MAYA NUT (*BROSIMUM ALICASTRUM* SW.) AND MAYA NUT-DERIVED INGREDIENTS FOR USE IN TRADITIONAL FOODS

GENERALLY RECOGNIZED AS SAFE (GRAS) SELF-AFFIRMATION REPORT

Table of Contents

[1] This report was filed using the trade name “Ramon Seed”

List of Tables and Figures

Appendices

Appendix I:
Certificates of Analysis for All Assays

Appendix II:
Estimated Daily Intake of Maya Nut Ingredients by the U.S. Population from Proposed Food Uses

Appendix III:
"*Cooking with Maya Nut: Delicious Recipes for a Healthy Family*"

Appendix IV:
Investigations of the Potential Allergenicity of Maya Nut Powders Using Radioallergosorbent Test Inhibition (RAST-inhibition)

MAYA NUT (*BROSIMUM ALICASTRUM* SW.) AND MAYA NUT-DERIVED INGREDIENTS FOR USE IN TRADITIONAL FOODS

GENERALLY RECOGNIZED AS SAFE (GRAS) SELF-AFFIRMATION REPORT

November 13, 2007

I. EXECUTIVE SUMMARY

This report contains and explains a determination that the use of Maya Nut and powder derived from Maya Nut, as sources of protein and other nutrients, is generally recognized (i.e., common knowledge by competent experts in the scientific community that has expertise in botanicals that are added to food) as being safe to a reasonable certainty; this determination is based upon a substantial history of use by a significant number of consumers (i.e., a common use) of these substances as or in food prior to January 1, 1958.

Maya Nut is harvested from the *B. alicastrum* tree, which is widely distributed throughout South and Central America. In addition to a high-quality protein content, the Maya Nut contains carbohydrates, with smaller amounts of fat and minor amounts of flavonoids. All constituents of the Maya Nut are, therefore, consumed daily as part of the habitual Western diet and are expected to undergo normal physiological pathways of metabolism following consumption.

This report is intended to comply with 21 C.F.R. § 170.30(c)(2) and 21 C.F.R. § 170.36. We acknowledge that C.F.R. § 170.36, the part of the FDA's proposed GRAS rule that sets out the information required for a GRAS Notice submission, requires that four general categories of information be submitted to FDA by means of a Notice of a GRAS exemption claim. Those four categories are:

(1) (i-iv): General information about the Notifier and the substance. Included in that information is the following:

> "(c) Notifier shall submit the following information:
>
> > (4). "A detailed summary of the basis for the notifier's determination that a particular use of the notified substance is expempt from the remarket approval requirements of the act because such use is GRAS. Such determination may be based either on scientific procedures or on common use in food."

(2) Detailed information about the substance.

(3) Any self-limiting levels of use of the substance.

(4) A detailed summary of the basis for the GRAS determination.

We also acknowledge that subsection (ii) of 21 C.F.R. § 170.30(c)(4) sets out three additional specific requirements for a GRAS determination based upon common use in food. Those requirements are:

> "(A) A comprehensive discussion of, and citations to, generally available data and information that the notifier relies on to establish safety, including evidence of a substantial history of consumption of the substance by a significant number of consumers;
>
> (B) A comprehensive discussion of any reports of investigations or other information that may appear to be inconsistent with the GRAS determination;
>
> (C) The basis for concluding, in light of the data and information described under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(ii)(A), and (c)(4)(ii)(B) of this section, that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use."

Finally, we acknowledge that para. 5-d on page 18950 of the 1997 Proposed GRAS rule discusses the basis for "concluding expert consensus" exists in a "Common Use GRAS Determination" setting. In addition to the general standard and the specific standards identified immediately above, the following standard must be met because the "common use" primarily occurred in Central and South America.

- "Corroboration of the principal source of the 'history of use' conclusion by information from a second independent source;
- That source must confirm the history and circumstances of use of Maya Nut.
- The information about the history of use must be widely available in the countries in which the substances will be harvested;
- The information must be readily accessible to interested qualified experts in the United States.

The authors of this report note that 21 C.F.R. 170.30(c)(2) provides that if a history of use is based on use in foreign countries, the GRAS Notice should be submitted to the FDA. We note that such language is only a recommendation and does not require any such submission. See 62 Federal Register 18944 (4-17-97).

II. INTRODUCTION

Brosimum alicastrum Sw. (Moraceae) (*B. alicastrum*) is a large evergreen tree with a history of human use as a food that extends from the classical Mayan period to the present. The *B. alicastrum* tree is widely distributed throughout South and Central America. Historically, every part of the *B. alicastrum* tree has been used by humans including the fruit, seed ("nut"), bark, wood, leaves, and latex. The seed in particular is widely recognized for its nutritious value. Additionally, various parts of the *B. alicastrum* plant also have been used in folk medicine, with use of the latex and/or seed decoction as a galactagogue (lactation stimulant) most commonly cited (Morton, 1977). The *B. alicastrum* seed, commonly called ramón or ramón "nut," continues to be a part of the habitual diet of native populations of Central and South America. The name *Brosimum* reportedly comes from the Greek word *brosimos*, meaning "edible."

The safety of the Maya Nut and powder derived from dried or roasted Maya Nut is primarily supported by a well-established and safe history of use of the ingredients as a traditional food in the diets of native populations of South and Central America. The primary source for this history of use determination is the chapter by Ortiz et al. entitled "The Corn Tree (Brosimum alicastrum): A Food Source for the Tropics" from the 1995 volume *Plants in Human Nutrition* (Ortiz M, Azañón Y, Melgar M, Elias L. The corn tree (Brosimum alicastrum): a food for the tropics. In Simopoulos, AP, ed. Plants in Human Nutrition, World Review of Nutrition and Diabetics. 1995. Basel, Switzerland: Karger Publishers. pp 134-146.) Important secondary sources include the work of Peters and Pardo-Tejeda, 1982; Puleston, 1971, 1982; and Ramirez-Sosa, 2006.

The safety of the Maya Nut and Maya Nut powder is further supported by the fact that all constituents of the seed are consumed as part of the normal human diet. Under the conditions of intended use, the Maya Nut would be consumed at levels that approximate those from the consumption of the Maya Nut as part of the diet of Central and South American populations.

Maya Nut and powder derived from the Maya Nut are intended for use as ingredients in traditional foods including baked goods and baking mixes, beverages and beverage bases, breakfast cereals, grain products and pastas, gravies and sauces, and milk products as a source of protein.

This report contains and explains a determination that the use of Maya Nut and powder derived from Maya Nut, as sources of protein, is generally recognized (i.e., common knowledge by competent experts in the scientific community that has expertise in botanicals that are added to food) as being safe to a reasonable certainty; this

determination is based upon a substantial history of use by a significant number of consumers (i.e., a common use) of these substances as or in food prior to January 1, 1958.

Maya Nut is harvested from the *B. alicastrum* tree, which is widely distributed throughout South and Central America. In addition to a high-quality protein content, the Maya Nut contains carbohydrates, with smaller amounts of fat and minor amounts of flavonoids. All constituents of the Maya Nut are, therefore, consumed daily as part of the habitual Western diet and are expected to undergo normal physiological pathways of metabolism following consumption.

This report is intended to comply with 21 C.F.R. § 170.30(c)(2) and 21 C.F.R. § 170.36. We acknowledge that C.F.R. § 170.36, the part of the FDA's proposed GRAS rule that sets out the information required for a GRAS Notice submission, requires that four general categories of information by submitted to FDA by means of a Notice of a GRAS exemption claim. Those four categories are:

(1) (i-iv): General information about the Notifier and the substance. Included in that information is the following:

> "(c) Notifier shall submit the following information:
>
> > (4). "A detailed summary of the basis for the notifier's determination that a particular use of the notified substance is expempt from the remarket approval requirements of the act because such use is GRAS. Such determination may be based either on scientific procedures or on common use in food."

(2) Detailed information about the substance.

(3) Any self-limiting levels of use of the substance.

(4) A detailed summary of the basis for the GRAS determination.

We also acknowledge that subsection (ii) of 21 C.F.R. § 170.30(c)(4) sets out three additional specific requirements for a GRAS determination based upon common use in food. Those requirements are:

> "(A) A comprehensive discussion of, and citations to, generally available data and information that the notifier relies on to establish safety, including evidence of a substantial history of consumption of the substance by a significant number of consumers;

(B) A comprehensive discussion of any reports of investigations or other information that may appear to be inconsistent with the GRAS determination;

(C) The basis for concluding, in light of the data and information described under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(ii)(A), and (c)(4)(ii)(B) of this section, that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use."

Finally, we acknowledge that para. 5-d on page 18950 of the 1997 Proposed GRAS rule discusses the basis for "concluding expert consensus" exists in a "Common Use GRAS Determination" setting. In addition to the general standard and the specific standards identified immediately above, the following standard must be met because the "common use" primarily occurred in Central and South America.

- "Corroboration of the principal source of the 'history of use' conclusion by information from a second independent source;
- That source must confirm the history and circumstances of use of Maya Nut.
- The information about the history of use must be widely available in the countries in which the substances will be harvested;
- The information must be readily accessible to interested qualified experts in the United States.

The authors of this report note that 21 C.F.R. 170.30(c)(2) provides that if a history of use is based on use in foreign countries, the GRAS Notice should be submitted to the FDA. We note that such language is only a recommendation and does not require any such submission. See 62 Federal Register 18944 (4-17-97).

Additional Information

The report was produced as cooperative project of Botanical Liaisons, LLC and qualified independent reviewers. Botanical Liaisons, LLC of Boulder, Colo. assembled primary literature on the history of use and safety of ramón and its use. The data were compiled in a report, which was submitted to other qualified experts for peer review. The comments of the peer-reviewers were considered in the preparation of the final document.

This report provides a literature-based review of the history of use and safety of the Maya Nut when consumed as a food or a food ingredient. To obtain the necessary information, comprehensive and detailed searches of the published scientific literature were performed utilizing multiple scientific databases, including Medline® and EMBASE®. The report also includes some primary ethnobotanical research and personal communications with leading ethnobotanists and other experts, as well as an original assay and analysis by a food allergy researcher.

The report summarizes the chemical and nutritional analyses of the seed, outlines the product specifications and manufacturing processes for the ingredients, compares the composition of the Maya Nut to other foods, defines the intended conditions of use in foods, provides estimates of exposure under the conditions of intended use, and summarizes the history of human use of the Maya Nut and ingredients from the Maya Nut, as well as other parts of the *B. alicastrum* plant, current background consumption data, and animal toxicology and human clinical data. Botanic and ethnographic descriptions of the plant also have been included. Information regarding potential product compositions and manufacturing process was supplied by Forest Trade.

III. TECHNICAL OVERVIEW

III.A. Details of the Raw Material

III.A.1 Common or Usual Name

Common names include ramón (Mexico, Guatemala), corn tree, cow tree, Maya nut, ujuxte (Guatemala), ojushte or ujushte (El Salvador), barúu (Venezuela), iximché, capomo, másico, ojoche, and ox.

The names “ramón” or *Brosimum alicastrum* (*B. alicastrum*) are used throughout this report.

III.A.2 Botanical Name

Brosimum alicastrum Sw. (Moraceae)

III.A.3 Description of *Brosimum alicastrum*

B. alicastrum is a medium to large evergreen tree that can attain a height of more than 40 meters (range: 18 to 45 meters) and a diameter of greater than 1 meter. The tree is characterized by a smooth or scaly, dark- to light-gray bark. The inner bark is fibrous and creamy yellow, containing large amounts of latex. The tree’s branches and leaves are first ascending and then drooping; leaves are typically oval-shaped with scars from fallen stipules, gray-green in color, and glabrous. Typical populations of *B.* alicastrum are dioecious, therefore, not all trees bear fruit (Peters and Pardo-Tejada, 1982). The male inflorescence contains an abundant number of cream-colored flowers that lack or have a barely visible perianth. Each flower has a single stamen. Female flowers are smaller and have a long style with 2 stigmas.

Fruiting occurs for a period of 50 to 75 days during December through July. The fruits are subglobose and about 2 to 2.5 cm in diameter. They have a fleshy pericarp and a surface covered with many white scales. The flesh is edible and sweet. The fruits are yellowish-green in color during maturation, turning orange when ripe. One kilogram of clustered fruit will contain approximately 190 individual fruits. Each fruit contains 1 seed approximately 1.2 cm in diameter. The seed is covered by a papery yellowish testa. The seed has two asymmetrical cotyledons that are greenish when fresh and yield large amounts of latex when cut. The seed accounts for about 80% of the weight of the dried fruit (Ortiz, 1995). Seeds are dispersed by bats and gravity (Ramirez-Sosa, 2006). The *B. alicastrum* plant produces abundant quantities of white latex with a slightly salty taste. The latex has a low viscosity and congeals slowly (Ortiz, 1995).

An adult ramón tree that is at least 8 years old and growing in a high forest may yield up to 75 kg fruit, 58 kg seed, and 400 kg green leaves. Trees can occur at a density of 250 trees per hectare in densely forested areas (Ortiz, 1995). One 3-year study of yields per hectare in Tikal, Guatemala, reported an average yield of 1,762 kg of seed per hectare, with a maximum yield of 2,616 kg per year (Ortiz, 1995).

There are approximately 28 species in the genus *Brosimum*, including at least 4 that occur in Guatemala (Ortiz, 1995). The name *Brosimum* reportedly comes from the Greek word *brosimos*, meaning “edible” (Ortiz, 1995).

III.A.4 Distribution and Natural Sources of *Brosimum alicastrum*

Distribution of *B. alicastrum* in Central and South America ranges from southern Mexico to Bolivia. It may be found in both northern and southern Guatemala and Belize, as well as the Caribbean and Hawaii. The tree prefers very humid, hot subtropical forests, and grows at altitudes ranging from 300 to 2,000 meters above sea level. Its most common habitat is at 125 to 800 meters above sea level, with an average temperature of 24.4º C, yearly rainfall of 1,500 to 2,000 mm, relative humidity of 80% to 96%, and 30% to 50% daylight. In El Salvador, the tree is found throughout the entire country, from sea level to around 1,000 meters above sea level.

III.A.5 Nutritional Value

Numerous studies reported high nutritional values for both humans and animals of ramón leaves, twigs, and seeds (Pardo-Tejada and Sanches-Munoz, 1980; Perez *et al.*, 1995; Santos Ricalde and Abreu Sierra, 1995). An early description and chemical analysis of the latex and bark of the "palo vaca" or cow tree (*Brosimum* sp.) published in 1837 by John Murray appears to refer not to *B. alicastrum*, but to *B. galactodendron*, formerly *Galactodendron utile* (Murray, 1837). Murray's report concludes, "Both the 'milk' and bark contain the elements of nutritious and wholesome food for man, and bread formed from its bark would be almost equal to the *cerealia*, or that made from corn,-‘the finest of the wheat'-for the immediate or proximate parts of wheaten flour are found in the bark of the Cow Tree—so that the 'Palo de Vaca' yields both bread and milk."

Analysis of the fruit pulp yields 84% water content, 2.5% protein, 0.5% ether extract, 1.2% fiber, and 10.9% free nitrogen extract (Ortiz, 1995). Fresh seeds may contain as much as 52.2% water (Ortiz 1995). After drying, water content varies from 4.60% to 12.17%. The whole dried fruit (analyzed for purposes of animal feed) yielded 12.3% crude protein, 8% water, and 15.5% ash. According to Ortiz *et al*., “it may be concluded that the seed is carbohydrate-rich; values ranging from 39.6% to 74.6% of nitrogen-free extracts have been reported.” Crude fiber content varies from 2.4% to 8.9%; total dietary

fiber varies from 16.6% to 23.6%. Factors affecting the composition of the material include degree of ripeness and harvesting season, among others (Ortiz, 1995).

Maya Nut is calorie-dense, with the caloric content varying from 3.59 to 4.16 kcal/g. Puleston *et al*. reported protein values for the seed ranging from 11.4% to 13.4% (as crude protein); data obtained by other researchers suggests somewhat lower values of 7.7% to 8.9%. For purposes of comparison, wheat, corn, and rice have an average protein content of 9.3%, 9.8%, and 7.2%, respectively. Analyses of the amino acids contained in Maya Nut indicate that it provides a high-quality protein. The seed contains both lysine and tryptophan, which are often limited in typical Central American diets. Lysine values of 2.34% to 4.0% and tryptophan values of 1.2% to 2.3% have been reported (Ortiz, 1995).

III.B Description of the Ingredient

The ingredients intended for use in traditional foods include the *B. alicastrum* seed and powder produced from *B. alicastrum* seed. Following drying, the whole or processed seed product can be sold or added to food. Alternatively, the dried seed can be ground into powder or can be further roasted and then ground to produce roasted powder. An overview of the manufacturing processes for the ingredients is presented below, followed by corresponding technical specifications.

III.B.1 Harvesting of the Seed

The Maya Nut is harvested from sites in Central America by indigenous wild harvesters, and the harvesting of the seeds is monitored for quality assurance by the purchasing companies.

The seeds are collected in clean bags. Because fresh seeds contain more than 50% water, they must be thoroughly dehydrated before storage to prevent mold growth and aflatoxin formation; therefore, solar dryers are constructed at the harvesting camps or in local communities for preliminary drying of the seeds, with subsequent implementation of additional drying methods (*e.g.*, mechanical, sun, or solar thermic drying at a central drying area) as needed. Gathered seeds are transferred from the bags to the preliminary solar drying system, and poor quality seeds (*i.e.*, unsound, broken, moldy, discolored, etc.) are removed and discarded. The seeds are allowed to dry in the sun for a period of 5 to 7 days, after which they are put into clean bags and transported to a central drying area for further drying in a solar and/or other acceptable dryer to achieve a moisture content of the seed of not more than 20%. After this second drying process, the seeds are sorted, poor quality seeds are removed, and good quality seeds are bagged for transport by covered trucks to a facility for further processing and export.

III.B.2 Methods of Manufacture

III.B.2.1 Whole Dried Seeds

The bagged Maya Nuts are received at the processing facility and stored in a clean, dry, cool, well-ventilated storage area until processing. For further drying of the seeds, the Maya Nuts are spread on a drying screen and hot air is blown through a duct to a dryer under stirring to ensure even drying of the seeds until moisture reaches the acceptable level of ≤12%. The dried seeds are put into a detailing machine to remove shell fragments and any extraneous/foreign matter that might still be present, and the cleaned seeds are bagged for export or further processing. Seeds for export are blended to produce a uniform lot, and following appropriate quality control procedures, are packaged in poly-woven bags and prepared for shipping.

III.B.2.2 Powder from Dried Seeds

Shelled, dried seeds not prepared for export could be subjected to grinding to produce Maya Nut powder. The powder is produced by grinding the seeds with a roller mill to produce a final powdered product, which is achieved to maximum quantity by regrinding anything left on the screens. The powder product is blended to produce a uniform lot, and following appropriate quality-control procedures, is packaged in poly-lined, 3-ply paper bags and prepared for shipping.

III.B.2.3 Powder from Roasted Seeds

Alternatively, the shelled dried seeds could be further processed by roasting in a roaster. Part way through the roasting process, a sample Maya Nut is removed, cracked open, and assessed for color compared to a roasted standard. Product that is not dark enough is subjected to further roasting until it meets the color specifications of roasted Maya Nut. Seeds that are dark enough are transferred to a rotating blender with cool air flowing underneath for cooling of the seeds, and once cooled, the seeds are transferred to clean bags and stored. Roasted seeds are blended to produce a uniform lot, which is then ground to specification using a roller mill. The roasted Maya Nut is more brittle than the unroasted seed and in some instances during grinding may splinter into small particles instead of being ground to a powder. Accordingly, the roasted Maya Nut may be reground a number of times to achieve the appropriate product yield (powder and tea bag cut). The powder and the tea bag cut are separated, blended as separate lots, and packaged in poly-lined 3-ply paper bags for shipping.

III.B.3 Product Specifications for the Ingredients

As the powder obtained from the dried seeds is produced mechanically solely by grinding of the Maya Nuts, without any additional processing, the ingredients (dried Maya Nut and dried Maya Nut powder) are expected to be essentially equivalent from a compositional standpoint, and hence will share the same product specifications. The product specifications for the dried Maya Nut and Maya Nut powder, including chemical and microbiological parameters, are presented in Table III.B.3-1. Details of the methods of analysis of the ingredients are presented in Appendix I.

Table III.B.3-1 Specification of Dried Maya Nut and Dried Maya Nut Powder

Specification Parameter	Specification	Method of Analysis
Moisture (%)	≤12	AOAC 925.40
Protein (g/100 g)	6 to 15	AOAC 991.20.1
Ash (g/100 g)	≤5	To standard
Fat (g/100 g)	<1.0, *trans* fat <0.10	To standard
Total heavy metals (as Pb, ppm)	<10	AAS; USP29/NF24
Standard plate count (CFU/g)	≤1,000,000	Compendium CHP 7 BAM
Yeasts (CFU/g)	≤10,000	Compendium 20, 54, 56 BAM CHP
Mold (CFU/g)	≤10,000	Compendium 20, 54, 56 BAM CHP
Salmonella (per 25 grams)	Negative	BAM CHP 5, AOAC 998.09
Escherichia coli (per gram)	Not detected (<3 MPN)	BAM CHP 4, AOAC CHP 17

CFU = Colony forming units; MPN = Most Probable Number.

The chemical and microbiological specifications for roasted Maya Nut powder are presented in Table III.B.3-2. Details of the methods of analysis of the ingredients are presented in Appendix I.

Table III.B.3-2 Specification of Roasted Maya Nut Powder

Specification Parameter	Specification	Method of Analysis
Moisture (%)	≤12	AOAC 925.40
Protein (g/100 g)	6-15	AOAC 991.20.1
Ash (g/100 g)	≤5	To standard
Fat (g/100 g)	<1.0, *trans* fat <0.10	To standard
Total heavy metals (as Pb, ppm)	<10	AAS; USP29/NF24
Standard plate count (CFU/g)	≤1,000,000	Compendium CHP 7 BAM
Yeasts (CFU/g)	≤10,000	Compendium 20, 54, 56

Table III.B.3-2 Specification of Roasted Maya Nut Powder		
Specification Parameter	**Specification**	**Method of Analysis**
		BAM CHP
Mold (CFU/g)	≤10,000	Compendium 20, 54, 56 BAM CHP
Salmonella (per 25 grams)	Negative	BAM CHP 5, AOAC 998.09
Escherichia coli (per gram)	Not detected (<3 MPN)	BAM CHP 4, AOAC CHP 17

CFU = Colony forming units; MPN = Most Probable Number.

III.B.4 Product Analysis

Sample lots of the dried Maya Nut were analyzed to verify that the manufacturing process produced consistent products in terms of composition and also conformed to the specifications set for heavy metal and microbiological contaminants. These data are summarized in Sections III.B.4.1 and III.B.4.2.

III.B.4.1 Compositional Analysis

The analytical data for 6 non-consecutive lots of unroasted Maya Nut powder (Lot Nos. US1361W-22-XLU, 2067, 2087, GT130-22B-7021, GT144-22-6101, and GT130-22-7021) and 5 non-consecutive lots of roasted Maya Nut powder (GT130-22RB-7021, GT144-22RA-6101, GT144-22RB-6101, GT154-22-7011, and GT130-22RA-7032) show conformity to the compositional product specifications. See Appendix I for certificates of analysis.

III.B.4.2 Microbiological Analysis

The analytical data for 3 non-consecutive lots (Lot Nos. GT130-22B-7021, GT144-22-6101, and GT130-22-7021) of unroasted Maya Nut, ground and unground, are presented in Table III.B.4.2-1. While 2 of the 3 lots were Maya Nut powder, Lot No. GT130-22-7021 was dried, whole seeds. Two of the 3 lots show conformity to the microbiological product specifications. The third lot (Lot No. GT144-22-6101) exceeds the specifications, but this was a small lot analyzed only for research purposes and never sold for human consumption.

Since the seed is only milled to produce unroasted seed powder and is not subject to further processing, analysis conducted on the whole seed is expected to be also reflective of the unroasted seed powder. See Appendix I for certificates of analysis. Moisture levels for all three batches were less than 12%.

III.B.4.2-1	Microbiological Analysis of Unroasted Maya Nut, Ground and Unground			
Specification Parameter	**Requirement**	**Manufacturing Lot**		
		GT130-22B-7021	GT144-22-6101[1]	GT130-22-7021[2]
Standard plate count (CFU/g)	≤1,000,000	900,000	1,900,000	32,000
Yeasts (CFU/g)	≤10,000	<10	<10	<10
Mold (CFU/g)	≤10,000	1,700	300	<10
Salmonella (per 25 grams)	Negative	Negative	Negative	Negative
Escherichia coli (per gram)	Not detected (<3 MPN)	Not detected	Not detected	Not detected

CFU = Colony forming units; MPN = Most Probable Number.

[1] A small lot analyzed only for research purposes and not sold for human consumption
[2] Whole Maya Nut (dry)

The analytical data for 5 non-consecutive lots (Lot Nos. GT144-22RA-6101, GT144-22RB-6101, GT154-22-7011, GT130-22RB-7021, and GT130-22RA-7032) of roasted Maya Nut, ground and unground, are presented in Table III.B.4.2-2 and show conformity to the microbiological product specifications. Four of the 5 lots were Maya Nut powder of varying sizes (including fine powder and tea bag cut) and Lot No. GT130-22-7011 was roasted whole seed. See Appendix I for certificates of analysis.

III.B.4.2-2	Microbiological Analysis of Roasted Maya Nut, Ground and Unground					
Specification Parameter	**Requirement**	**Manufacturing Lot**				
		GT144-22RA-6101[1]	GT144-22RB-6101	GT154-22-7011[2]	GT130-22RB-7021	GT130-22RA-7032[3]
Standard plate count (CFU/g)	≤1,000,000	8,000	8,000	20,000	2,900	200
Yeasts (CFU/g)	≤10,000	<10	<10	NA	<10	20
Mold (CFU/g)	≤10,000	70	70	300	380	<10
Salmonella (per 25 grams)	Negative	Negative	Negative	Negative	Negative	Negative
Escherichia coli (per gram)	Not detected (<3 MPN)	Not detected	Not detected	Not detected	Not detected	Not detected

CFU = Colony forming units; MPN = Most Probable Number; NA = Not analyzed.

[1] TBCMaya Nut (roasted)

[2] Whole Maya Nut (fire dried)

[3] Granulated Maya Nut (tea bag cut) (roasted)

III.B.5 Additional Chemical Characterization

III.B.5.1 Compositional Analysis of the Dried Maya Nut and Comparison with Other Foods

The constituents of the dried Maya Nut are present in a wide range of other foods consumed as part of the normal Western diet. The nutrient profile of the dried Maya Nut was compared with that of other commonly consumed beans and grains in the United States. The levels of individual constituents of the dried Maya Nut and other foods (*i.e.*, cornmeal, oats, pinto bean, rice flour) are presented in Table III.B.5.1-1.

Table III.B.5.1-1 Nutrient and Compositional Comparison of Maya Nut to Other Foods

Component	Amount per 100 g Sample				
	Ramón seed[1]	Other Foods[2]			
		Cornmeal (whole grain, yellow)	Oats	Pinto beans (mature, raw)	Rice flour (brown)
Calories (kcal)	345.9	362	389	347	363
Carbohydrate (g)	76.2	76.89	66.27	62.55	76.48
Fiber, dietary (g)	18.94	7.3	10.6	15.5	4.6
Sugars (g)	9.35	0.64	N/A	2.11	0.85
Fat, total (g)	0.44	3.59	6.90	1.23	2.78
Protein (g)	9.28	8.12	16.89	21.42	7.23
Ash (g)	3.14	1.13	1.72	3.46	1.54
Moisture (g)	10.95	10.26	8.22	11.33	11.97
Total (g/100 g)	100.01	99.99	100	99.99	100

[1] Source: Silliker Laboratories, Chicago, IL.
[2] Source: USDA Nutrient Database (www.ars.usda.gov/nutrientdata)

Additional analyses of the vitamin, mineral, sugar, and fatty and amino acids composition in 1 lot of dried Maya Nut also were conducted. Summaries of the additional chemical analyses conducted on the single lot of the dried Maya Nut (Lot No. US1361W-22-XLU) are presented in Tables III.B.5.1.1-1, III.B.5.1.2-1, III.B.5.1.3-1, and III.B.5.1.4-1. Certificates of analysis and details of the analytical methodologies are presented in Appendix I. Wherever available, the composition of the dried Maya Nut is compared with other foods based on data obtained from the USDA's Nutrient Database.

III.B.5.1.1 Amino Acid Composition

The amino acids present in the dried Maya Nut were assessed using Method MSS2 (1993) of the USDA. As a percent of total amino acid content, aspartic acid, tryptophan,

and glutamic acid were determined to be present in the greatest amounts, while leucine, praline, and arginine were the next most abundant amino acids (see Table III.B.5.1.1-1).

Table III.B.5.1.1-1 Levels of Amino Acids Present in Lot No. US1361W-22-XLU[1] of Dried Maya Nut and Other Foods

Amino Acid	Amount (g) per 100 g Sample				
	Maya Nut	Other Foods[3]			
		Cornmeal (whole grain, yellow)	Oats	Pinto beans	Rice flour (brown)
Aspartic acid	1.18	0.565	1.448	2.268	0.677
Tryptophan	1.12	0.057	0.234	0.237	0.092
Glutamic acid	0.89	1.525	3.712	3.027	1.473
Leucine	0.57	0.996	1.284	1.558	0.598
Proline	0.50	0.709	0.934	1.072	0.339
Arginine	0.49	0.405	1.192	1.096	0.548
Serine	0.40	0.386	0.750	1.171	0.374
Valine	0.37	0.411	0.937	0.998	0.424
Phenylalanine	0.36	0.399	0.895	1.095	0.373
Glycine	0.32	0.333	0.841	0.796	0.356
Threonine	0.30	0.305	0.575	0.810	0.265
Alanine	0.29	0.608	0.881	0.872	0.422
Isoleucine	0.28	0.291	0.694	0.871	0.306
Tyrosine	0.25	0.330	0.573	0.427	0.271
Lysine	0.22	0.228	0.701	1.3566	0.276
Histidine	0.22	0.248	0.405	0.556	0.184
Methionine	0.05	0.170	0.312	0.259	0.163
Cystine	0.03	0.146	0.408	0.187	0.088
Hydroxyproline	<0.01	--[2]	--	--	--

[1] Certificate of Analysis (COA) No.: CHG-31806354-1; COA Date: 6/20/07
[2] Not available
[3] USDA Nutrient Database (www.ars.usda.gov/nutrientdata)

III.B.5.1.2 Sugar Composition

Sugars identified in the dried Maya Nut included sucrose and fructose, with a lesser amount of lactose (see Table III.B.5.1.2-1).

Table III.B.5.1.2-1 Levels of Sugars Present in Lot No. US1361W-22-XLU[1] of Dried Maya Nut

Sugar	Amount (g) per 100 g Sample
Fructose	0.72
Lactose	<0.10
Sucrose	2.93
Glucose	<0.10
Maltose	5.70

[1] Certificate of Analysis (COA) No.: CHG-31806354-1; COA Date: 6/20/07

III.B.5.1.3 Fatty Acid Composition and Cholesterol Content

As a percent of total fatty acids, the individual fatty acid composition of the ingredient was assessed using gas chromatography and was determined to comprise primarily linoleic acid (37.76%) and palmitic acid (30.62%), which are long-chain fatty acids naturally present in the human diet. Additionally, linolenic and oleic acids were identified to be present at >5% of the total fatty acid content of the ingredient. A summary of the fatty acid composition of dried Maya Nut is presented in Table III.B.5.1.3-1.

Table III.B.5.1.3-1 Levels of Fatty Acids Present in Lot No. US1361W-22-XLU[1] of Dried Maya Nut

Fatty Acid	% Total Fatty Acids
4:0, Butanoic acid (Butryic)	1.97
14:0, Tetradecanoic acid (Myristic)	0.545
16:0, Hexadecanoic acid (Palmitic)	30.615
17:0, Heptadecanoic acid (Margaric)	0.483
18:0, Octadecanoic acid (Stearic)	3.578
18:1, Octadecenoic acid (Oleic)	7.740
18:2, Octadecadienoic acid (Linoleic)	37.763
20:0, Eicosanoic acid (Arachidic)	3.376
18:3, Octadecatrienoic acid (Linolenic)	9.21
20:3, 5,8,11-Eicosatrienoic acid	4.210

[1] Certificate of Analysis (COA) No.: CHG-31704000-0; COA Date: 4/16/07

In addition to analysis of the fatty acid levels in the dried Maya Nut, the level of cholesterol was assessed using standard methodology and was determined to be <1 mg/100 g of sample.

III.B.5.1.4 Vitamin and Mineral Composition

The vitamin and mineral levels in the product were analyzed using various methods of the Association of Analytical Chemists (AOAC). The vitamin and mineral composition of the dried Maya Nut is summarized in Table III.B.5.1.4-1.

Table III.B.5.1.4-1 Vitamins and Minerals Present in Lot No. US1361W-22-XLU[1] of Dried Maya Nut and Other Foods

Vitamin/Mineral	Amount per 100 g of Sample				
	Ramón seed	Other Foods			
		Cornmeal (whole grain, yellow)	Oats	Pinto beans	Rice flour (brown)
Calcium (mg)	140	6	54	113	11
Copper (mg)	0.54	0.193	0.626	0.893	0.230
Folic acid (µg)	26.90	25	56	525	16
Iron (mg)	1.2	3.45	4.72	5.07	1.98

Table III.B.5.1.4-1 Vitamins and Minerals Present in Lot No. US1361W-22-XLU[1] of Dried Maya Nut and Other Foods					
Vitamin/Mineral	**Amount per 100 g of Sample**				
	Ramón seed	**Other Foods**			
		Cornmeal (whole grain, yellow)	**Oats**	**Pinto beans**	**Rice flour (brown)**
Magnesium (mg)	143	127	177	176	112
Manganese (mg)	0.29	0.498	4.916	1.148	4.013
Niacin (mg)	1.4	3.362	0.961	1.174	6.340
Pantothenic acid (mg)	0.70	0.425	1.349	0.785	1.591
Phosphorus (mg)	105	241	523	411	337
Potassium (mg)	1,110	287	429	1,393	289
Riboflavin (mg)	0.070	0.201	0.139	0.212	0.080
Selenium (mg)	<0.050	15.5	--[2]	27.9	--
Sodium (mg)	12.9	35	2	12	8
Thiamine (mg)	0.11	0.385	0.763	0.713	0.443
Vitamin A (IU)	74	214	0	0	0
Vitamin B_6 (mg)	0.23	0.304	0.119	0.474	0.736
Vitamin C (mg)	<1.0	0	0	6.3	0
Zinc (mg)	1.4	1.82	3.97	2.28	2.45

[1] Certificate of Analysis (COA) No.: CHG-31806354-1; COA Date: 6/22.280/07

[2] Not available.

Additionally, the dried Maya Nut also was analyzed for beta-carotene and retinol content, which were identified at levels of 74 and <5 IU, respectively. In comparison, beta-carotene also was identified in cornmeal at levels of 97 IU, but was not a constituent of oats, pinto beans, or brown rice flour. Retinol was not present in any of these other foods.

III.B.5.2 Mycotoxin Analysis

Results of the aflatoxin analysis for unroasted and roasted Maya Nut powder are summarized in Table III.B.5.2-1. Aflatoxin in samples of unroasted and roasted Maya Nut powder was identified at levels below the current action level[2] for aflatoxin (*i.e.*, 20 ppb) in foods for human consumption as set by the U.S. FDA (2000). For certificates of analysis, see Appendix I.

[2] Action levels and tolerances represent limits at or above which FDA will take legal action to remove products from the market.

Table III.B.5.2-1 Summary of Aflatoxin Levels in Unroasted and Roasted Maya Nut Powder								
Manufacturing Lot	Unroasted Maya Nut Powder				Roasted Maya Nut Powder			
	GT130-22B-7021	GT144-22-6101	GT130-22-7021[1]	GT144-22RA-6101[1]	GT144-22RB-6101	GT154-22-7011[2]	GT130-22RB-7021	GT130-22RA-7032[3]
Aflatoxin level (ppb)	<15 ppb	<15 ppb	<5 ppb	<5 ppb	<5 ppb	<5 ppb	<15 ppb	<0.2 ppb

III.B.5.3 Pesticides

Screening for a series of pesticides was conducted on 1 sample lot of each organic-certified and conventional (uncertified) Maya Nut (see Appendix I for Certificates of Analysis). No detectable levels of pesticides were identified in either the organic-certified or conventional sample of Maya Nut.

IV. PROPOSED FOOD USES AND ESTIMATED INTAKE OF MAYA NUT, RAMÓN POWDER, AND ROASTED RAMÓN POWDER FROM INTENDED FOOD-USES

IV.A Proposed Food-Uses and Use Levels of Maya Nut Ingredients in Conventional Foods

The ingredients, Maya Nut, Maya Nut powder, and powder derived from roasted Maya Nut are intended for use in various food categories including baked goods and baking mixes, beverages and beverage bases, breakfast cereals, grain products and pastas, gravies and sauces, and milk products. Food codes representative of each proposed food use were chosen from the National Center for Health Statistics' (NCHS) 2003-2004 National Health and Nutrition Examination Surveys (NHANES 2003-2004) (CDC, 2006; USDA, 2006) and grouped in food use categories according to Title 21, §170.3 of the Code of Federal Regulations (CFR) (U.S. FDA, 2007a). Serving sizes were assigned according to Title 21, §101.12, Reference Amounts Customarily Consumed Per Eating Occasion (RACC) (U.S. FDA, 2007b). The individual proposed food-uses and use levels of Maya Nut ingredients are summarized in Table IV.A-1.

Table IV.A-1 Summary of the Individual Proposed Food Uses and Use Levels for Maya Nut Ingredients in the U.S.

Food Category	Proposed Food Uses	Use Levels (% w/w)
Baked Goods and Baking Mixes	Biscuits	81.8
	Brownies	47.5
	Cakes (Light-weight)	8.2
	Cakes (Medium-weight)	17.5
	Cookies	13.3
	Cornbread and Tortillas	29.1
	Crackers	33.3
	Cereal Bars	47.5
	Energy bars	87.5
	Grain-Based Crackers	33.3
	Pancakes	37.5
	Pie Crust	46.7
	Rolls	76.0
	Quick Breads	76.0

Table IV.A-1 Summary of the Individual Proposed Food Uses and Use Levels for Maya Nut Ingredients in the U.S.		
Food Category	**Proposed Food Uses**	**Use Levels (% w/w)**
	Sliced Bread	76.0
	Waffles (Dry mix)	36.5
Beverages and Beverage Bases	Coffee	0.7
	Tea	0.7
Breakfast Cereals	Ready to Eat Breakfast Cereals (light, medium, heavy weights)	13.3, 6.7, 3.6
Grain Products and Pastas	Macaroni and Noodle Products	29.1
Gravies and Sauces	Gravies	1.7
Milk Products	Flavored Milk and Milk Drinks	4.2

IV.B Estimated Dietary Consumption of Maya Nut Ingredients from Proposed Food Uses

The consumption of the Maya Nut ingredients from all proposed food uses was estimated using the NCHS' NHANES 2003-2004 (CDC, 2006; USDA, 2006). NHANES are conducted as continuous, annual surveys, and are released in 2-year cycles. Each year, about 7,000 people from 15 different locations across the U.S. are interviewed, and approximately 5,000 complete the health examination component of the surveys. Any combination of consecutive years of data collection is a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Anderson, 1988). Because two 24-hour dietary recalls administered on two non-consecutive days (Day 1 and Day 2) are available from the NHANES 2003-2004, these data were used to generate estimates for the current intake analysis. The surveys provide the most appropriate data for evaluating food use and food consumption patterns in the United States, containing 2 years of data on individuals selected *via* stratified multistage probability sample of civilian, non-institutionalized population of the United States.

The NHANES 2003-2004 data were collected from individuals and households *via* 24-hour dietary recalls administered on two non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSU), which were counties throughout the United States. Small counties were combined to attain a minimum population size. These PSU were segmented and households were chosen within each

segment. One or more participants within each household were interviewed. Fifteen PSU are visited each year. For the NHANES 2003-2004, 12,761 individuals were selected for the sample, and 10,122 were interviewed (79.3%).

In addition to collecting information on the types and quantities of foods being consumed, the NHANES 2003-2004 collected socioeconomic, physiologic, and demographic information from individual participants in the survey, such as sex, age, height, weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Sample weights were incorporated with NHANES 2003-2004 to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2006).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of the Maya Nut ingredients by the U.S. population. Estimates for the daily intake of the Maya Nut ingredients represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2003-2004, and these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. All-person intake refers to the estimated intake of Maya Nut ingredients averaged over all individuals surveyed, regardless of whether they consumed food products intended to contain the ingredient, and therefore includes “zero” consumers (those who reported no intake of food products intended to contain the Maya Nut ingredients during the two survey days). All-user intake refers to the estimated intake of Maya Nut ingredients by those individuals consuming food products intended to contain the ingredients, hence the “all-user” designation. Individuals were considered users if they consumed 1 or more food products intended to contain the Maya Nut ingredients on either Day 1 or Day 2 of the survey.

Calculations for the mean and 90th percentile all-person and all-user intakes and percent consuming were performed for each of the individual identified food uses of the Maya Nut ingredients. Similar calculations were used to determine the estimated total intake of Maya Nut ingredients from all identified food uses combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

- infants, ages 0 to 2;
- children, ages 3 to 11;

- female teenagers, ages 12 to 19;
- male teenagers, ages 12 to 19;
- female adults, ages 20 and up;
- male adults, ages 20 and up; and
- total population (all population and gender groups combined).

The estimated total intake of the Maya Nut ingredients from all proposed food uses in the U.S. by population group is summarized in Table IV.B-1. Table IV.B-2 presents these data on a per kilogram body weight basis. A complete description of the consumption analysis is presented in Appendix II.

In total, approximately 96.9% of the total U.S. population was identified as potential consumers of Maya Nut ingredients from the proposed food uses (8,011 actual users identified). The individual surveyed population groups were all observed to contain a significant number of consumers of products in which the Maya Nut ingredients are proposed for use, with the percentage of identified users ranging between 75.8% in infants and 99.9% in children. Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates. Consequently, only the all-user intake results are discussed in detail.

Table IV.B-1 Summary of the Estimated Daily Intake of Maya Nut Ingredients from All Proposed Food Categories in the U.S. by Population Group (2003-2004 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)
Infants	0 to 2	75.8	705	28.53	65.02	34.28	66.02
Children	3 to 11	99.9	1,286	66.77	108.08	66.84	108.08
Female Teenagers	12 to 19	99.6	988	63.11	115.38	63.36	115.38
Male Teenagers	12 to 19	99.6	995	84.23	151.36	84.72	151.36
Female Adults	20 and Up	99.2	2,113	61.36	111.67	61.84	111.67
Male Adults	20 and Up	99.7	1,924	83.64	154.07	83.97	154.63
Total Population	All Ages	96.9	8,011	69.70	127.12	70.56	127.35

Table IV.B-2 Summary of the Estimated Daily per Kilogram Body Weight Intake of Maya Nut Ingredients from All Proposed Food Categories in the U.S. by Population Group (2003-2004 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (g/kg)	90th Percentile (g/kg)	Mean (g/kg)	90th Percentile (g/kg)
Infants	0 to 2	75.8	705	2.30	5.05	2.76	5.26
Children	3 to 11	99.9	1,286	2.55	4.73	2.55	4.73
Female Teenagers	12 to 19	99.6	988	1.11	2.15	1.11	2.15
Male Teenagers	12 to 19	99.6	995	1.33	2.38	1.33	2.39
Female Adults	20 and Up	99.2	2,113	0.87	1.63	0.88	1.63
Male Adults	20 and Up	99.7	1,924	0.99	1.89	1.00	1.90
Total Population	All Ages	96.9	8,011	1.23	2.52	1.24	2.53

On an all-user basis, the mean intake of Maya Nut ingredients by the total population from all proposed food-uses was estimated to be 70.56 g/person/day or 1.24 g/kg body weight/day. The heavy consumer (90th percentile) all-user intake of Maya Nut ingredients by the total population from all proposed food-uses was 127.35 g/person/day or 2.53 g/kg body weight/day. On a per person basis, male teenagers displayed the highest mean intake of Maya Nut ingredients at 84.72 g/day (1.33 g/kg body weight/day), while male adults displayed the highest 90th percentile intake of Maya Nut ingredients at 154.63 g/day (1.90 g/kg body weight/day). On a kilogram body weight basis, the highest mean and 90th percentile intakes were observed to occur in infants (2.76 and 5.26 g/kg body weight/day, respectively).

IV.C Conclusion

Consumption data and information pertaining to the individual proposed food uses of Maya Nut ingredients were used to estimate the all-person and all-user intakes of the ingredients for specific demographic groups and for the total U.S. population. This type of methodology is generally considered to be 'worst case' in terms of potential intake as a result of several conservative assumptions made in estimating consumption. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate consumption of food products that are consumed relatively infrequently.

The mean and 90th percentile intakes of ramón nut ingredients by the total population from all proposed food uses were estimated to be 70.56 g/person/day (1.24 g/kg body weight/day) and 127.35 g/person/day (2.53 g/kg body weight/day), respectively.

V. HUMAN USE OF INGREDIENT

The safety of the Maya Nut and powder derived from dried or roasted Maya Nut is primarily supported by a well-established and safe history of use of the ingredients as a traditional food in the diets of native populations of South and Central America. Additionally, the Maya Nut and other constituents of the *B. alicastrum* tree have a long history of use in traditional folk medicine, which has extended into present day.

V.A Traditional Human Uses

V.A.1 History of Use as a Food

The history of human use of Maya Nut as a food and/or food ingredient extends from the classical Mayan period to the present. Maya Nut has a long history of traditional food use in a number of South and Central American nations, including Guatemala, Mexico, El Salvador, Honduras, Nicaragua, and Venezuela (Lizarralde, 1997; Ortiz, 1995; Ramirez-Sosa; Yates, 2004; Lundell, 1938). According to sources, every part of the *B. alicastrum* tree has been used by humans, including the fruit, seed, bark, wood, leaves, and latex (Ortiz, 1995; Morton, 1977).

Maya Nut is reported to have a flavor similar to that of the potato. Numerous sources report that the tree was an important food source for the Maya and was widely cultivated as such (Gomez-Pompas, 1982; Ortiz, 1995; Peters, 1982; Puleston, 1971; Puleston, 1982). One source states, "Corn and *Brosimum alicastrum* were probably the main food sources for the Mayas of the classical period, one of the most advanced ancient civilizations of the American continent" (Ortiz, 1995). Some anthropologists suggest that the Maya managed their forests in order to maintain a constant source of *B. alicastrum*. Large assemblages of this plant species have been found in the old ruins of city-states in the Yucatan penisula, Chiapas, and Tabasco states of Mexico, as well as in Guatemala and Honduras (Gillespie, 2004). Others have argued that *B. alicastrum* primarily was used as a subsistence food by the Maya during times of famine and that the high concentration of trees around archaeological sites is due to seed dispersal by bats and optimal growth conditions on ruins, not cultivation practices (Lambert, 1982; Miksicek, 1981; Peters, 1983).

B. alicastrum trees are also widely found in the Mexican *pet kot* (plural *pet kotoob*)—a type of walled, man-made tropical forest garden created by Mayan ancestors of local populations living in the Yucatan and Quintana Roo (Gomez-Pompa, 1978). The typical *pet kot* is a tall stand of vegetation that contrasts significantly with surrounding lower-growing vegetation. Local Maya farmers told researchers studying *pet kotoob* that these areas were made by their recent ancestors as well as by the "old" Maya and that their locations "were chosen for the protection and cultivation of useful trees." The

researchers stated, "We believe [the *pet kot*] to be an artificial forest garden constructed with 'wild' species coming from the local forests or from distant and more humid forests of the Maya region, or from other kitchen gardens from nearby or remote areas" (Gomez-Pompa, 1978). The inclusion of *B. alicastrum* trees along with other tree species of food value both in modern home gardens and in *pet kotoob* lends further support to the historical value of this plant as a food source for humans and animals.

In a recent interview in the *New Scientist*, a biologist working in Guatemala recounted seeing "almost all the forest animals" eating fallen Maya Nuts, which "formed a carpet on the forest floor" (Richardson, 2006). The biologist's guide, an indigenous Guatemalan, told her that his ancestors, the Maya, used to eat Maya Nuts, and made her "some nut [seed] soup, which was delicious." However, the local people seem to consider the seeds more appropriate food for animals than people. She reported that in some places, there was a kind of stigma attached to the consumption of Maya Nuts among those who remembered it as a famine food. She also noted the existence of old stories explaining that the people stopped eating ramón after outsiders from Mexico introduced corn to the region.

In an interview of El Salvadorian adolescents, interviewers also noted fairly widespread knowledge of the use of the Maya Nut as an alternative food source to maize during the armed revolution in the 1980s (Ramirez-Sosa, 2006). In addition, "anecdotal information from elders tells that people consumed *ujushte* tortillas in hard times during World War II." In the Yucatan penisula of Mexico, older interviewees recalled previous generations using the Maya Nut as a substitute or supplement for maize, and at least one remembered eating ramón as a child (Gillespie, 2004). "Fruits were eaten or used to prepare tortillas or fritters as well as being mixed with beans." Traditional knowledge of use as a famine food was widespread; one interviewee remembered consumption of ramón 60 years earlier when locusts destroyed a maize crop. These uses are consistent with the earliest reports of ramón being boiled or made into powder (flour) for bread or tortillas (Lundell, 1938).

V.A.2 Traditional Medicinal Uses

Various plant parts of *B. alicastrum* also have been used as folk medicine. Use of decoctions prepared from the seeds of the *B. alicastrum* as a galactagogue (to increase milk production in nursing mothers) is most commonly cited (Morton, 1977; Peters, 1982; Ortiz, 1995). The latex also has been used medicinally as a galactagogue (Morton, 1977; Peters, 1982; Ortiz, 1995). Preparations derived from various parts of the *B. alicastrum* tree (*i.e*., leaf decoction or latex mixed with water) also are reported by ethnobotanists to have been used for the treatment of asthma and bronchitis (Morton, 1977; Peters, 1982; Ortiz, 1995).

V.A.3 Traditional Methods of Preparation

Traditional preparation methods vary among local populations. The seeds may be roasted like chestnuts or boiled and used as a potato substitute. Roasted and milled Maya Nuts may be steeped in boiling water to make a beverage or made into flour for use either alone or mixed with corn in foods such as tortillas (Ortiz, 1995).

In Mexico, *B. alicastrum* fruits have been eaten raw or made into juice or marmalade, after which the seeds were dried, ground into flour, and mixed with corn to make tortillas. Maya Nuts were also reportedly boiled, mashed, and eaten as a substitute for root vegetables, and a dessert was made by combining ground seeds with honey (Peters, 1982). A report from 1938 describes seeds being boiled or made into flour for bread or tortillas in Mexico (Lundell, 1938).

In El Salvador, the processing and cooking of Maya Nut is reported to be the same as for maize (Ramirez-Sosa, 2006); *B. alicastrum* seeds are boiled with lime (ashes), ground to a paste, and cooked on a flat surface as tortillas (Ortiz, 1995). In Venezuela, the seeds are reportedly boiled with salt before consumption (Ramirez-Sosa, 2006).

Another source suggests that to prepare Maya Nut for further processing, the seed should first be cooked in an alkaline medium to facilitate removal of the skin. As an alternative, the seed may be roasted, which enhances its flavor in addition to making it easier to remove the skin. To make tortillas, the seed is then washed, milled, kneaded, and cooked (Ortiz, 1995). To incorporate Maya Nut in bread, the seed is prepared by the roasting method just described, ground, and the dough mixed in a ratio of no more than 1 part ramón flour to 1 part wheat flour. This is then baked as typical wheat bread. The quality of such a bread reportedly diminishes if ramón flour is included in more than the 1:1 ratio specified here (Ortiz, 1995). Before final cooking, the dough may be diluted with water and boiled to make a beverage. This drink, to which sugar, salt, and/or cinnamon are typically added, is called *atol* (Ortiz, 1995).

Rosita Arvigo, a traditional healer and midwife in Belize, recounts the following modern (local) uses and preparation methods for Maya Nut:

> “I do not know of any published recipes, but do remember how the Mexican women used ramón nuts [seeds]. The outer shell is sweet and tastes a bit like tart apricots. The inner shell is boiled in two changes of water until soft, ground in a hand mill or on a stone *metate*, and then mixed with corn to make a flat cake. I've seen some people just eat them boiled and whole as if they were small potatoes, which is the way I like them the best. Others make them into a hot cereal by grinding and combining with a bit of corn masa, salt, and sugar...quite tasty. Some can

make tortillas with just the ramón without corn, but it requires skill, as the dough tends to fall apart easily. I once made the flat cakes with ramón and cooked green plantain and they were good as well…

The leaves are fed to animals who have just given birth—cows, horses, goats, sheep—to increase their milk production. Also, Don Elijio said that sometimes when babies were born at chicle camp and the mother did not survive, they were able to squeeze a milky substance out of the young leaves and mix that with water to keep the baby alive until it was removed from the camp" (Arvigo, personal communication, 2007).

V.B Current Background Intake and Other Uses

V.B.1 Current Natural Occurrence of the Maya Nut in the Diet

While the tree is commonly found in household gardens in rural areas, it appears that the seeds are currently more a specialty item than a subsistence food for humans. In Mexico, "The ready availability of and low price of government-subsidized maize may be partially responsible for the limited use, but in many regions there also appears to be a sociological stigma associated with consumption of its seeds" (Peters, 1982). *B. alicastrum* trees are commonly included in Mayan home gardens of the Yucatan penisula of Mexico, primarily for use as fodder for animals (Gillespie, 2004).

Nonetheless, Maya Nut remains in current use mainly in Mexico, El Salvador, and Guatemala, notably among certain Guatemalan communities of Mayan descent (Ortiz, 1995; Vohman, 2007). In 1975, the U.S. National Academy of Sciences included *B. alicastrum* in a group of 36 plants "considered to be underexploited, while having a high economic potential for tropical regions" (Ortiz, 1995). The most consistent modern use persists in Guatemala, Mexico (Michoacan), and El Salvador, where women still sell boiled, salted Maya Nuts as a roadside snack near Jiquillisco (Vohman, 2007).

Although current use of *B. alicastrum* seeds as a food for humans is more limited than in the past, sources document substantial modern ethnobotanical knowledge of the plant and its uses among indigenous populations in Central America (Ramirez-Sosa, 2006; Yates, 2004; Gillespie, 2004; Ortiz, 1995). Most adults interviewed for one survey in El Salvador knew about and had eaten *ujushte* (ramón), although there was some evidence of generational loss of knowledge (Yates, 2004). Today in El Salvador, ramón tortillas are generally eaten mainly in times of scarcity (Ramirez-Sosa, 2006). Among El Salvadorian adolescents interviewed for an ethnobotanical survey, rural adolescents had greater knowledge of and experience with *B. alicastrum* than urban adolescents (Ramirez-Sosa, 2006). Knowledge of the plant among adolescents appears to have declined about 20% between 1995 and 2005 (Ramirez-Sosa, 2006).

The Equilibrium Fund, a nonprofit corporation founded in 2001 by American, Guatemalan, and Nicaraguan professionals to help indigenous and marginalized women find ways to produce food, earn income, and raise healthy families without destroying their environment, provides a cookbook ("*Cooking with Maya Nut: Delicious Recipes for a Healthy Family"*) containing a variety of recipes incorporating Maya Nut (see Appendix III). Recipes for fresh Maya Nut include mock potato salad, ramón dough, croquettes, tamales, soup, tortillas, puree, dumplings, fritters, and quiche. Recipes for roasted Maya Nut include pancakes, bread, banana bread, pudding, cereal, ice cream, cake, cookies, and a coffee-like beverage. The cookbook instructs the reader to prepare Maya Nut for consumption by boiling with ash (1 cup to 1 gallon of water) for at least 2 hours ("until it is soft and tastes like potatoes"), rinsing well with water, and removing the skin from around the seeds. According to the cookbook, "Now you can substitute it for any recipe that calls for corn masa" (The Equilibrium Fund).

The first certified organic, industrial Maya Nut processing plant in Guatemala was established by ForesTrade, Inc. and Cafinter, S.A. in 2005. An example of an emerging trend in community-based processing is that of Alimentos Nutri-Naturales, which inaugurated a processing plant in December 2006 with help from numerous nongovernmental and governmental national and international organizations. Alimentos Nutri-Naturales, "a Guatemalan biodiversity-based business," was a finalist for the 2007 Equator Initiative Prize. The women-owned and operated cooperative, selected from over 130 competitors as one of the 10 best new businesses in Guatemala, was granted $10,000 from the president of Guatemala to help implement their business plan to produce and distribute ramón-based snacks to rural schools in order to improve the nutritional status of school-age children in Guatemala. Bread and cookies are among the products currently being manufactured at the facility.

To date, ramón has been incorporated into only a few commercial products with limited local distribution. A beverage mix containing Maya Nut, called Café de Mojo, is reportedly produced and sold locally by a small cottage business in Mexico City. No details on product formulation are available.

V.B.2 Current Background Intake

V.B.2.1 Current Production Estimates

Based on projected figures for 2008, national production estimates for Maya Nut in Guatemala, Honduras, Mexico, and Nicaragua have been set at 5,000, 1,000, 500, and 10,000 pounds, respectively, for a total of 16,500 pounds.

Amounts of Maya Nut exported by one producer in Guatemala were 18,230 kg in 2004, 960 kg in 2006, and 17,313 kg in 2007.

V.B.2.2 Current Per Capita Intakes

Manuel Lizzaralde, an ethnobotanist who wrote his dissertation on *B. alicastrum*, estimated that "a person might collect about 1 to 2 kilogram of Maya Nuts on a hunting expedition and share it with the family in the hunting camp." Of the 1995 field trip during which he made this observation, he said, "There were about 5 or 6 families in that camp (late April 1995) and I believe everyone was eating [ramón] that night" (Lizzaralde, 1995).

Average weekly consumption of Maya Nut in one community on the southern coast of Guatemala was estimated to be approximately 1 pound per family of 4 to 6 people (Vohman, 2007) or approximately 76 to 113 g/person/week (approximately 11 to 16 g/person/day).

V.B.3 Ongoing Special Nutritional and Clinical Programs

As part of a feeding program in Guatemala designed to improve nutritional status in school children, children are reportedly consuming 1 cookie and 1 glass of Maya Nut beverage with milk per day on school days (Vohman, 2007). This program involves at least 500 children in Peten, Guatemala, and another 500 on the southern coast. In another 4-month project in Guatemala, malnourished children were provided daily snacks that were prepared with Maya Nut to improve nutritional status (Ujexte Nutrition Project, 2006; see Section VI.B for study description). The Maya Nut-containing snacks provide daily intakes of ramón for these children.

In San Pedro del Norte, Nicaragua, pregnant women are routinely prescribed 1 pound per week of Maya Nut (approximately 65 g/woman/day) by the health clinic director as a means of increasing neonatal birth weight, with good results reported (Cruz, La Bendicon Guatemala, 2007).

According to another report, a typical quantity of the Maya Nut consumed as food by pregnant women or sick people in need of supplemental nutrition may be up to 12 ounces per day (approximately 340 g/person/day), in the form of a thick drink made with milk or water and sugar (Vohman, 2007).

There have been no reports of adverse effects associated with consumption of Maya Nut.

V.B.4 Non-Human Uses

In much of Central America, the most common current use of the Maya Nut is as animal fodder (Ortiz, 1995; Polito, 1998). *B. alicastrum* leaves, twigs, and seeds also are currently used widely throughout Central America as animal fodder (Ortiz, 1995; Polito, 1998). Animal husbandry professionals and scientists in the region recognize the nutritional value of the plant material (Gillespie, 2004; Santos Ricalde, 1995; Sandoval-Castro, 2005.) In animal husbandry, use of tree fodders as feed supplements can improve the rumen environment, leading to increased forage uptake. In a tree fodder preference assessment in cattle, beneficial effects of including tree fodder in the diet on dry matter intake were seen only with *B. alicastrum* and *Leucaena leucocephala.* In this study, *B. alicastrum* was deemed "the tree fodder with the highest digestibility both *in vitro* and *in situ*" (Sandoval-Castro, 2005).

Another dietary preference study involving spiny pocket mice (*Heteromys desmarestianus*) compared consumption rates and dietary values of a variety of seeds and fruits from the tropical-deciduous forest of Costa Rica, including *B. alicastrum*, over a 5-day period (Martinez-Gallardo, 1993). Dietary values (average change in body weight/average consumption rate) indicated that *B. alicastrum* was among the most valuable food species to spiny pocket mice. Earlier studies involving another tropical spiny pocket mouse species (*Liomys pictus*) have shown that the mice selectively consume certain fruits and seeds of plant species that produce large crops and avoid others containing toxic compounds (Martinez-Gallardo, 1993). Field observations of *L. pictus* "indicated a complex foraging behavior of rapid-individual learning to discriminate toxic seeds from nontoxic seeds and a tendency to harvest large amounts of seeds by making consecutive trips from rich seed sources to the burrows" (Martinez-Gallardo, 1993).

In forages, condensed tannins are significant because they influence voluntary intake and nutritive value through effects on ruminal digestibility. Values of free condensed tannins (FCT) 60 g/kg^{-1} or higher are reported to be less palatable and less digestible than forages with lower concentrations of FCT (Lopez, 2004). In an analysis of humid tropical fodder crops and their *in vitro* activity, *B. alicastrum* yielded a total condensed tannin (TCT) value of 25.8±0.8 g/kg^{-1}. Tannins bound to fiber (CTF) were the major component found. CTF were present at a concentration of 20.1$\pm$0.8 g/kg^{-1} (77.8% of TCT), while condensed tannins bound to protein (CTP) were present at 7.7$\pm$0.0 g/kg^{-1} (22.2% of TCT). No FCT were detected (Lopez, 2004).

Substitution of sorghum with 50% Maya Nut in chicken feed with 23% protein did not significantly affect weight gain after 30 days; however, 100% substitution did decrease weight gain (Lozano, 1978).

In Mexico, other current uses of Maya Nut include feeding boiled seeds to pigs and cows to increase milk production (Gillespie, 2004). Other present-day medicinal applications of *B. alicastrum* for livestock include use as a treatment for respiratory problems, as an anti-diarrheal, and as a toothache remedy (Gillespie, 2004).

VI. DATA TO SUPPORT SAFETY

Although the safety of the Maya Nut is primarily based on its long history of safe use, some animal and human safety data were identified. The results of a 4-month clinical trial conducted with children serve to further support its safe use as an ingredient in food for human consumption. Results of a few animal studies cited by review authors have been included only for completeness.

VI.A Animal and Other Relevant Studies

An LD_{50} of 47.3 cm^3/kg, reportedly demonstrating a low order of acute toxicity, was cited by Chappaz (1961) for *B. alicastrum* (preparation not specified), although neither the route of administration nor the test organism were reported.

As cited by Chappaz (1961), a *B. alicastrum* tincture was administered to a group of 6 pigeons aged 2.5 months by subcutaneous injection at a dose level of 10 m^3 for a period of 6 days. The authors concluded that *B. alicastrum* tincture stimulated lactation in a similar manner to prolactin in the treated birds.

An animal study performed to screen some medicinal plants of Mexico for selected biological activity concluded that a 40% extract of Maya Nut and leaf demonstrated antiatherogenic effects in male rats with induced hypercholesterolemia who were administered test extracts in water at 100 mg/kg via an intraperitoneal route. Extracts that reduced the serum cholesterol level in more than half the animals by at least 30% were considered active (Jiu, 1966). In one assay, the seed and leaf extract also demonstrated some central nervous system depressant activity (assay and effect unspecified) (Jiu, 1966).

One source discussed indigenous use of ash derived by burning *B. alicastrum* wood (and wood of several other trees) to disinfect utensils and the oral cavity (Farina, 1999). The ash exhibited activity against gram-negative bacilli, but not against gram-positive cocci.

VI.B Observations in Humans

Chappaz (1961) discussed the reports of a human study designed to assess *B. alicastrum*'s potential as a galactagogue. Twenty women who gave birth were treated for an unspecified period of time with 30 oral drops of *B. alicastrum* tincture three times a

day. No adverse effects or digestive troubles were reported by any of the subjects in this study. With respect to the ability of *B. alicastrum* to act as a galactagogue, 4 of the 20 women reported no effects, while 5 had modest results and 11 reported excellent outcomes; the authors concluded that *B. alicastrum* is a good galactagogue.

In a recently completed project, snacks containing Maya Nut were provided to children in 10 communities in southwest Guatemala and on the southern coast of Guatemala to determine the potentially beneficial effects of Maya Nut on nutritional status (Ujexte Nutrition Project, 2006). The project, a collaborative undertaking of the Equilibrium Fund and Association Amigas del Sol in Guatemala, included 164 children who were malnourished or in danger of malnutrition after Hurricane Stan. One group of 132 children received daily snacks containing Maya Nut or flour (*i.e*., cake, bread, soup, pancakes, and beverages, including *atol,* for which the community mothers had received recipes and preparation instructions). A control group of 32 children did not receive ramón snacks. All of the children were tested for weight, height, and hemoglobin levels at the beginning and again at the end of the project, which lasted approximately 4 months.

Some problems with study implementation, data collection, and data analysis likely affected the results of this study, which must be considered preliminary. Results from only 51 of the 132 children consuming Maya Nut-containing snacks were included in the analysis; results from the other 81 children were excluded for one of several reasons (*i.e*., missing height, weight, or hemoglobin data from either first or second test; lack of control group in the southwest region; or the final test was performed at an inappropriate interval, 2 months after study period ended). Shortcomings of the study included lack of training for data gatherers, possible errors in data collection, and a need to apply more rigorous statistical tools (such as a correlation coefficients and standard deviations) to analyze the data collected.

The available results suggested that children who received the snacks prepared with Maya Nut experienced better growth, weight gain, and improvements in nutritional status than children in the control group. When the data was adjusted to exclude outliers, the children receiving ramón-containing snacks grew on average 0.883 centimeters more than the control group during the project. When outliers were excluded, children in the ramón group gained on average 1.775 pounds more than children in the control group. Children consuming ramón snacks also had 0.723 higher hemoglobin levels than control children. No adverse effects of ramón consumption were reported in this study.

VI.C Additional Documentation Pertaining to Safety

VI.C.1 Potential Allergenicity

Nut allergies are among the most prevalent and commonly recognized food allergies. The Maya Nut is not closely related botanically to any edible tree nuts, but is in the same botanical order (Rosales) as almonds. However, because Maya Nut has been traditionally referred to as a "nut," the potential allergenicity of Maya Nut powders was investigated. Specifically, the potential for cross-reactivity between unroasted and roasted Maya Nut powder and allergens [immunoglobulin E (IgE)-binding proteins] from almonds and walnuts, commonly consumed tree nuts, was evaluated *in vitro* using radioallergosorbent test (RAST) inhibition, a competitive radioimmune assay (see Appendix IV for the full study report). Samples of unroasted and roasted Maya Nut powder were evaluated using sera from almond- or walnut-allergenic individuals. In the almond RAST inhibition assay, the unroasted Maya Nut powder sample exhibited weak IgE-binding, indicating that there could be a potential for cross-reactivity in individuals allergic to almonds. However, IgE-binding disappeared when roasted powder was assayed. Since roasting of almonds does not reduce IgE-binding, the IgE-binding observed with unroasted Maya Nut powder could be mediated by cross-reactivity of non-clinically significant and non-specific carbohydrate epitopes. Results of the walnut RAST inhibition assay showed that neither unroasted nor roasted Maya Nut powder cross-reacts with walnut protein, thus indicating that consumption of Maya Nut powder is unlikely to causes any allergic reactions in individuals with walnut allergies.

In a chemical analysis of commercial wood species, *B. alicastrum* wood was determined to contain 2,6-dimethoxy-p-benzoquinone, a naturally occurring quinone that exhibits strong bacteriostatic activity against microorganisms, but also may be a potential sensitizer that can cause allergic contact dermatitis. The compound was isolated and detected *via* both infrared and ultraviolet spectroscopy in *B. alicastrum* wood (not seed) (Hausen, 1978). No reports of contact dermatitis related to any part of *B. alicastrum* are reported in the literature.

VI.C.2 Phenylalanine

Maya Nut contains phenylalanine (Ortiz, 1995). Phenylalanine is an amino acid that should not be consumed by individuals with phenylketonuria (inability to metabolize phenylalanine) or pregnant women with high plasma levels of phenylalanine (hyperphenylalanine). However, phenylalanine is not unique to the Maya Nut and is a natural constituent of many protein-rich foods. Several of the foods included in the list of proposed food-uses for Maya Nut already contain background levels of this amino acid.

VII. SUMMARY AND CONCLUSION

The existing scientific evidence and history of safe use indicates that addition of the Maya Nut or powder derived from roasted or dried Maya Nuts to food would not be expected to result in any adverse effects.

Following harvesting, Maya Nut is dried and distributed for use in food without further processing or is ground to produce a powdered ingredient (granules or flour). Alternatively, the Maya Nut also may be roasted prior to grinding. Maya Nut and Maya Nut (dried or roasted) powder are proposed for use in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, grain products and pastas, gravies and sauces, and milk products at use levels of up to 87.5%. Under conditions of intended use, assessment of the estimated intake of Maya Nut ingredients on an all-user basis provided a mean intake by the total U.S. population of 70.56 g/person/day (34.28 to 84.72 g/person/day), while the 90th percentile intake of the Maya Nut ingredients was estimated at approximately 127.35 g/person/day (66.02 to 154.63 g/person/day). On a per kilogram body weight basis, mean and 90th percentile all-user intakes of 1.24 and 2.53 g/kg body weight/day, respectively, were estimated for the total U.S. population.

Maya Nut is harvested from the *B. alicastrum* tree, which is widely distributed throughout South and Central America. In addition to a high-quality protein content, the Maya Nut also contains carbohydrates, with smaller amounts of fat and minor amounts of flavonoids. All constituents of the Maya Nut are, therefore, consumed daily as part of the habitual Western diet and are expected to undergo normal physiological pathways of metabolism following consumption.

The assessment of safety of the Maya Nut and Maya Nut powder is based on the long history of use of Maya Nut and other parts of the *B. alicastrum* tree. Reports in the literature indicate that the incorporation of the Maya Nut into the daily diet of humans extends back to the classic Mayan period. Although the popularity of the Maya Nut as a staple food has declined over the years, it continues to be used as a specialty food item in Central and South America, with national production estimates for 2008 forecasted at approximately 16,500 lbs. Traditional uses of the Maya Nut as part of the diet of native communities provide weekly intakes in the range of 100 g/person (approximately 10 to 20 g/person/day).

The Maya Nut is recognized for its high nutrient content and is used to supplement the diets of undernourished children and ill individuals requiring nutrient supplementation. Current supplemental and therapeutic adult uses of Maya Nut in Central and South America result in daily intakes up to 340 g/person. Based on the long history of use and continued use of the Maya Nut, addition of Maya Nut or Maya Nut powder to foods under the intended conditions of use described herein, which is estimated to result in intake

levels several-fold lower than those from existing human uses of the Maya Nut, would not produce any adverse effects on human health.

The safety of the Maya Nut is further corroborated by reports of low acute toxicity in animals. Furthermore, no adverse effects were reported in children following daily consumption of Maya Nut-containing snacks for a period of 4 months. Based on measurements of height and weight and hemoglobin values, children in the group receiving snacks prepared with Maya Nut exhibited better nutritional status than those in the control group.

The safety of the Maya Nut and Maya Nut powder is supported by the fact that all constituents of the seed are consumed as part of the normal human diet. Furthermore, the Maya Nut has a well-established history of consumption and, under the conditions of intended use, would be consumed at levels that are within the range of intakes resulting from the background traditional uses of the Maya Nut by Central and South American populations. Furthermore, preliminary human trials, as well as use of the Maya Nut for supplemental and therapeutic uses, have demonstrated that the Maya Nut and food prepared with ingredients derived from the Maya Nut are well-tolerated and without adverse effects. The data and information summarized in this report support the conclusion that Maya Nut and Maya Nut powder, meeting appropriate food grade specifications and manufactured in accordance with current GMP, would be GRAS based on all the information presented herein.

VIII. REFERENCES

Anderson, S.A. (Ed.). 1988. Estimation of Exposure to Substances in the Food Supply. Federation of American Societies for Experimental Biology (FASEB), Life Science Research Office (LSRO); Bethesda, MD. [Contract No. FDA 223-84-2059].

Arvigo R. Personal communication, January 8, 2007.

Berg CC. 1972. Olmedieae, Brosimeae (Moraceae) by CC Berg. Flora Neotropica Vol. 7. The New York Botanical Press. Bronx, NY.

CDC. 2006. Analytical and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES). Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS); Hyattsville, Maryland. Available from: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf.

Chappaz G. Trials of a new galactogenic agent. Brosimum alicastrum. Bulletin de la Federation des societes de gynecologie et dobstetrique de langue francaise (1961); 13: 481-484.

Cravioto RO, GH Massieu, Guzman Garcia J, de la Torre JC. Nutritive value of food plants from Yucatan. Nutricion 1952;31:328-39. [In Spanish]

Cruz O. Community health care promoter, La Benedicion Guatemala, personal communication, March 2007.

Farina C, Goglio A, Kamliot N, Mainetti O, Tognoni G. Antibacterial activity of Nicaraguan vegetal extracts used in folk medicine. Igiene Moderna 1999; 111(1): 89-103. [In Italian with English abstract]

Gillespie AR, Bocanegra-Ferguson DM, Jimenez-Osornio JJ. The propagation of Ramon (Brosimum alicastrum Sw.; Moraceae) in Mayan homegardens of the Yucatan peninsula of Mexico. New Forests 2004; 27(1) 25-38.

Gomez-Pompa A, Flores JS, and Sosa V. The “Pet Kot”: a man made tropical forest of the Maya. Interciencia 1978;12(1):10-15.

Gomez-Pompa, Arturo. Ecology of the Vegetation of Veracruz. In: Graham A, ed. Vegetation and Vegetational History of Northern Latin America. 1973. Amsterdam: Elsevier. 73-148.

Hausen BM. Sensitizing capacity of naturally occurring quinones. Contact Dermatitis 1978; 4: 204-213.

Jiu J. A survey of some medicinal plants of Mexico for selected biological activities. Lloydia 1966;29(3):250-259.

Lambert JDH, Arnason JT. Ramon and Maya ruins: an ecological, not an economic, relation. Science 1982;216(4543):298-299.

Lentz DL, Ramirez-Sosa CR. Ceren plant resources: abundance and diversity. In: Sheets P, ed. Before the Volcano Erupted. The Ancient Ceren Village in Central America. 2002. Austin, Tex: University of Texas Press.

Lizarralde M. Perception, knowledge and the use of the rainforest: Ethnobotany of the Barí of Venezuela. 1997. Ph.D. Dissertation. University of California, Berkeley. CA.

Lizzaralde M. Personal communication, January 16, 2007.

Lopez J. Tejada O. Vasquez C. Condensed tannins in humid tropical fodder crops and their in vitro biological activity: Part 1. Journal of the Science of Food and Agriculture 2004;84(4):291-294.

Lozano O, Shimada AS, Avila E. Nutritional value of Maya Nut (Brosimum alicastrum) for chickens and pigs. Tecnica Pecuaria en Mexico 1978; 34:100-104.

Martinez-Gallardo R, Sanchez-Cordero V. Dietary value of fruits and seeds to spiny pocket mice, Heteromys desmarestianus (Herteromyidae). Journal of Mammology 1993;74(2):436-442

Miksicek CH, Elsesser KJ, Wuebber IA, Bruhns KO, Hammond N. Rethinking Ramon: a comment on Reina and Hill's lowland maya subsistence. American Antiquity 1981; 46(4):916-919.

Morton JF. Some folk-medicine plants of Central American markets. Quart J Crude Drug Res 1977;15:165-192.

Murray J. A descriptive account of the Palo Vaca, cow tree of the Caracas with chemical analysis of the milk and the bark. 1837. Effingham Wilson, Royal Exchange, London.

Nicholson MS, Arzeni CB. The market medicinal plants of Monterrey, Nuevo Leon, Mexico. Economic Botany 1993; 47:184-192.

Ortiz M, Azañón Y, Melgar M, Elias L. The corn tree (Brosimum alicastrum): a food for the tropics. In Simopoulos, AP, ed. Plants in Human Nutrition, World Review of Nutrition and Diabetics. 1995. Basel, Switzerland: Karger Publishers. pp 134-146.

Pearce F. The new life for Maya nut. Cahiers Agricultures 2006;15(4):387 [In French]

Peters CM. Observations on Maya subsistence and the ecology of a tropical tree. American Antiquity 1983; 48(3):610-615

Peters CM, Pardo-Tejada E. *Brosimum alicastrum* (Moraceae) uses and potentials in Mexico. Economic Botany 1982; 36(2): 166-175.

Polito JC, Devall MS. Brosimum alicastrum Sw. In: Burns RM, Mosquera MS, Whitmore JL, eds. Useful Trees of the Tropical Region of North America. 1988. Washington, DC: North American Forestry Commission Publication Number 3.

Puleston DE. An experimental approach to the function of classic Maya chultuns. American Antiquity 1971;36(3):322-335.

Puleston DE. The role of ramón in Maya subsistence. In: Flannery KV, ed. Maya Subsistence: Studies in Memory of Dennis E. Puleston. 1982. New York, NY: Academic Press, pp 353-366.

Ramirez-Sosa CR. Quantitative ethnobotany in El Salvador, Central America: a model to study ethnobotanical knowledge dynamics. 2006. Proceedings of the IVth International Congress of Ethnobotany (CEWB 2005).

Ramírez-Sosa CR. Ethnobotany of Brosimum alicastrum Sw. (Moraceae) in El Salvador.

Ramirez-Sosa CR, Lentz D. In review. Brosimum alicastrum SW. (Moraceae) torillas in El Salvador, C.A.

Richardson A. A gift from the Mayans. New Scientist 2006; 190:48-9.

Sandoval-Castro CA, Solorio-Sanchez FJ, Lizarraga-Sanchez HL. Assessment of tree fodder preference by cattle using chemical composition, in vitro gas production and in situ degradability. Animal Feed Science and Technology 2005; 123-124:277-289.

Santos Ricalde RH, Abreu Sierra JE. Evaluación nutricia de la *Leucaena leucocephala* y del *Brosimum alicastrum* y su empleo en alimentación de credos. Veterinaria México 1995; 26: 51-57.

Turner II, BL, Miksicek CH. Economic Species Associated with Prehistoric Agriculture in the Maya Lowlands. 1981. Paper presented at the 13th International Botanical Congress, Sydney.

Report on the Ujuxte Nutrition Project, Association Amigas del Sol, Gutemala, 2006.

USDA. 2006. What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2003-2004. U.S. Department of Agriculture (USDA); Riverdale, Maryland. Available from: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release.

U.S. FDA. 2000. Action levels for poisonous or deleterious substances in human food and animal feed. U. S. Food and Drug Administration (FDA). August 2000. Available from: http://www.cfsan.fda.gov/~lrd/fdaact.html#afla.

U.S. FDA. 2007a. Part 170—Food additives. Section §170.3—Definitions. In: U.S. Code of Federal Regulations Title 21: Food and Drugs. Food and Drug Administration (FDA). U.S. Government Printing Office (GPO); Washington DC, pp. 5-9. Available from: http://a257.g.akamaitech.net/7/257/2422/26mar20071500/edocket.access.gpo.gov/cfr_2007/aprqtr/pdf/21cfr170.3.pdf.

U.S. FDA. 2007b. Part 101—Food labeling. Sections §101.12— Reference amounts customarily consumed per eating occasion. In: U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration). U.S. Food and Drug Administration (U.S. FDA). U.S. Government Printing Office (GPO); Washington, DC, pp. 46-56. Available from: http://a257.g.akamaitech.net/7/257/2422/26mar20071500/edocket.access.gpo.gov/cfr_2007/aprqtr/pdf/21cfr101.12.pdf.

Vohman E. ¡Cooking with Maya Nut! Delicious Recipes for a Healthy Family. 2003. The Equilibrium Fund: www.TheEquilibriumFund.org

Vohman E. Personal communication, March 13, 2007.

Yates S, Ramirez-Sosa CR. Ethnobotanical knowledge of Brosimum alicastrum (Moraceae) among urban and rural El Salvadorian adolescents. Economic Botany 2004; 58(1):72-77.

Appendix I

[Analytical results]

Four Certificates of Analysis from Silliker Labs

Nutritional analysis (COA-26-April-07-31718714-0.pdf)

Nutritional analysis (COA-16-April-07-31704000-0.pdf)

Caffeine, gluten, heavy metals (COA-16-April-07-31704009-0.pdf)

Heavy metals, as lead (COA-19-April-07-31707347-0.pdf)

Appendix II

Estimated Daily Intake of Maya Nut Ingredients by the U.S. Population from Proposed Food-Uses

Appendix III

¡Cooking with Maya Nut! Delicious Recipes for a Healthy Family by Erica Vohman, Equlibrium Fund

Appendix IV

Investigations of the Potential Allergenicity of Maya Nut Powders Using Radioallergosorbent Test Inhibition (RAST-inhibition)

Appendix VI

Comments of Peer Reviewers

Credentials of Primary Reviewer

Credentials of Peer Reviewers

[To come]