (Gaunt *et al.* 1976) that rats with a reduced body weight have a delayed onset of glomerulonephrosis. This was evident in the present study, since the incidence of animals with marked glomerulonephrosis was lower at the highest dietary level than in the controls. This difference could account for the lower water intake during the last 6 months of the study, the higher urinary specific gravity at wk 78 and the lower kidney weights. The urinary specific gravity of the treated rats at wk 78 was in fact similar to that recorded in both treated and control groups at earlier examinations, whereas the control value had decreased by wk 78. One result of nephrosis is left ventricular hypertrophy (Berg, 1967) and thus the delayed onset of the renal changes could account also for the lower heart weights in the treated rats. Snell (1967) showed that parathyroid hyperplasia occurs in animals with chronic nephrosis and since the parathyroid glands are included in the thyroid weights it is possible that a delay in this hyperplasia accounts for the differences recorded in the thyroid weights.

The lower body weights and associated findings in the rats fed 3% quillaia are ascribable to reduced food consumption, which was probably due to the unpalatability of the diet. The test of preference showed that diets containing quillaia extract were avoided and, in a 90-day experiment, Gaunt *et al.* (1974) found that the food intakes of rats given 2 or 4% quillaia extract were markedly reduced on the first day of treatment.

Table 4. *Mean renal concentration values following 16-hr water deprivation in rats fed diets containing 0–3% quillaia extract for 13, 24 or 78 wk*

Dietary level (%)	Urine values† At wk 13 Specific gravity	At wk 13 Volume (ml)	At wk 24 Specific gravity	At wk 24 Volume (ml)	At wk 78 Specific gravity	At wk 78 Volume (ml)
			Males			
0	1·078	0·1	1·072	0·2	1·055	1·1
3·0	1·063	0·1	1·075	0·2	1·075**	0·7
			Females			
0	1·084	0·1	1·065	0·2	1·067	0·7
3·0	1·082	0·1	1·090	0·1	1·078	0·2

†For 4-hr samples collected after a 16-hr period without water. Results are means for groups of ten rats. The value marked with asterisks differs significantly ($P < 0·01$; White, 1952) from the control value.

Tests for glucose, bile salts, blood and ketones were negative. Cell excretion rates, protein levels and microscopic constituents were similar in control and test samples.

Table 5. *Leucocyte counts in rats fed diets containing 0–3% quillaia extract for 15, 25, 52 or 108 wk*

Dose level (%)	No. of rats	Leucocytes: Total (10^3/mm^3)	Differential (%): N	E	L	M
Wk 15						
Males						
0	10	13·8	12	1	86	1
1	10	19·5*	—	—	—	—
3	10	23·0**	17**	1	80**	2
Females						
0	10	12·4	15	0	83	2
1	10	13·8	—	—	—	—
3	10	12·4	12	0	86	0
Wk 25						
Males						
0	10	13·8	21	1	78	0
1	10	12·2	—	—	—	—
3	10	18·5**	17	1	80	0
Females						
0	10	12·4	21	1	77	0
1	10	13·4	—	—	—	—
3	10	13·2	16	0	84	0
Wk 52						
Males						
0	10	14·1	20	0	77	1
1	10	13·8	—	—	—	—
3	10	14·8	21	0	76	2
Females						
0	10	10·2	15	1	82	1
1	10	10·3	—	—	—	—
3	10	10·3	12	1	86	1
Wk 108						
Males						
0	29	5·0	38	0	58	1
0·3	30	5·0	—	—	—	—
1	22	4·6	—	—	—	—
3	37	3·9*	37	1	61	0
Females						
0	31	3·3	36	0	62	0
0·3	33	3·5	—	—	—	—
1	37	3·0	—	—	—	—
3	34	2·6*	35	1	64	0

N = Neutrophils E = Eosinophils L = Lymphocytes M = Monocytes

Values marked with asterisks differ significantly (Student's *t* test) from those for the corresponding controls: $*P < 0.05$; $**P < 0.01$. The arcsin transformation was used for percentage values. Basophils did not account for more than 0·5% of the leucocytes in any group.

a finding confirmed in the present study when 3% extract was given in the diet. These factors support the suggestion that the diet was unpalatable and the changes observed cannot be considered to be a sign of toxicity.

The observed significantly increased incidence of mortality in male animals given 1% extract in the diet during wk 87–91 was not accompanied by any consistent pathological changes either during that time or in the subsequent treatment period. Furthermore, female rats in either the 1 or 3% group showed no increased mortality. Therefore, this increase in male mortality is considered to be unrelated to treatment.

At the end of the study the total white cell counts of all groups including the controls were less than the earlier values. This is because the latter counts were performed on peripheral blood obtained from the tail veins whereas at wk 108 the blood was taken from the aorta. This difference has been observed in other studies (Evans, Butterworth, Gaunt & Grasso, 1977; Mason, Gaunt, Hardy *et al.* 1976). The differences in total leucocyte counts between the treated and control groups are not readily interpretable. The reduction in the total counts seen at wk 108 in animals of both sexes given 3% extract in the diet is probably a reflection of the decreased growth rate, as reported previously (Gaunt *et al.* 1976; Oishi, Oishi & Hiraga, 1979). The statistically significant increases at wk 15 and 25 were confined to the male animals and were not recorded at subsequent treatment periods. Furthermore, this finding at wk 15 is at variance with the results of an earlier 90-day study (Gaunt *et al.* 1974). In the light of these inconsistencies, these findings are not considered to be related to treatment.

The relative liver weights were lower in males fed 1% quillaia extract and higher in females given the 3% dietary level. This lack of consistency again supports the view that these findings cannot be attributed to treatment. The same comment can be made about the significant increases in weights of stomach, small intestine and caecum (full and empty) in the females since these occurred at the lowest and highest dietary levels, while the intermediate dose level showed no change from the controls, and no corresponding differences were observed in the males. Microscopic examination provided no evidence of irritation of the alimentary tract.

The slight increases in the incidence of heart fibrosis and dilatation of the glands of the gastric mucosa were evident only at the lowest treatment level in the females. This lack of any dose relationship, despite a ten-fold increase in the amount of test material given at the highest dietary level, and the lack of similar effects in the males, suggests that these are fortuitous variations of normal incidence rather than any effect of treatment.

It is relevant that the types of tumours seen in our treated rats have been reported elsewhere in untreated animals (Table 9). Furthermore no dose-related incidence of tumours was found in this study, and with one exception (the thyroid adenomas in the females fed 1% quillaia) there were no statistically significant increases in the numbers of any type of tumour compared with the control incidence. Even this exception was not dose-related, fewer being found at the higher dose level in the female rats while the incidence in all the treated male groups was lower than that in the controls. The total incidence of thyroid adenomas in both sexes fed 1% quillaia extract in their diet was not statistically different from the total control incidence. This evidence together with the known spontaneous occurrence of these tumours (Table 9) indicates that this minor increase was not related to quillaia treatment. The finding of haemangiomas and haemangiosarcomas of the lymph nodes was unusual, since these

Table 6. *Organ weights and relative organ weights of rats fed diets containing 0–3% quillaia extract for 2 yr*

Dietary level (%)	No. of rats examined	Organ													Terminal body weight (g)
		Brain	Heart	Liver	Spleen	Kidneys	Stomach	Small intestine	Caecum Full	Caecum Empty	Adrenals†	Gonads‡	Pituitary†	Thyroid†	
Organ weight (g)															
Males															
0	32	2·11	1·53	15·18	1·23	3·88	2·58	9·57	3·59	1·29	62	3·37	10·9	27·8	594
0·3	32	2·12	1·55	15·83	1·26	3·88	2·54	9·89	3·49	1·33	62	3·54	10·7	25·9	616
1·0	23	2·12	1·49	14·57	1·34	3·84	2·54	9·22	3·34	1·24	62	3·63	10·4	25·7*	633
3·0	39	2·10	1·44*	14·47	1·15	3·32*	2·41	9·69	3·49	1·37	57	3·55	10·7	23·5*	583
Females															
0	32	1·93	1·12	10·55	0·85	2·36	1·75	7·33	2·69	0·87	67	74	11·7	19·0	395
0·3	35	1·94	1·18	11·50	0·84	2·52	1·97*	8·15*	3·25*	1·09***	68	68	12·5	21·0	392
1·0	38	1·94	1·10	11·07	0·94	2·27	1·78	7·43	2·91	0·91	69	75	13·8	19·1	421
3·0	34	1·89	1·07	11·42	0·82	2·30	1·95	8·17**	3·45**	1·11***	61	68	12·7	21·2	377
Relative weight (g/100 g body weight)															
Males															
0	32	0·37	0·27	2·61	0·21	0·69	0·46	1·65	0·62	0·22	11	58·2	1·9	4·8	
0·3	32	0·36	0·26	2·56	0·21	0·66	0·43	1·68	0·58	0·22	10	59·6	1·8	4·4	
1·0	23	0·34	0·24	2·33*	0·21	0·62	0·41	1·48	0·54	0·20	10	58·1	1·7	4·2	
3·0	39	0·37	0·25	2·49	0·20	0·58	0·42	1·67	0·60	0·24	10	61·6	1·8	4·1	
Females															
0	32	0·51	0·30	2·71	0·22	0·62	0·46	1·92	0·70	0·23	18	19·0	3·0	5·0	
0·3	35	0·52	0·31	3·00	0·22	0·67	0·53	2·13	0·86*	0·28***	18	17·4	3·2	5·7	
1·0	38	0·48	0·27	2·66	0·23	0·56	0·43	1·80	0·72	0·22	17	18·3	3·3	4·6	
3·0	34	0·51	0·29	3·05*	0·22	0·63	0·54*	2·22**	0·94**	0·30***	16	18·0	3·4	5·6	

†Absolute and relative weights of this organ are expressed in mg and mg/100 g body weight, respectively.
‡Absolute and relative weights of female gonads are expressed in mg and mg/100 g body weight, respectively.
Values are the means for the numbers of rats shown and those marked with asterisks differ significantly (Student's t test) from the control value: *$P < 0.05$; **$P < 0.01$; ***$P < 0.0001$.

Table 7. *Statistically significant histopathological findings (excluding tumours) in rats fed diets containing 0–3% quillaia extract for up to 2 yr*

Tissue and histological finding	Dietary level (%)...	Incidence of finding† Males 0	0·3	1·0	3·0	Females 0	0·3	1·0	3·0
Liver	*No. examined...*	*40*	*33*	*36*	*44*	*42*	*45*	*46*	*46*
Hepatocyte vacuolation and fatty change									
—mild/moderate		22	22	19	18	23	24	26	15*
—severe		3	2	2	3	4	7	3	1
Kidney	*No. examined...*	*39*	*36*	*38*	*42*	*42*	*45*	*46*	*44*
Glomerulonephrosis									
—mild/moderate		23	19	17	21	23	23	31	30
—severe		13	13	12	7	6	12	1	2
Calcium deposits		0	0	0	0	9	9	3*	4
Heart	*No. examined...*	*40*	*34*	*37*	*42*	*40*	*44*	*43*	*44*
Fibrosis		11	6	8	7	0	4*	2	2
Lung	*No. examined...*	*38*	*35*	*37*	*44*	*40*	*44*	*46*	*45*
Peribronchiole cuffing		0	0	0	0	6	6	4	1*
Spleen	*No. examined...*	*40*	*35*	*37*	*43*	*43*	*43*	*41*	*44*
Pigment		1	0	0	0	23	18	9**	18
Stomach	*No. examined...*	*40*	*33*	*37*	*43*	*42*	*42*	*44*	*44*
Glandular dilatation		1	4	0	1	2	9*	6	6
Parathyroid	*No. examined...*	*37*	*28*	*37*	*39*	*39*	*40*	*45*	*42*
Enlarged		5	3	4	3	8	5	1**	4
Vacuolated		0	0	1	0	1	0	0	0
Adrenal	*No. examined...*	*38*	*34*	*37*	*41*	*40*	*44*	*44*	*43*
Vascularization/haemorrhage		0	0	0	0	5	0*	0*	1
Harderian gland	*No. examined...*	*34*	*32*	*33*	*39*	*35*	*43*	*42*	*44*
Pigmentation		0	0	0	0	14	15	14	7*

†Incidence is expressed as the number of animals affected among the number examined (italicized). Values marked with asterisks differ significantly (chi-square test) from that for the corresponding control: $*P < 0.05$; $**P < 0.01$. No allowances were made for time of incidence.

Table 8. *Incidence of tumours in rats fed diets containing 0–3% quillaia extract for up to 2 yr*

Organ/tumour	Dietary level (%)...	No. of animals with tumours Males 0	0·3	1·0	3·0	Females 0	0·3	1·0	3·0
Liver	*No. examined...*	*40*	*33*	*26*	*44*	*42*	*45*	*46*	*46*
Small adenoma		0	0	0	0	0	0	0	1
Lung	*No. examined...*	*38*	*35*	*37*	*44*	*40*	*44*	*46*	*45*
Adenoma		0	0	0	0	0	1	0	0
Adenomatosis		0	0	0	0	0	1	0	0
Pancreas	*No. examined...*	*33*	*33*	*36*	*39*	*34*	*33*	*44*	*40*
Islet-cell adenoma		3	1	1	1	0	2	2	1
Adenocarcinoma (mucoid)		0	1†[a]	0	0	0	1	0	0
Exocrine adenoma		1	0	1	1	0	0	0	0
Pituitary	*No. examined...*	*30*	*29*	*34*	*35*	*36*	*39*	*40*	*42*
Adenoma		8	9	7	6	23	21	22	18
Carcinoma		0	0	0	1	0	0	1	3†[b]
Ileum	*No. examined...*	*39*	*32*	*35*	*42*	*41*	*44*	*44*	*45*
Adenocarcinoma		0	1	0	1	0	0	0	0
Leiomyoma		1	0	0	0	0	0	0	0
Other tumour		0	0	0	0	0	0	0	1
Adrenal	*No. examined...*	*38*	*34*	*37*	*41*	*40*	*44*	*44*	*43*
Medullary adenoma		0	1	0	3	0	0	0	1
Medullary carcinoma		0	0	1	1	0	0	0	0
Phaeochromocytoma		1	1	0	0	0	0	0	0
Cortical adenoma		0	0	0	1	0	0	0	0
Thyroid	*No. examined...*	*37*	*28*	*37*	*39*	*39*	*40*	*45*	*42*
Adenoma		5	3	3	3	0	2	5*	4
Carcinoma		1	0	1	0	0	0	1	0
Parathyroid adenoma		0	0	0	0	0	1	0	0

[*contd*]

Table 8 (*continued*)

Organ/tumour	Dietary level (%)...	No. of animals with tumours							
		Males				Females			
		0	0·3	1·0	3·0	0	0·3	1·0	3·0
Skin, subcutaneous tissue and mammary gland‡	*No. examined...*	*40*	*37*	*38*	*44*	*43*	*45*	*46*	*46*
Haemangioma		0	1	1	0	0	0	0	0
Fibroma		3	2	2	4	1	0	3	4
Fibrosarcoma		0	0	2	0	1	0	0	1
Lipoma		0	1	0	0	0	0	0	1
Adenocarcinoma		0	0	0	0	3	0	2	2
Osteosarcoma		1	0	0	0	0	0	0	0
Reticulum-cell sarcoma		0	0	1†[c]	0	0	0	0	0
Fibroadenoma/adenoma		0	0	1	0	15	14	8	11
Carcinoma—undifferentiated		0	0	0	0	0	0	2	0
—basal-cell		0	0	0	0	0	1	0	0
—squamous-cell		0	0	0	0	1	0	1	0
Thymus	*No. examined...*	*4*	*16*	*18*	*18*	*5*	*8*	*12*	*11*
Thymoma		1	0	0	0	0	0	0	0
Lymphoma		0	0	0	0	0	0	1	0
Lymphosarcoma		0	0	0	0	1	1	0	1
Harderian gland	*No. examined...*	*34*	*33*	*32*	*39*	*35*	*33*	*42*	*44*
Carcinoma		0	0	1†[c]	0	0	0	0	0
Uterus	*No. examined...*					*34*	*44*	*41*	*43*
Adenocarcinoma		—	—	—	—	0	2	1	1
Leiomyoma		—	—	—	—	0	0	1	0
Haemangioma		—	—	—	—	0	1	0	0
Testis	*No. examined...*	*37*	*34*	*36*	*43*				
Interstitial-cell tumour		1	3	0	0	—	—	—	—
Kidney	*No. examined...*	*39*	*36*	*38*	*42*	*42*	*45*	*46*	*44*
Lipoma		0	0	0	0	0	0	1	0
Leiomyosarcoma		1	0	0	0	0	0	0	0
Nephroblastoma		0	0	0	0	1	0	0	0
Peritoneal cavity‡	*No. examined...*	*44*	*37*	*38*	*44*	*43*	*45*	*46*	*46*
Fibrosarcoma		1	0	0	0	0	0	0	0
Other sarcomas		0	1	0	0	0	0	1	1†[d]
Salivary gland	*No. examined...*	*40*	*35*	*34*	*40*	*42*	*43*	*45*	*42*
Adenoma		0	0	0	0	1	0	0	0
Brain	*No. examined...*	*40*	*35*	*38*	*43*	*41*	*43*	*46*	*45*
Astrocytoma		0	0	1	0	0	0	0	0
Other tumour		0	0	0	0	1	0	0	0
Lymph node	*No. examined...*	*33*	*29*	*32*	*38*	*35*	*41*	*41*	*36*
Haemangioma		1	2	1	1	1	0	1	2
Haemangiosarcoma		1	1	0	0	0	0	0	0
Lymphoma		0	2	0	0	0	0	0	0
General Lymphosarcoma		0	0	0	1†[c]	0	0	0	0

†Secondary deposits—(a) in salivary gland, adrenal gland, lymph node, heart, lung and Harderian gland; (b) invading brain, in one rat; (c) in lung; (d) in spleen; (e) in lung, liver, spleen and kidney.

‡Lesions identified at autopsy in the number of animals shown.

The figure marked with an asterisk differs significantly ($P < 0.05$ by chi-square test) from the corresponding control value. No allowances were made for the time of incidence.

tumours have occurred rarely in previous studies. However, the incidence of these tumours was noted in both control and treated animals.

This study, in which quillaia extract was fed at levels up to 3% in the diet for 2 yr, confirmed the results of the previous study in mice (Phillips *et al.* 1979) which failed to detect any carcinogenic effects. The no-untoward-effect level established in this rat study was 3% in the diet, which is equivalent to an intake of approximately 1·5 g/kg/day. This is considerably in excess of the possible intake from the maximum permitted level of quillaia extract (200 ppm) in soft drinks. The difference between the no-untoward-effect level in the present study and that of 0·7 g/kg/day obtained in the long-term study in mice (Phillips *et al.* 1979) suggests a species difference, while the wide difference between this result in rats and the no-untoward-effect level of 0·4 g/kg/day deduced from the short-term rat study (Gaunt *et al.* 1974) was probably due to the rats' becoming accustomed to the quillaia extract over the prolonged feeding period.

Table 9. *Literature references to the spontaneous occurrence of tumours in the rat*

Organ	Tumour	References*
Thyroid	Adenocarcinoma	1, 5, 7
Pancreas	Adenocarcinoma	1, 5, 7
Adrenal	Medullary-cell types	5, 6
Lung	Adenocarcinoma	1, 3
Salivary gland	Various types	1, 4, 5, 7
Uterus	Adenocarcinoma	1, 4, 5
	Leiomyoma	2
Peritoneum	Reticulum-cell sarcoma	2
Brain	Astrocytoma	4
Lymph nodes	Sarcoma	5, 6
Subcutaneous tissue	Lipoma	6

*(1) Brantom, Gaunt, Hardy *et al.* (1973); (2) Evans *et al.* (1977); (3) Gaunt, Brantom, Grasso *et al.* (1972); (4) Gaunt, Carpanini, Grasso & Lansdown (1972); (5) Gaunt, Butterworth, Hardy & Gangolli (1975); (6) Gaunt *et al.* (1976); (7) Snell (1965).

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