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February 27, 2009

Via Federal Express

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835
Att: Robert L. Martin, Ph.D.

Re: GRAS Notification for SweetLeaf Stevia submitted by Wisdom Natural Brands

Dear Dr. Martin:

Please find enclosed the GRAS ASSESSMENT OF HIGH PURITY STEVIOL GLYCOSIDES - SWEETLEAF™ STEVIA Notice submitted by United American Industries, Inc. dba Wisdom Natural Brands. We are including three copies of this document bearing original signatures of James A. May, President and CEO of Wisdom. We have also included a digital copy of this document on a CD which is in .PDF format for your further reference.

If you have any questions regarding this Notice, please do not hesitate to contact me or Jim May at 480-921-1373. Thank you.

Very truly yours,

Steven E. Weinberger
General Counsel

/SEW
Enclosures

cc: Jennifer Thomas, CFSAN (by e-mail w/o enclosure)
Dr. Toni Mattia, CFSAN (by e-mail w/o enclosure)
Dr. Laura Tarantino, CFSAN (by e-mail w/o enclosure)

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MAR 3 2009
BY:

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GRAS ASSESSMENT

OF

HIGH PURITY STEVIOL GLYCOSIDES - SWEETLEAF® STEVIA

Food Usage Conditions for General Recognition of Safety

For

WISDOM NATURAL BRANDS

Gilbert, AZ

Evaluation by GRAS Expert Panel

Richard C. Kraska, Ph.D., DABT
Robert S. McQuate, Ph.D.
James R. Coughlin, Ph.D.
Robin C. Guy, M.S., DABT
Michael C. Falk, Ph.D.
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February 26, 2009

CONFIDENTIAL

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

High purity steviol glycosides, meeting the specifications for SweetLeaf® Stevia as described below, have been determined to be Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act.* This determination was made by an appropriately convened panel of experts who are qualified by scientific training and experience; the GRAS evaluation is based on scientific procedures as described in the following sections; and the evaluation accurately reflects the conditions of the stevia-derived sweetener's intended use in foods.

Signed:

2/27/09

James A. May
President & CEO
Wisdom Natural Brands

Date

B. Name and Address of Notifier

Wisdom Natural Brands
1203 W. San Pedro Street
Gilbert, AZ 85233

As the notifier, Wisdom Natural Brands ("WNB") accepts responsibility for the GRAS determination that has been made for the SweetLeaf® Stevia high purity steviol glycosides as described in the subject notification; consequently, the high purity steviol glycosides preparation meeting the conditions described herein is exempt from pre-market approval requirements for food ingredients.

[1] See 62 FR 18938 (17 April 1997).

C. Common Name and Identity of the Notified Substance

High purity steviol glycosides; also see Section III.A.

D. Conditions of Intended Use in Food

The high purity steviol glycosides preparation is intended to be added as a general purpose non-nutritive sweetener into various food categories at per serving levels that reflect good manufacturing practices principles in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, the SweetLeaf® Stevia high purity steviol glycosides have been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR 97702-3074.

II. INTRODUCTION

A. Objective

At the request of Wisdom Natural Brands (WNB), GRAS Associates, LLC (GA) has undertaken an independent safety evaluation of WNB's high purity steviol glycosides preparation that is identified as SweetLeaf® Stevia. SweetLeaf® Stevia is composed of approximately equal parts of two steviol glycosides, namely stevioside and rebaudioside (Reb) A. The purpose of the evaluation is to ascertain whether the intended food uses of SweetLeaf® Stevia as a general purpose non-nutritive sweetener is generally recognized as safe, i.e., GRAS, under the intended conditions of use.

B. Foreword

WNB provided GA with substantial background information needed to enable the GRAS assessment to be undertaken. In particular, the information provided addressed the safety/toxicity of steviol glycosides; the history of use of stevia in food; and compositional details, specifications, and method of preparation of SweetLeaf® Stevia. WNB was asked to provide adverse reports, as well as those that supported conclusions of safety.

Safety/toxicity studies performed with animals were noted to have value, along with available human testing. WNB was also asked to supply past and present human food use information. Knowing how much steviol glycosides has been safely consumed, i.e., the so-called "doses" or use levels, is critical in extrapolating to safe exposures for SweetLeaf® Stevia when consumed as a food ingredient. The composite safety/toxicity studies, in concert with exposure information, ultimately provide the specific scientific foundation for the GRAS determination.

WNB supplied the requested safety/toxicity studies and consumption/exposure information, along with other related documentation. This was augmented with an independent search of the scientific and regulatory literature extending through July 2008. A GRAS assessment based primarily on the composite safety information, that is, based on scientific procedures, with supportive information provided by common use in food, was undertaken. Those references that were deemed pertinent to the objective at hand are listed in Section VIII.

C. Summary of Regulatory History of Stevia and Stevia-Derived Sweeteners

Stevia derived-sweeteners are permitted as a food additive in South America and in several countries in Asia, including China, Japan, and Korea. As discussed more fully below, over the past few months, the subject sweeteners have received approvals in Australia, New Zealand, and Switzerland, and the US FDA has issued two "no objection letters" in response to the GRAS notifications filed on behalf of Reb A food uses.

In the US, steviol glycosides have been used as a dietary supplement since 1995 (Geuns, 2003). No application for dietary supplement use of purified Reb A is known to have been made. At least two GRAS petitions seeking authorization for the addition of stevioside or steviol glycosides to foods had been submitted to FDA since 1989, yet no authorizations had been issued by FDA in response to these filings, presumably because the previously available safety data---including purity considerations---for stevia, stevioside, or steviol glycosides were viewed as being inadequate. These petitions were subsequently withdrawn.

Individual GRAS nofitications were submitted by Merisant and Cargill to FDA in May, 2008 for Reb A, both more highly purified forms of the steviol glycosides.[2] FDA issued "no objection" letters for each of the GRAS notices on December 17, 2008.

The Food Standards Australia New Zealand (FSANZ) has completed evaluation of an application for use of steviol glycosides in foods and has recommended to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) to amend the Australia New Zealand Food Standards Code to allow its use in food (FSANZ, 2008).

Steviol glycosides have been under a lengthy review by the Joint FAO/WHO Expert Committee on Food Additives ("JECFA"). The original review was published in 2000 (WHO, 2000). A draft monograph was reviewed at the 51st, 63rd and 68th JECFA meetings. A temporary ADI (acceptable daily intake) of 0-2 mg/kg bw (on a steviol basis) was established at the 63rd meeting (WHO, 2006). In addition, food grade specifications were made final by JECFA (FAO, 2007a). At the 69th meeting, the temporary status of the ADI was removed and the ADI was raised to 0-4 mg/kg bw/day (on a steviol basis) as a result of the JECFA review of recently completed clinical studies with steviol glycosides (JECFA, 2008). A final monograph on steviol glycosides is expected from JECFA.

In August 2008, Switzerland's Federal Office for Public Health cited the favorable actions of JECFA in issuing its approval for the use of stevia as a sweetener (Switzerland Office of Public Health, 2008).

The stevia-derived sweeteners are not presently permitted as an ingredient in conventional food in the EU, UK, Hong Kong, or Canada (Hawke, 2003). This likely reflects a lack of review of new data on the sweeteners rather than a continuing concern about safety.

Hong Kong maintains that stevia is not permitted as a sweetener, as cited on the government website (Hong Kong Government, 2002). The Hong Kong Government was reported to be waiting for the JECFA determination on the safety of steviol glycosides. However, no further official actions have been noted since JECFA's final resolution was reported in June 2008.

Other international bodies have investigated the safety aspects of stevia and steviol glycosides use in foods. In 1999 in the EU, the Scientific Committee on Food (SCF) for the European

[2] GRAS notification 252 which was submitted by Merisant and GRAS notification 253 which was submitted by Cargill are listed on FDA's website at http://www.cfsan.fda.gov/~rdb/opa-grsn.html, along with the respective December 17, 2008 FDA "no objection" letters.

Commission concluded that "there are no satisfactory data to support the safe use of these stevia plants and leaves," as reported in a five-page opinion dated June 17, 1999 (European Commission, 1999a). The Committee also reiterated "its earlier opinion that stevioside is not acceptable as a sweetener on the presently available data," in a seven-page opinion also dated June 17, 1999 (European Commission, 1999b). Unconfirmed reports indicate that the European Food Safety Authority (EFSA) is reexamining the safety of steviol glycosides in light of JECFA's 2008 findings and in response to a June 2008 request by the European Commission for EFSA to evaluate steviol glycosides (EFSA, 2008). EFSA's Food Additives & Nutrient Sources (ANS) Panel has been given a deadline of March 31, 2010 to answer this request.

On September 24, 1998 in the UK, the Advisory Committee on Novel Foods and Processes for the Ministry of Agriculture, Fisheries and Food rejected an application for use of steviol glycosides as a sweetener in herbal teas because "the applicant had not provided all of the information necessary to enable an assessment to be made."[3]

D. FDA Regulatory Framework

Stevia, also known as steviol glycosides or steviosides, has been used in dietary supplements in the US since 1995 (Geuns, 2003) and is widely available to consumers in the US through retail outlets and Internet purchases (Al-Achi, 2000).

In accordance with FDA regulation of foods, however, dietary supplements cannot legally be added to conventional foods. Such ingredients must undergo premarket approval by FDA as food additives or, alternatively, the ingredients to be incorporated into conventional foods must be determined to be generally recognized as safe (GRAS). The authority to make GRAS determinations is not restricted to FDA. In fact, GRAS determinations may be provided by experts who are qualified by scientific training and experience to evaluate the safety of food and food ingredients under the intended conditions of use.[4]

In 1997, FDA altered the GRAS determination process by eliminating the formal GRAS petitioning process and replacing the petitioning process with a notification procedure.[5] While outlining the necessary content to be considered in making a GRAS determination, FDA encouraged that such determinations should be provided to FDA in the form of a notification. However, notifying FDA of such determinations is strictly voluntary.

[3] See http://www.maff.gov.uk/food/novel/980924.html.
[4] See 21 CFR 170.3(i)(3).
[5] See Footnote 1 or http://www.cfsan.fda.gov/~lrd/fr970417.html.

III. CHEMISTRY AND MANUFACTURE OF HIGH PURITY STEVIOL GLYCOSIDES AND SWEETLEAF® STEVIA

A. Common or Usual Name

High purity steviol glycosides is the common or usual name of the non-nutritive sweetener derived from *Stevia rebaudiana Bertoni* that is the subject of the GRAS evaluation, and the specific substance that is the subject of this safety evaluation is SweetLeaf® Stevia as produced and sold by WNB. The compositional features of the subject high purity steviol glycosides (i.e., SweetLeaf® Stevia) are described in more detail elsewhere in this Section.

JECFA adopted the term, steviol glycosides, for the family of chemical substances with sweetness properties that are derived from stevia. The term, stevia, is used more narrowly to describe the plant or crude extracts of the plant, while stevioside is the common name for one of the specific glycosides that is commonly extracted from stevia.

B. Chemistry of Steviol Glycosides

The following description is taken from the original JECFA monograph (WHO, 2000).

> Stevioside is a glycoside of the diterpene derivative steviol (ent-13-hydroxykaur-16-en-19-oic acid). Steviol glycosides are natural constituents of the plant *Stevia rebaudiana Bertoni*, belonging to the Compositae family. The leaves of *S. rebaudiana Bertoni* contain eight different steviol glycosides, the major constituent being stevioside (triglucosylated steviol), constituting about 5-10% in dry leaves. Other main constituents are rebaudioside A (tetraglucosylated steviol), rebaudioside C, and dulcoside A. *S. rebaudiana* is native to South America and has been used to sweeten beverages and food for several centuries. The plant has also been distributed to Southeast Asia. Stevioside has a sweetening potency 250-300 times that of sucrose and is stable to heat. In a 62-year-old sample from a herbarium, the intense sweetness of *S. rebaudiana* was conserved, indicating the stability of stevioside to drying, preservation, and storage (Soejarto et al., 1982; Hanson & De Oliveira, 1993).

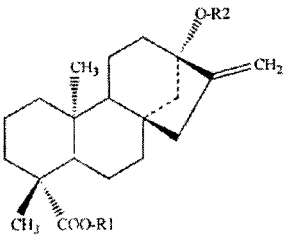
The predominant sweetener components of stevia extracts have been identified as stevioside and Reb A. The chemical identities and key chemical identifiers for the two major components are shown below.

Common Name:	Stevioside
Chemical Name:	13-[2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
Chemical formula:	$C_{38}H_{60}O_{18}$
Formula Weight:	804.88
CAS Number:	57817-89-7

Common Name: Rebaudioside A
Chemical Name: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy] kaur-6-en-8-oic acid, β-D-glucopyranosyl ester
Chemical Formula: $C_{44}H_{70}O_{23}$
Formula Weight: 967.03
CAS Number: 58543-16-1

In the most recent Chemical and Technical Assessment (FAO, 2007b), JECFA identified the sweetener components. They updated the list of common glycosides and their chemical stuctures, which are slightly different from compounds depicted in older publications (Nanayakkara et al., 1987; Suttajit et al., 1993). They are shown in Figure 1.

Figure 1. Chemical Structures of Various Steviol Glycosides[a]



	Compound name	C.A.S. No.	R1	R2
1	Steviol	471-80-7	H	H
2	Steviolbioside	41093-60-1	H	β-Glc-β-Glc(2→1)
3	Stevioside	57817-89-7	β-Glc	β-Glc-β-Glc(2→1)
4	Rebaudioside A	58543-16-1	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
5	Rebaudioside B	58543-17-2	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
6	Rebaudioside C (dulcoside B)	63550-99-2	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
7	Rebaudioside D	63279-13-0	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
8	Rebaudioside E	63279-14-1	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
9	Rebaudioside F	438045-89-7	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
10	Rubusoside	63849-39-4	β-Glc	β-Glc
11	dulcoside A	64432-06-0	β-Glc	β-Glc-α-Rha(2→1)

[a] From FAO, 2007b.

The structures of the components of steviol glycosides were also described in reviews by Kinghorn (2002), Kennelly (2002), and Geuns (2003). Non-sweet elements include the labdane diterpenes, triterpenes, sterols and flavonoid glycosides. Gas chromatography, infrared spectroscopy and mass spectroscopy have also revealed 31 constituents of volatile oil (Kinghorn, 2002). The structures, stereochemistry, and absolute configurations of steviol and isosteviol were established through a series of chemical reactions and analyses over a 20-year period after the seminal work of Bridel and Lavieille (1931) in France. Support for the proposed structures was achieved by the synthetic transformation of steviol into stevioside by Ogawa et al. in 1980 (cited in Brandle et al., 1998). Two of the other sweet diterpenoid glycosides, Reb A and B, were obtained from methanol extracts of stevia leaves, along with the major sweet principle constituent, stevioside, and steviolbioside, a minor constituent, which was first prepared from stevioside by alkaline hydrolysis by Wood et al. in 1955 (cited in Brandle et al., 1998). It was suggested subsequently that Reb B was an artifact formed from Reb A during isolation (Brandle et al., 1998; Kennelly, 2002). Furthermore, stevioside can be converted both chemically and enzymatically to Reb A. Further fractionation led to the isolation and identification of three other sweet glycosides respectively named Reb C, D, and E. It was reported that Reb A and D could be converted to Reb B by alkaline hydrolysis showing that only the ester functionality differed (Brandle et al., 1998). Dulcosides A and B were also described by Kobayashi et al. (1977). Dulcoside B was subsequently shown to have the identical structure as Reb C.

C. Accepted Identity Specifications for Food Grade Steviol Glycosides

The composition of extracts of *Stevia rebaudiana Bertoni* depends upon the composition of the harvested leaves, which, in turn, are influenced by soil, climate, and the manufacturing process itself (FAO, 2007b).

In 2007, JECFA recommended that food grade specifications for steviol glycosides consist of a minimum of 95% on a dried weight basis of seven specific steviol glycosides (FAO, 2007a, see Appendix A). The JECFA recommendation included appropriate analytical methods. Stevioside and Reb A are the component glycosides of interest for their sweetening property. The five other associated glycosides found in preparations of steviol glycosides accepted by the JECFA specification for the 95% requirement are Reb C, dulcoside A, rubusoside, steviolbioside, and Reb B. These, however, are typically found at lower levels than the stevioside or Reb A.

D. Manufacturing Processes

Manufacturing processes have been described in the scientific and patent literature as noted below, and WNB's SweetLeaf® Stevia manufacturing process is also provided.

1. Scientific and Patent Literature

Typically, steviol glycosides are obtained by extracting leaves of *Stevia rebaudiana Bertoni* with hot water or alcohols (ethanol or methanol); the obtained extract is a dark particulate

solution containing all the active principles plus leaf pigments, soluble polysaccharides, and other impurities. Some processes remove the "grease" from the leaves with solvents such as chloroform or hexane before extraction occurs (Kinghorn, 2002). There are dozens of patents for the extraction of steviol glycosides. Kinghorn (2002) has categorized the extraction patents into those based on solvent, solvent plus a decolorizing agent, adsorption and column chromatography, ion exchange resin, and selective precipitation of individual glycosides. Methods using ultrafiltration, metallic ions, supercritical fluid extraction with CO_2, and extract clarification with zeolite are found within the body of newer patents.

At the 68th JECFA meeting in 2007, steviol glycosides were defined as the products obtained from the leaves of *Stevia rebaudiana Bertoni*. As cited by JECFA, the typical manufacture starts with extracting leaves with hot water, and the aqueous extract is then passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with methanol to release the glycosides, and the product is recrystallized with methanol. Ion-exchange resins may be used in the purification process. The final product is commonly spray-dried.

2. Process for SweetLeaf® Stevia

The WNB process extracts the desired glycosides with membrane deionized water only, yielding a stevioside to Reb A ratio of about 50:50. The aqueous extract is purified to 60-65% using a membrane filtration system. Mechanical separation takes place at this juncture, and stevioside and Reb A are screened from large molecular weight and low molecular weight impurities. The resulting extract is finally polished with food grade ion exchange resin to achieve minimum glycoside specification. The polished extract is then concentrated to 30% Brix for spray drying. Following pasteurization at 85°C for 55 minutes, the pasteurized product is spray dried. An inlet temperature of 210°C is utilized, and the outlet temperature is 95°C to reduce moisture to specification.

E. Product Specifications and Supporting Methods

1. JECFA Specifications

JECFA finalized food grade specifications at the 68th JECFA meeting, which were then published in FAO JECFA Monograph 4 (FAO, 2007a). Steviol glycosides are described as a white to yellow powder, odorless to having a slight characteristic odor, and exhibiting a sweetness that is 200-300 times greater than sucrose. It is freely soluble in water and ethanol with a pH between 4.5 - 7.0 (1 in 100 solution). The product should not have more than 1% ash with no more than a 6% loss on drying at 105°C for 2 hours. Residual solvents (methanol) should not exceed 200 mg/kg (ppm). Arsenic levels should not exceed 1 mg/kg (determined by the atomic absorption hydride technique). Lead levels should not exceed 1 mg/kg. The complete JECFA specifications including recommended analytical methods are contained in Appendix A.

2. Specifications for SweetLeaf® Stevia

WNB has adopted product specifications for SweetLeaf® Stevia to meet or exceed JECFA recommendations. The specifications provided by WNB for the final spray dried product are given in Table 1, along with observed ranges seen with recent production batches. A report of an analytical study measuring the steviol glycoside content of the several batches of the WNB's high purity steviol glycosides product is given in Appendix B. Typical data on heavy metals and pesticide residues are also given in Appendix B.

Table 1. Specifications for SweetLeaf® Stevia

PARAMETER	WNB SPECIFICATIONS	WNB OBSERVED RANGES
Appearance	Off-white to white powder	Off-white to white powder
CHEMICAL SPECIFICATIONS		
Total Glycosides (as St, Reb A, Reb B, Reb C Dulc A, Rub, and SB) on dry weight basis	> 95%	97.1% - 98.4%
Phenolics on dry weight basis	2 - 4%	0.12 % - 0.18 %
Polysaccharides, Organic Acids, Inorganic Salts on dry weight basis	1-3%	< 2.5%
Moisture (loss on drying)	< 6%	3.4% - 4%
Ash	< 1%	0.5%
Solubility	Freely soluble in water and ethanol	Soluble in both solvents
pH (1% solution)	4.5 - 7.0	4.7 - 6.2
Lead	< 1 ppm	< 0.5 ppm
Arsenic	< 1 ppm	< 0.5 ppm
MICROBIOLOGICAL SPECIFICATIONS		
Aerobic Plate Count	< 2000 cfu/g	610 cfu/g
Mold	< 100 cfu/g	20 cfu/g
Yeast	< 100 cfu/g	< 10 cfu/g
Salmonella	Negative	Negative
Total *E. coli*	< 3 npm/g	< 3 npm/g
Fecal *E. coli*	< 3 npm/g	< 3 npm/g

Abbreviations: St = Stevioside. Reb A = Rebaudioside A. Reb B = Rebaudioside B. Reb C = Rebaudioside C. Dulc A = Dulcoside A. Rub = Rubusoside. SB = Steviolbioside.

F. Stability Data for Stevioside and Rebaudioside A

1. Scientific Literature

Stevioside is a stable molecule over the pH range 3 - 9 and can be heated at 100°C for 1 hour, but rapidly decomposes at pH levels greater than 9 under otherwise comparable conditions (Kinghorn, 2002). It is speculated that steviobioside produced from stevioside by alkaline hydrolysis would be the major decomposition product obtained at pH 10 (Kinghorn, 2002).

Chang and Cook (1983) tested the stability of pure stevioside and Reb A in carbonated phosphoric and citric acidified beverages and reported some degradation of both sweetening components after 2 months of storage at 37°C; however, there was no significant change at room temperature or below following 5 months storage of stevioside and 3 months storage of Reb A. They also reported that exposure to 1 week of sunlight did not affect stevioside, but it did result in approximately 20% loss of Reb A. Heating at 60°C for 6 days resulted in 0 - 6% loss of sweeteners.

As summarized below, extensive stability testing results were compiled for inclusion in both the Merisant and Cargill GRAS notifications.

Detailed stability testing was conducted by Merisant on Reb A as a powder, as a pure sweetener in solution, and on both cola-type and citrus carbonated beverages. No degradation was detected when the powder was stored at 105°C for 96 hours, and it was concluded that the powder was stable when stored for 26 weeks at 40±2°C with relative humidity of 75±5%. When considering Merisant's results of the stability investigations, there was considerable degradation noted after 13 hours at 100°C for carbonated beverage solutions and pure sweetener solutions (Merisant, 2008).

Cargill (2008) conducted detailed stability testing on Reb A as a powder under various storage conditions and under a range of pH and temperatures. In addition, Cargill assessed Reb A stability in several representative food matrices at room temperature and elevated temperatures. Stability profiles were created for table top sweetener applications, mock beverages including cola, lemon-lime, and root beer, yogurt, thermally processed beverages, and white cake. The stability testing revealed some degradation products that had not been detected in bulk Reb A. However, it was noted that these degradation products were structurally related to the steviol glycosides that are extracted from the leaves of *Stevia rebaudiana Bertoni.* The degradation products all share the same steviol aglycone backbone structure as found in stevioside and Reb A, but they differ by virtue of the glucose moities present.

Cargill (2008) also conducted photostability studies on the dry powder and mock beverages to ascertain Reb A behavior under defined conditions of fluorescent and near UV light exposure. Reb A was determined to be photostable under the defined conditions of analysis.

From the stability testing reported, it was concluded that Reb A is stable in various food matrices following several days or weeks of storage. The extent and rate of degradation is dependent on pH, temperature, and time. When placed in beverages, Reb A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, 2008).

2. Stability of SweetLeaf® Stevia

WNB reports that the dry high purity steviol glycosides product is stable when moisture is maintained below 8%; it exhibits a shelf life of 1 year as indicated by preservation of the glycoside profile and absence of caking. A two-year test of shelf life is in progress. It has been noted that the glycosides profile and caking stability for two years are reasonable when stored inside sealed polyethylene bags in cool, dry environments with similar products.

Regarding stability in water, WNB indicated that the sweetener products are stable in deionized water when the pH is less than 7. Above 7, it is unstable. If applied in non-deionized water at a pH above 5.5 in the final application, the product is not stable for long periods.

WNB reported that SweetLeaf® Stevia is stable in most foods as stability will likely be inversely related to water activity of the individual food. WNB also maintains that SweetLeaf® Stevia is stable in foods at cooking temperatures, and the observed stability at elevated temperatures correlates with water activity of the food.

The stability testing noted for SweetLeaf® Stevia, along with the stability test profile for stevioside and the more extensive stability testing presented by Merisant and Cargill for the chemically similar Reb A, supports the position that the subject high purity steviol glycosides are well-suited for the described intended food uses.

IV. INTENDED DIETARY USES

A. Intended Uses

WNB intends to market its SweetLeaf® Stevia high purity steviol glycosides for incorporation into various food categories as a general purpose sweetener, including specific food uses as reported in other countries and as depicted in Table 2a. SweetLeaf® Stevia will function as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19). The use levels will vary by food category, but the levels are self-limiting due to organoleptic factors and consumer taste considerations. However, the amounts of SweetLeaf® Stevia to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation.[6]

B. Food Uses as Addressed by JECFA, Merisant and Cargill

Many estimates of possible consumption of stevia-based sweeteners have been published. Both JECFA and FSANZ reviewed estimates of possible consumption of mixed steviol glycosides as part of their safety deliberations. In addition, Merisant and Cargill estimated the consumption of Reb A in their submissions to FDA.

Estimated maximum use levels in various foods as evaluated by JECFA are summarized in Table 2a.

Table 2a. Food Uses of Steviol Glycosides Reported to JECFA with Calculated Steviol Equivalents

FOOD USES	MAXIMUM USE LEVEL REPORTED[a] (mg STEVIOL GLYCOSIDES / kg OF FOOD)	MAXIMUM USE LEVEL CALCULATED[b] (mg STEVIOL EQUIVALENTS / kg OF FOOD)
Desserts	500	200
Cold confectionery	500	200
Pickles	1000	400
Sweetcorn	200	80
Biscuits	300	120
Beverages	500	200
Yogurt	500	200
Sauces	1000	400
Delicacies	1000	400
Bread	160	64

[a] From WHO, 2006. [b] Calculated by Expert Panel by multiplying by ratio of molecular weight of steviol to molecular weight of stevioside.

[6] See 21 CFR 182.1(b)(1).

Merisant listed expected levels of use for Reb A for various food applications in their GRAS Notification. Their consumer estimates were largely based on food consumption survey data from 2003-2004 NHANES, a resource that reflects food intake over a two-day time period. Statistically weighted values were utilized to provide reliable quantitative findings that are representative of food consumption of actual "users" within the US population. The 2-day food surveys are known to overestimate actual consumption levels when compared to longer term food surveys, such as those based on 14-day surveys. On a per user basis, the mean daily consumption of Reb A was calculated to be 2.0 mg/kg bw/day, and that for the 90th percentile consumer was found to be 4.7 mg/kg bw/day. Specific food uses and use levels are given in Table 2b.

Table 2b. Proposed Uses and Levels of Rebaudioside A by Merisant (2008)

FOOD USES	REB A (PPM)
Tabletop sweeteners	30,000[a]
Sweetened ready-to-drink teas	90-450
Fruit juice drinks	150-500
Diet soft drinks	150-500
Energy drinks	150
Flavored water	150
Cereals (oatmeal, cold cereal, cereal bars)	150

[a] Reb A content of sachet prior to dilution and not representative of "as consumed."

Cargill utilized a different approach in estimating dietary intake figures for Reb A when incorporated as a general sweetener in a broad cross-section of processed foods (Cargill, 2008). Cargill reasoned that Reb A uses and use levels would be rather comparable to aspartame uses in the US with a few minor exceptions. They performed a side-by-side consumption analysis for Reb A versus aspartame, using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008). Their findings are considered further in Section IV.C.

C. Estimated Daily Intake

WNB intends to incorporate SweetLeaf® Stevia into a broad selection of foods as a general-purpose sweetener, but specific consumption estimates (i.e., numbers of servings or firm use levels) for the individual food categories have not been provided. Instead, the very conservative

consumer intake estimates provided by JECFA (as shown above in Table 2a) were utilized to gauge the potential human exposures of the subject steviol glycosides in foods as reported in the US and in other countries.

JECFA evaluated information on exposure to steviol glycosides as submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana Bertoni* plants and leaves that was prepared for the European Commission by the Scientific Committee on Food.

JECFA used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that **steviol glycosides would replace all dietary sugars**, at the lowest reported relative sweetness ratio for steviol glycosides and sucrose which is 200:1. The intakes ranged from 1.3 mg/kg bw/day with the African diet to 3.5 mg/kg bw/day with the European diet.

JECFA also estimated the per capita exposure derived from disappearance (poundage) data supplied by Japan and China. The Committee evaluated exposures to steviol glycosides by assuming full replacement of all dietary sugars in the diets for Japan and the US. Table 3 summarizes the exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee.

Table 3. Summary of Estimates of Exposure to Steviol Glycosides (as Steviol)

ESTIMATE	EXPOSURE (mg/kg BW/DAY)
GEMS/Food (International)[a]	1.3 -3.5 (for a 60 kg person)
Japan, Per Capita	0.04
Japan, Replacement Estimate[b]	3
US, Replacement Estimate[b]	5

[a] WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme.
[b] These estimates were prepared in parallel to those for the international estimates; it was assumed that all dietary sugars in diets in Japan and the US would be replaced by steviol glycosides on a sweetness equivalent basis, at a ratio of 200:1.

JECFA concluded that the replacement estimates were highly conservative---that is, the calculated dietary exposure overestimates likely consumption---and that true dietary intakes of steviol glycosides (as steviol) would probably be **20 – 30% of these values or 1.0 - 1.5 mg/kg bw/day on a steviol basis.**

FSANZ (2008) similarly estimated steviol glycoside dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario which resulted in estimated exposures of 0.3 - 1.0 mg/kg bw/day for the mean and 90th percentile consumer, respectively. FSANZ examined consumption in other age groups and concluded there was no safety concern in children of any age.

Merisant also calculated a dietary estimate for Reb A of 2.0 mg/kg bw/day for the average consumer of the foods listed in Table 2b and 4.7 mg/kg bw/day for a 90th percentile consumer. On a steviol equivalent basis, the Merisant estimates would be 0.7 and 1.6 mg/kg bw/day, respectively.

In another recent consumption estimate review conducted on behalf of Cargill and included in their GRAS notification, the intake of Reb A when used as a complete sugar replacement was estimated at 1.3 - 3.4 mg/kg bw/day when calculated as Reb A or 0.4 - 1.1 mg/kg bw/day on a steviol basis (Renwick, 2008).

In concert with the JECFA intake estimates, the anticipated human exposures as projected independently and with different approaches by both Merisant and Cargill in compiling their GRAS dossiers for Reb A (Merisant, 2008 and Cargill, 2008) tended to converge to yield estimated daily intakes (EDIs) in the range 0.4 - 1.6 mg/kg bw/day on a steviol basis or 1.3 - 4.7 mg/kg bw/day on a Reb A basis

The actual daily intakes of stevioside from products such as WNB's SweetLeaf® Stevia, and from Reb A from products such as SweetLeaf® Stevia and Merisant's and Cargill's Reb A products, cannot yet be determined with accuracy. The extent that stevia-based sweeteners will penetrate the US food supply and the extent the market will select mixed steviol glycoside products versus Reb A products is uncertain. Furthermore, many competing non-caloric sweeteners are currently available to consumers which have been successful in the marketplace, most notably aspartame and sucralose.

In light of the above considerations, the intake estimates presented here are viewed as being overestimates of actual consumption. When comparing these EDI assessments for steviol glycosides, we see that total daily consumption of the steviol glycosides and Reb A for defined food uses and as a general purpose sweetener is expected to be substantially less than the acceptable daily intake values discussed more fully in Section VI.B.1.

D. Other Information on Human Exposure to Stevia

The predominant use of stevia as a food ingredient presently occurs in Brazil and Japan.[7] It is reported that 40% of the artificial sweetener market in Japan is stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999).

Stevia usage as a dietary supplement is presently permitted in the US, Canada, Australia and New Zealand. It has wide use in China and Japan in food and in dietary supplements.
In the US, stevia is available in packets containing 60 - 90 mg steviol glycoside for home supplement uses, such as in beverages or other foods. It is estimated that sales of stevia in the US reached $45 million in 2005 (The Food Institute Report, 2006). No estimates are available on

[7] See Raintree Nutrition Tropical Plant Database (www.rain-tree.com/stevia.htm).

the daily consumption levels of steviol glycosides consumed in the US via dietary supplements in the form of capsules, softgels, tablets, etc.

In South America, stevia is commonly used as a treatment for type 2 diabetes (Hawke, 2003). However, elevated doses in the range of 1 gram per person per day or more were reported to be necessary to achieve this therapeutic effect (Gregersen et al., 2004).

V. SAFETY DATA FOR STEVIOL GLYCOSIDES

A. Safety Data on Steviol Glycosides: Reviews by Expert Bodies and Other Scientists

Because SweetLeaf® Stevia is a mixed glycoside product composed of two major ingredients, stevioside and reb A, the data on each component are relevant to this review. The biological, toxicological and clinical data on stevia and steviol glycosides have been assessed by a number of reviewers (Brusick, 2008a; Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002) and most notably through the on-going evaluation by JECFA (WHO, 2000, 2006, 2007, 2008) and a review by Food Standards Australia New Zealand (FSANZ, 2008) for use in food. Most of these reviews completed primarily focused on mixtures of steviol glycosides. The early reviews tracked the development of better toxicology studies on purer samples of steviol glycosides. These reviews followed with keen interest whether effects of concern seen in various toxicology studies, such as the decrease in fertility with crude stevia preparations and the mutagenic activity of the principle metabolite steviol, would be manifest in comprehensive studies using modern test protocols with pure test materials. In addition, JECFA encouraged the further elucidation of clinical effects on blood pressure and glucose metabolism in hypertensive and diabetic individuals, respectively, in comparison to normal human subjects. By 2006, sufficient favorable data were generated for JECFA to yield a temporary ADI which was finalized at an elevated level in 2008. More details on the JECFA reviews are discussed in Section V.A.1. The key toxicology and clinical data on steviol glycosides (primarily stevioside), more recently developed data on Reb A, and data on the principle metabolite, steviol, as reviewed by JECFA and other reviewers are summarized in Sections V.B, V.C and V.D, respectively.

1. Summary of Interim and Final JECFA Reviews

In 1999, the 51st meeting of JECFA (WHO, 2000) expressed the following reservations about the safety data available at that time for steviol glycosides:

> The Committee noted several shortcomings in the information available on stevioside. In some studies, the material tested (stevioside or steviol) was poorly specified or of variable quality, and no information was available on other constituents or contaminants. Furthermore, no studies of human metabolism of stevioside and steviol were available. In addition, data on long-term toxicity and carcinogenicity were available for stevioside in only one species. The mutagenic potential of steviol has been tested sufficiently only *in vitro*.

Additional data were requested and subsequently provided on the metabolism of steviol glycosides. These data clarified that the common steviol glycosides are converted to steviol by intestinal bacteria and then rapidly converted to glucuronides that are excreted. The Committee now had a molecular basis to become comfortable with studies on test materials which consisted of variable composition but were relatively high purity mixtures of the common steviol glycosides. The Committee came to the conclusion that steviol glycosides are not mutagenic and that steviol is mutagenic only in *in vitro* studies but not *in vivo*. The Committee also became convinced that 1) purified steviol glycosides did not impair reproductive performance as did crude preparations of stevia and 2) there were sufficient chronic studies in rats with adequate no observed adverse effect levels

(NOAELs) to support a reasonable temporary acceptable daily intake (ADI) in the range of doses that would be encountered by the use of steviol glycosides as a sugar substitute. However, JECFA wanted more clinical data to rule out pharmacological effects at the expected doses. The following excerpt is taken from the report of the 63rd meeting (WHO, 2006):

> The Committee noted that most of the data requested at its fifty-first meeting, e.g., data on the metabolism of stevioside in humans, and on the activity of steviol in suitable studies of genotoxicity *in vivo*, had been made available. The Committee concluded that stevioside and rebaudioside A are not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*.
>
> The NOEL for stevioside was 970 mg/kg bw/day in a long-term study (Toyoda et al., 1997) evaluated by the Committee at its fifty-first meeting. The Committee noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses corresponding to about 12.5–25 mg/kg bw/day (equivalent to 5–10 mg/kg bw/day expressed as steviol). The evidence available at present was inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g., those with hypotension or diabetes).
>
> The Committee therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg/kg bw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970 mg/kg bw/day (or 383 mg/kg bw/day, expressed as steviol) in the 2-year study in rats and a safety factor of 200. This safety factor incorporates a factor of 100 for inter- and intra-species differences and an additional factor of 2 because of the need for further information. The Committee noted that this temporary ADI only applies to products complying with the specifications.
>
> The Committee required additional information, to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics.

At the 68th meeting in 2007, JECFA apparently felt that sufficient progress had been made on the clinical studies and extended the temporary ADI until 2008 (WHO, 2007). Furthermore, sufficient data had been received to revise and finalize food additive specifications for steviol glycosides (FAO, 2007a). The Chemical and Technical Assessment report, written after the 2007 meeting, explained the Committee's thinking which resulted in flexibility in the identity specifications (FAO, 2007b).

> In response to the call for data on "stevioside" for the 63rd meeting of the Committee, submissions from several countries showed that the main components of the commercially available extracts of stevia are stevioside and rebaudioside A, in various amounts ranging from about 10-70% stevioside and 20-70% rebaudioside A. The information indicated that most commercial products contained more than 90% steviol glycosides with the two main steviol glycosides comprising about 80% of the material. The 63rd JECFA required that the summed content of stevioside and rebaudioside A was not less than 70% and established a minimum purity of 95% total steviol glycosides. Analytical data showed that most of the remaining 5% could be accounted for by saccharides other than those associated with the individual steviol glycosides.
>
> Noting that the additive could be produced with high purity (at least 95%) and that all the steviol

> glycosides hydrolyze upon ingestion to steviol, on which the temporary ADI is based, the 68th JECFA decided it was unnecessary to maintain a limit for the sum of stevioside and rebaudioside content. The Committee recognized that the newly revised specifications would cover a range of compositions that could include, on the dried basis, product that was at least 95% stevioside or at least 95% rebaudioside A.

At the 69th meeting in 2008, JECFA came to a final evaluation (WHO, 2008) based on their satisfaction with the completed clinical studies, and the Committee actually raised the ADI to 0 – 4 mg/kg bw/day and removed the "temporary" designation. A final toxicology monograph will likely be published soon. The summary of the Committee's key conclusions are as follows:

> ADI of 0–4 mg/kg bw expressed as steviol, based on a NOEL of 970 mg/kg bw per day from a long-term experimental study with stevioside (383 mg/kg bw per day expressed as steviol) and a safety factor of 100. The results of the new studies presented to the Committee showed no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw per day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks.
>
> Some estimates of high-percentile dietary exposure to steviol glycosides exceeded the ADI, particularly when assuming complete replacement of caloric sweeteners with steviol glycosides. The Committee recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI.

2. Summary of FSANZ Review of Steviol Glycosides

Food Standards Australia New Zealand (FSANZ) completed a review of the safety of steviol glycosides for use as a sweetener in foods in 2008. The risk assessments undertaken by FSANZ concluded that steviol glycosides are well-tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day. The FSANZ review discussed the adequacy of the existing database and several new studies, including the clinical studies reviewed by JECFA in the summer of 2007, most notably the work of Barriocanal et al. (2008).

In their early reviews, FSANZ expressed the opinion that new data in humans provide a basis for revising the uncertainty factors that were used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened, so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. FSANZ anticipated that JECFA would raise the ADI at the summer meeting in 2007. At that 2007 meeting, JECFA elected to extend its current temporary ADI for another year, apparently awaiting the completion of the clinical studies. Therefore, FSANZ established an ADI of 4 mg/kg bw/day, derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study (FSANZ, 2008). It is important to note that JECFA and FSANZ arrived at the same ADI.

B. Safety Data on Stevioside and Stevia Extracts that are Predominantly Stevioside

This Section summarizes studies on stevioside or stevia extracts that were identified compositionally as predominantly stevioside. Related studies on Reb A are discussed in Section V.C, Safety Data on Rebaudioside A.

1. Absorption, Distribution, Metabolism and Excretion (ADME) Studies

Many studies in rats (Wingard et al., 1980; Nakayama et al., 1986; Koyama et al., 2003a) and other animal models, including chickens (Geuns et al., 2003a), hamsters (Hutapea et al., 1999), and pigs (Geuns et al., 2003b) indicate that stevioside is not readily absorbed from the GI tract.

There is evidence from *in vitro* metabolism studies that bacteria in the colon of rats and humans can transform various stevia glycosides into steviol (Gardana et al., 2003). Steviol was shown to be more readily transported with *in vitro* intestinal preparations than various steviosides (Geuns, 2003, Koyama et al., 2003b). Slow absorption of steviol was indicated by detection in the plasma of rats given oral stevioside (Wang et al., 2004). However, Sung (2002) did not detect plasma steviol after oral doses of steviosides when administered to rats. In studies with human and rat liver extracts, it was demonstrated that steviol can be converted to various glucuronides (Koyama et al., 2003b).

Excretion of metabolites of stevioside after oral doses has been shown in urine and feces in rats (Sung, 2002) and hamsters (Hutapea et al., 1999). Oral doses in pigs led to the detection of metabolites in feces but not in urine (Geuns et al., 2003b).

In a study using 10 healthy human subjects, blood, urine and fecal metabolites were measured after subjects received 3 doses of 250 mg of purified stevioside (>97%) 3 times a day for 3 days. Urine was collected for 24 hours on day 3 and blood and fecal samples were also taken on day 3. Free steviol was detected in feces but not in blood or urine. Steviol glucuronide was detected in blood, urine and feces. 76% of the total steviol equivalents dosed were recovered in urine and feces. Based on these measurements, the authors concluded that there was complete conversion of stevioside in the colon to steviol, which was absorbed and rapidly converted to the glucuronide (Geuns et al., 2006).

2. Acute Toxicity Studies

Studies of the toxicity of stevioside (purity, 96%) given as single oral doses to rodents are summarized in Table 4. No lethality was seen within 14 days after administration, and no clinical signs of toxicity or morphological or histopathological changes were found, indicating that stevioside is very non-toxic.

Table 4. Acute Toxicity of Stevioside (Purity, 96%) Given Orally to Rodents

SPECIES	SEX	LD_{50} (g/kg bw)	REFERENCE
Mouse	Male and Female	>15	Toskulkao et al., (1997)
Mouse	Male	> 2	Medon et al., (1982)
Rat	Male and Female	>15	Toskulkao et al., (1997)
Hamster	Male and Female	>15	Toskulkao et al., (1997)

3. Subchronic Toxicity Studies

Three published subchronic studies with oral administration of stevioside have been conducted in rats. In addition, a reproduction study in hamsters included subchronic phases on the F_0, F_1 and F_2 generations. These are summarized in Table 5.

One of these studies was particularly important because it served as a range-finding study for two subsequent chronic studies. A 13-week toxicity study was carried out in Fischer 344 rats given doses of 0, 0.31, 0.62, 1.25, 2.5, or 5% in the diet (equivalent to 160, 310, 630, 1300, and 2500 mg/kg bw/day) to determine the appropriate doses for a two-year study for carcinogenicity. The rats were randomly allocated to six groups, each consisting of 10 males and 10 females. None of the animals died during the administration period, and there was no difference in body-weight gain between the control and treated groups during administration or in food consumption in the latter part of the study. The activity of lactic dehydrogenase and the incidence of single-cell necrosis in the liver were increased in all groups of treated males. The authors considered these effects to be nonspecific, because of the lack of a clear dose-response relationship, the relatively low severity, and their limitation to males. Other statistically significant differences in hematological and biochemical parameters were also considered to be of minor toxicological significance. The authors concluded that a concentration of 5% in the diet was a suitable maximum tolerable dose of stevioside for a two-year study in rats (Aze et al., 1991).

In earlier 3-month rat studies reviewed by Geuns (2003)---the sample purity, doses, strain of rat were not reported---a no effect level was determined to be in excess of 2500 mg/kg bw/day and 7% of the diet, apparently due to lack of effects at the highest dose tested in both studies (Akashi and Yokoyama, 1975).

Table 5. Subchronic Studies on Stevioside

STUDY	ANIMAL MODEL/ GROUP SIZE	TEST MATERIAL/ SAMPLE PURITY	DOSES / DURATION	AUTHOR ASSIGNED NOAEL (mg/kg bw/day)	RESULTS AND REMARKS
Aze et al., 1991[a]	F344 rat/ 10 females and 10 males in each of 6 groups	Stevioside/ Not reported	0, 0.31, 0.62, 1.25, 2.5, 5% in diet/13 weeks	Not reported	No effects observed on mortality, body weight or food consumption. Clinical chemistry investigation revealed increased LDH levels and histopathological investigation indicated increased incidence of single-cell liver necrosis in all male treated groups, but not in a clear dose-response relationship. Invstigators did not consider these changes to be treatment related due to the small magnitude and low severity of changes, the lack of a clear dose relationship and the limitation to males only. Organ weights, urine chemistry and gross necropsy not discussed. Authors concluded that 5% stevioside in diet is a tolerable dose for a 2 year study.
Yodyingyuad and Bunyawong, 1991[a]	Hamster/ four groups of 20 (10 male, 10 female)	Stevioside/ 90%	0, 0.5, 1.0, 2.5 g/kg bw/day/ duration unclear/ 3 months	2500	F_0, F_1 and F_2 generations in reproductive study were dosed for 90 days. Histological examination showed no effect at any dose. Weights of organs, blood analysis, urine chemistry and gross necropsy not discussed. The F_1 and F_2 hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents).
Mitsuhashi, 1976[b]	Rat (strain not reported)	Stevioside/ Not reported	Dietary concentrations up to 7%/ 3 months	Not reported	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy and histopathology not discussed.
Akashi and Yokoyama, 1975[b]	Rat (strain not reported)	Stevioside/ Not reported	Oral doses up to 2500 mg/kg bw/3 months	2500	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy and histopathology not discussed.

[a] Abstract only. [b] As reported by Geuns, 2003.

4. Chronic Toxicity Studies

There have been three chronic rat studies conducted on stevioside which are summarized in Table 6. No treatment-related increase in tumor incidence was seen in any of these studies. In the most recent and best-documented study (additional study details were presented to JECFA in 2006), the apparent no observed adverse effect level (NOAEL) in F344 rats was the dietary level of 2.5% (test sample purity 96%, Toyoda et al., 1997). At 5% of the diet, statistically significant decreases in body weight, percent survival and kidney weight were seen. The author attributed these effects to various factors. The decrease in body weight was attributed to an inhibition of glucose utilization. The decrease in survival seemed to have been caused by an unusual late onset of large granular lymphocyte leukemia in high dose males. The authors reported that this tumor is

rather common in F344 rats and that the overall incidence in male rats was actually within the historical control range experienced in the particular laboratory. The authors attributed the decrease in kidney weight as probably due to a decrease in chronic inflammation found in the histopathological examination relative to control animals.

Table 6. Chronic Toxicity Studies on Stevioside

STUDY	ANIMAL MODEL/ GROUP SIZE	TEST MATERIAL/ SAMPLE PURITY	DOSES / DURATION	AUTHOR ASSIGNED NOAEL (mg/kg bw/day)	RESULTS AND REMARKS
Toyoda et al., 1997	F344 rat/ 50 per sex per group	95.6% Stevioside	*Ad libitum* 0,2.5, 5% of diet/~24 months (104 weeks)	Author did not assign a NOAEL. (Mid-dose calculates to 970 in males; JECFA, 2006)	A significant decrease in survival rates in males receiving 5%. General condition, body weight, food intake, mortality, hematological, histopathological and organ weights were observed. Body weight gains dose-dependently decreased in both sexes. Kidney weights were significantly lower in 5% males and ovary, kidney and brain weights were significantly increased in 5% females. Tumors and non-neoplastic lesions found in all groups, and were not correlated to treatment. Conclusion was that stevioside is not carcinogenic under these experimental conditions.
Xili et al., 1992[a]	Wistar rat/ 45 per sex per group	85% Stevioside	0, 0.2, 0.6, 1.2 % of diet/24 months	794 (high dose)	After 6, 12 and 24 months five rats from each group were sacrificed for analysis. No effects observed on growth, food utilization, general appearance, mortality or lifespan. No changes in hematological, urinary or clinical biochemical values. Histopathological analysis showed that the neoplastic and non-neoplastic lesions were unrelated to the level of stevioside in the diet.
Yamada et al., 1985	F344 rat/ 70 per sex per group, 30 per sex per group in low-dose	95.2% Steviol glycosides (75% stevioside; 16% Reb A)	0.1, 0.3, 1% of diet/22 months for males, 24 months for females	550 (high dose)	At 6 and 12 months, 10 males and 10 females were sacrificed for analysis. General behavior, growth and mortality were same among groups throughout the experiment. At 6 months, proteinurea was significantly increased in females, and blood glucose was increased in both sexes, although urinary glucose not detected. Weights of liver, kidney, heart, prostate and testes were increased in males at 6 months, and weight of ovaries was decreased in females in dose-dependent manner. Histopathological examination showed differences in various organs at 6 months that were unrelated to stevioside dose. These differences were not found at 12 months. Authors concluded that there were no significant changes after 2 years.

[a] Only abstract available.

5. Reproductive and Developmental Toxicity Studies

S. rebaudiana has been used by Indians in Paraguay as an oral contraceptive (Planas and Kuc, 1968; Schvartzman et al., 1977). Crude stevia leaf extract has been shown to inhibit fertility in rats (Planas and Kuc, 1968). Several reproductive toxicity studies have been

conducted with orally administered purified stevioside as tabulated in Table 7. No effect on fertility or reproductive parameters was seen in a three-generation study in hamsters at doses up to 2500 mg/kg/day (Yodyingyuad and Bunyawong, 1991). There was an absence of statistically significant effects at doses up to 3% (equivalent to 3000 mg/kg bw/day; sample purity 96%; Mori et al., 1981). Similar results were observed in an additional rat study that was reviewed by Geuns (2003) where limited information is available in English (Usami et al., 1995). Further details on these studies to the extent available are given in Table 7.

Table 7. Reproductive Toxicity Studies on Steviol Glycosides

Study	Animal Model/ Group size	Test Sample Purity Stevioside (Unless Otherwise Noted)	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results and Remarks
Usami et al., 1995[a]	Wistar Rat/4 groups of 25 or 26 pregnant rats	95.6%[b]	0, 250, 500, 1000 mg/kg bw/10 days	1000	Pregnant rats given doses of stevioside by gavage once a day on days 6-15 of gestation and were sacrificed on day 20 of gestation. Fetuses were examined for malformations in addition to maternal and fetal body weight, number of live fetuses, sex distribution, and numbers of resorptions or dead fetuses. No treatment-related effects observed. Authors concluded that orally administered stevioside is not teratogenic in rats.
Yodyingyuad and Bunyawong, 1991	Hamster/ 10 male, 10 female per group (40 total)	90%	0, 500, 1000, 2500 mg/kg bw/day/ duration unclear/ 3 months	2500	Males from each group were mated to females from respective dose group. Each female was allowed to bear 3 litters during the course of experiment. Stevioside had no effect on pregnancies of females at any dose. The F_1 and F_2 hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents); showed normal growth and fertility. Histological examination showed no effect on reproductive organs at any dose.
Oliveira-Filho et al., 1989[a]	Rat/number not reported	Not reported (Dried Stevia Leaves)	0 or 0.67 g dried leaves /ml, 2 ml twice per day/ 60 days	Not reported	Prepubertal rats (25-30 days old) tested for glycemia; serum concentrations of thyroxine; tri-iodothyroxine; available binding sites in thyroid hormone-binding proteins; binding of ^{3}H-methyltrienolone (a specific ligand of androgen receptors) to prostate cytosol; zinc content of prostate, testis, submandibular salivary gland, and pancreas; water content of testes and prostate; body-weight gain; and final weights of testes, prostate, seminal vesicle, submandibular salivary gland, and adrenal. Only difference due to treatment was seminal vesicle weight, which fell to 60% compared to control.
Mori et al., 1981	Rat/11 male, 11 female per group (44 total)	96%	0, 0.15, 0.75 or 3 % of feed/60 days	2000	Males given stevioside dose in diet for 60 days before and during mating with females who received same diet (as mated male) 14 days before mating and 7 days during gestation. No effect due to treatment on fertility or mating performance, and no effect of fetal development. Rats of each sex had slightly decreased body weight gain at highest dose with non-significant increase in number of dead and resorbed fetuses at highest dose.
Planas and Kuc, 1968[c]	Rat/14 per group (28 total)	Not reported (Crude stevia extract)	0 or 5% Crude stevia extract /18 days	Not reported	Extract given orally to adult female rats for 12 days, who were mated with untreated males during the last 6 days. Fertility reduced to 21% of fertility in control rats and remained reduced in a 50-60 day recovery. Histological examination, weights of organs, blood analysis, urine chemistry and gross necropsy not discussed.

[a] Only abstract available. [b] As reported by European Commision, 1999b.

6. Mutagenicity and Genotoxicity Studies

Mutagenicity and genotoxicity studies on stevia and stevioside are summarized in Table 8. All studies were negative with the exception of a comet assay done in rats (Nunes et al., 2007a). The methodology used in this study and the resulting conclusions have been questioned (Geuns, 2007; Nunes et al., 2007b and 2007c; Williams, 2007; and Brusick, 2008b).

Table 8. Mutagenicity and Genotoxicity Studies on Stevia Extracts and Stevioside

End-Point	Test System	Material	Purity (%)	Concentration / Dose	Result	Reference
In Vitro						
Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Stevioside	83	5 mg/plate[a] 1 mg/plate[b]	Negative	Matsui et al. (1996)
Reverse mutation	*S. typhimurium* TA98, TA100	Stevioside	99	50 mg/plate	Negative[c]	Suttajit et al. (1993)
Forward mutation	*S. typhimurium* TM677	Stevioside	83	10 mg/plate	Negative[c]	Matsui et al. (1996)
Forward mutation	*S .typhimurium* TM677	Stevioside	NS	10 mg/plate	Negative[c]	Pezzuto et al. (1985)
Forward mutation	*S. typhimurium* TM677	Stevioside	NS	Not specified	Negative[c]	Medon et al. (1982)
Gene mutation	Mouse lymphoma L5178Y cells, TK-locus	Stevioside	NS	5 mg/mL	Negative[c,d]	Oh et al. (1999)
Gene mutation (umu)	*S. typhimurium* TA1535/pSK1002	Stevioside	83	5 mg/plate	Negative[c]	Matsui et al. (1996)
Gene mutation	*B. subtilis* H17 rec+, M45 rec-	Stevioside	83	10 mg/disk	Negative[c]	Matsui et al. (1996)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	83	8 mg/mL 12 mg/mL	Negative	Matsui et al. (1996)
Chromosomal aberration	Human lymphocytes	Stevioside	NS	10 mg/mL	Negative	Suttajit et al. (1993)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	85	12 mg/mL	Negative[a]	Ishidate et al. (1984)
In Vivo						
DNA damage (comet assay)	Wistar rats; liver, brain and spleen	Stevioside	88.62	4 mg/L (estimated to be 80 - 500 mg/kg bw/day) in drinking water for 45 days	Positive in all tissues examined, most notably in liver	Nunes et al. (2007a)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevioside, 52; Reb A, 22	250 - 2000 mg/kg bw	Negative[e]	Sekihashi et al. (2002)
DNA damage (comet assay)	Male ddY mouse stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	Stevia	NS	2000 mg/kg bw	Negative[e]	Sasaki et al. (2002)
Micronucleus formation	ddY mouse bone marrow and regenerating liver	Stevioside	NS	62.5 - 250 mg/kg bw	Negative	Oh et al. (1999)
Mutation	*D. melanogaster* Muller 5 strain	Stevioside	NS	2% in feed	Negative	Kerr et al. (1983)

NS = Not specified. [a] Without metabolic activation. [b] As calculated by Williams, 2007. [c] With and without metabolic activation (source not specified in original monograph). [d] Inadequate detail available. [e] Sacrificed at 3 hours and 24 hours.

7. Clinical Studies and Other Reports in Humans

Several pharmacological and biochemical effects have been reported for crude extracts of stevia leaves and purified steviol glycosides. These include effects on glucose uptake, insulin secretion and blood pressure (Geuns, 2003). Stevioside is used in South America as a treatment for type 2 diabetes. The effects of purified steviol glycosides on glucose metabolism and blood pressure have been explored further in clinical studies (Hawke, 2003; Gregersen et al., 2004).

Aqueous extracts of 5 g of *S. rebaudiana* leaves were administered to 16 healthy volunteers at 6-hour intervals for three days, and glucose tolerance tests were performed before and after administration. Another six volunteers were given an aqueous solution of arabinose in order to eliminate possible effects of stress. The extract increased glucose tolerance and significantly decreased plasma glucose concentrations during the test and after overnight fasting in all volunteers (Curi et al., 1986).

In a multi-center randomized, double blind, placebo-controlled trial of hypertensive Chinese men and women (aged 28 - 75 years), 60 patients were given capsules containing 250 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 750 mg of stevioside per day (equivalent to 11 mg/kg bw/day as calculated by FSANZ, 2008). Follow-up evaluations occurred at monthly intervals for one year. Forty-six patients were given a placebo. After 3 months, systolic and diastolic blood pressure in men and women receiving stevioside decreased significantly and the effect persisted over the year. Blood biochemistry parameters, including lipids and glucose, showed no significant changes. Three patients receiving stevioside and one receiving the placebo withdrew from the study as a result of side effects (nausea, abdominal fullness, dizziness). In addition, four patients receiving stevioside experienced abdominal fullness, muscle tenderness, nausea and asthenia within the first week of treatment. These effects subsequently resolved and the patients remained in the study (Chan et al., 2000).

A follow-up multi-center randomized, double blind, placebo-controlled trial was conducted in hypertensive Chinese men and women (aged 20 - 75 years). Eighty-five patients were given capsules containing 500 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 1500 mg of stevioside per day (equivalent to 21 mg/kg bw /day, as calculated by FSANZ, 2008). Eighty-nine patients were given a placebo. Three patients in each group withdrew during the course of the study. There were no significant changes in body mass index or blood biochemistry parameters throughout the study. In the group receiving stevioside, mean systolic and diastolic blood pressure was significantly decreased compared with the baseline, commencing from about 1 week after the start of treatment. After 2 years, 6 out of 52 patients (11.5%) in the group receiving stevioside had left ventricular hypertrophy compared with 17 of 50 patients (34%) in the group receiving the placebo ($p < 0.001$). Eight patients in each group reported minor side effects (nausea, dizziness and asthenia), which led 2 patients in each group to withdraw from the study. Four patients in the group receiving stevioside experienced abdominal fullness, muscle tenderness, nausea and asthenia within the first week of treatment.

These effects subsequently resolved and the patients remained in the study (Hsieh et al., 2003).

In a paired cross-over study, 12 patients with type 2 diabetes were given either 1 g of stevioside (stevioside, 91%; other stevia glycosides, 9%) or 1 g of maize starch (control group), which was taken with a standard carbohydrate-rich test meal. Blood samples were drawn at 30 minutes before and for 240 minutes after ingestion of the test meal. Stevioside reduced postprandial blood glucose concentrations by an average of 18% and increased the insulinogenic index by an average of 40%, indicating beneficial effects on glucose metabolism. Insulin secretion was not significantly increased. No hypoglycemic or adverse effects were reported by the patients or observed by the investigators. Systolic and diastolic blood pressures were not altered by stevioside administration (Gregersen et al., 2004).

Forty-eight hyperlipidemic volunteers were recruited to a randomized, double blind trial designed to investigate the hypolipidemic and hepatotoxic potential of steviol glycoside extract. The extract used in this study was a product containing stevioside (73 ± 2%), Reb A (24 ± 2%) and other plant polysaccharides (3%). The subjects were given two capsules, each containing 50 mg of steviol glycoside extract or placebo, twice daily (i.e., 200 mg/day, equivalent to 3.3 mg/kg bw/day assuming an average body weight of 60 kg), for 3 months. One volunteer receiving placebo and three volunteers receiving steviol glycoside failed to complete the study for personal reasons, not related to adverse reactions. At the end of the study, both groups showed decreased serum concentrations of total cholesterol and of low-density lipoproteins. Analyses of serum concentrations of triglycerides, liver-derived enzymes and glucose indicated no adverse effects. The authors questioned the subjects' compliance with the dosing regime, in view of the similarity of effect between treatment and placebo (Anonymous, 2004a). In a follow-up study, 12 patients were given steviol glycoside extract in incremental doses of 3.25, 7.5 and 15 mg/kg bw/day, for 30 days per dose. Preliminary results indicated no adverse responses in blood and urine biochemical parameters (Anonymous, 2004b).

A new clinical study that was reported to JECFA and reviewed in 2007 was subsequently published in a peer reviewed journal (Barriocanal et al., 2008). The study group consisted of 76 subjects (30 with Type 2 diabetes, 16 with Type 1 diabetes and 30 without diabetes and normal/low-normal BP levels). At doses of stevioside of 11 mg/kg bw/day for a 3-month period in type 1 and 2 diabetics and non-diabetics with normal/low BP, no significant differences were observed in mean BP between control and treated subjects for all three groups. In the same study, no effects on blood glucose or insulin concentrations were observed with administration of oral doses of stevioside at 11 mg/kg bw/day in normal healthy human subjects, in type 1 or 2 diabetics, or in those with mild to moderate hypertension.

C. Safety Data on Rebaudioside A

Only limited studies were available on Reb A during the JECFA reviews. Several toxicology studies have been recently reported on purified Reb A (published in Food and Chemical Toxicology, Supplement 1, July 2008), although it is uncertain whether or not these studies were reviewed as pre-publication manuscripts by JECFA during its 2008 deliberations. These studies include additional mutagenicity data, comparative pharmacokinetic studies with stevioside in rats and humans, several subchronic studies in rats and one in dogs, and additional reproductive and developmental toxicity studies in rats as well as additional clinical studies.

1. Absorption, Distribution, Metabolism and Excretion (ADME) Studies

Three recently completed studies have shed light on the absorption and fate of Reb A in rats and humans.

The toxicokinetics and metabolism of Reb A, stevioside, and steviol were examined in rats for comparative purposes to determine whether toxicological studies conducted previously with stevioside would be applicable to the structurally-related glycoside, Reb A (Roberts and Renwick, 2008). Single, oral doses of the radiolabelled compounds were extensively and rapidly absorbed, with plasma concentration-time profiles following similar patterns for stevioside and Reb A. Elimination of radioactivity from plasma was essentially complete within 72 hours. All plasma samples had similar metabolite profiles; the predominant radioactive component in all samples was steviol, with lower amounts of steviol glucuronide(s) and low levels of one or two other metabolites. Reb A, stevioside, and steviol were metabolized and excreted rapidly, with the majority of the radioactivity eliminated in the feces within 48 hours. Urinary excretion accounted for less than 2% of the administered dose for all compounds in both intact and bile duct-cannulated rats, and the majority of the absorbed dose was excreted *via* the bile and the radioactivity in the feces was identified primarily as steviol. The predominant radioactive compound detected in the bile was steviol glucuronide(s), indicating de-conjugation in the lower intestine. The authors concluded that the overall data on toxicokinetics and metabolism indicate that Reb A and stevioside are handled in an almost identical manner in the rat after oral dosing.

This randomized, double-blind, cross-over study assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of Reb A and stevioside in healthy adult male subjects (Wheeler et al., 2008). Steviol glucuronide appeared in the plasma of all subjects after administration of Reb A or stevioside, with median T_{max} (time to maximum concentration) values of 12.0 and 8.00 hours post-dose, respectively. The $t_{1/2}$ values for elimination of steviol glucuronide were approximately 14 hours when either compound was given. Administration of Reb A resulted in a significantly (approximately 22%) lower steviol glucuronide geometric mean C_{max} (concentration maximum) value (1472 ng/ml) than administration of stevioside (1886 ng/mL). Steviol glucuronide was excreted primarily in the urine of the subjects during the 72 hour collection period, accounting for 59% and 62% of the Reb A and stevioside doses, respectively. No steviol glucuronide was detected in feces. Pharmacokinetic analysis indicated that both

Reb A and stevioside were hydrolyzed to steviol in the gastrointestinal tract prior to absorption. The majority of circulatory steviol was in the form of steviol glucuronide, indicating rapid first-pass conjugation prior to urinary excretion. Only a small amount of steviol was detected in urine (Reb A: 0.04%; stevioside: 0.02%). The authors concluded that Reb A and stevioside underwent similar metabolic and elimination pathways in humans with steviol glucuronide excreted primarily in the urine and steviol in the feces. No safety concerns were noted as determined by reporting of adverse events, laboratory assessments of safety or vital signs.

Another pharmacokinetic investigation was done as a toxicokinetic (TK) phase of a dietary study to determine the potential toxicity of Reb A in rats at levels up to 2000 mg/kg bw/day (Sloter, 2008). Reb A and total steviol were detected in peripheral blood of rats during daily administration of 2000 mg/kg bw/day of Reb A at extremely low levels, with mean plasma concentrations of approximately 0.6 and 12 µg/mL, respectively. Estimates of absorbed dose for Reb A and total steviol were approximately 0.02% and 0.06%, respectively, based on the amounts measured in urine collected over 24 hours in comparison to daily administered dietary dose to rats. Mean fecal Reb A and measured hydrolysis products expressed as Total Reb A Equivalents compared to daily administered dose resulted in an estimate of percentage of dose recovered ≈ 84%.

2. Subchronic Toxicity Studies

Two repeated-dose studies were conducted by the oral route in Wistar rats (Curry and Roberts, 2008). In a 4-week study, Reb A (97% purity) was administered at dietary concentrations of 0, 25,000, 50,000, 75,000 and 100,000 ppm. The NOAEL, including an evaluation of testes histopathology, was determined to be 100,000 ppm. In the 13-week study, Wistar rats were administered Reb A at dietary concentrations of 0, 12,500, 25,000 and 50,000 ppm. Reductions in body weight gain attributable to initial taste aversion and lower caloric density of the diet were observed in high-dose male and female groups. Inconsistent reductions in serum bile acids and cholesterol were attributed to physiological changes in bile acid metabolism due to excretion of high levels of Reb A via the liver. All other hepatic function test results and liver histopathology were within normal limits. Significant changes in other clinical pathology results, organ weights and functional observational battery test results were not observed. Macroscopic and microscopic examinations of all organs, including testes and kidneys, were unremarkable with respect to treatment-related findings. The NOAEL in the 13-week toxicity study was considered to be 50,000 ppm or approximately 4,161 and 4,645 mg/kg bw/day in male and female rats, respectively.

Reb A (99.5% purity) was administered in the diet at target exposure levels of 500, 1000, and 2000 mg/kg bw/day to Sprague-Dawley rats for 90 days (Nikiforov and Eaton, 2008). There were no treatment-related effects on the general condition and behavior of the animals as determined by clinical observations, functional observational battery, and locomotor activity assessments. Evaluation of clinical pathology parameters revealed no toxicologically relevant, treatment-related effects on hematology, serum chemistry, or

urinalysis. Macroscopic and microscopic findings revealed no treatment-related effects on any organ evaluated. Lower mean body weight gains were noted in males in the 2000 mg/kg bw/day group throughout the study, which was considered by the authors to be test article related; however, given the small magnitude of the difference as compared to controls, this effect was not considered to be adverse.

A 90-day dietary toxicity study was conducted in Crl:CD(SD) rats with Reb A (99.5% purity) doses of 500, 1000 and 2000 mg/kg bw/day (Eapen, 2007). Each group consisted of 20/animals/sex. There were no treatment-related effects on clinical observations, food consumption, and functional observational or locomotor activity parameters. There were no treatment-related macroscopic, organ weight, or microscopic findings. Significantly lower body weight gains were noted in the 2000 mg/kg bw/day group in males but not females. The body weight in males was 9.1% lower than the control group at the end of the dosing period (study week 13). The investigators did not consider this result to be adverse due to the small magnitude of difference from the control group value, and they suggested that the reduction was most likely due to the large proportion of the diet represented by the test material. The assigned NOAEL was ≥ 2000 mg/kg bw/day.

A 6-month dietary toxicity study in Beagle dogs was conducted to evaluate the potential toxic effects of Reb A (97.5% purity) at dosage levels of 0, 500, 1000 or 2000 mg/kg bw/day (Eapen, 2008). All groups consisted of 4 males and 4 females. During the course of the study, there were no unscheduled deaths. No treatment-related clinical observations were noted. Home cage, open field observations and functional observations and measurements were unaffected by the administration of Reb A. There were no differences in hematology findings, serum chemistry findings, or urinalysis findings between groups. In addition, no treatment related gross necropsy observations, alterations in final body weight, alterations in organ weights, or histological changes were noted. Based on the results of this study, the authors concluded that no systemic toxicity of Reb A was observed at dosage levels up to 2000 mg/kg bw/day and the assigned NOAEL was ≥2000 mg/kg bw/day.

3. Reproductive and Developmental Toxicity Studies

Reb A (97% purity) was administered via the diet to male and female Han Wistar rats at 0, 7,500, 12,500, and 25,000 ppm for two generations (Curry et al., 2008). Reb A treatment was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. No treatment-related effects of Reb A were observed in either the F_0 or F_1 generations on reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology. The survival and general condition of the F_1 and F_2 offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation, were not adversely affected by Reb A treatment. The NOAEL for reproductive effects was 25,000 ppm and the NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm or 2,048 to 2,273 mg/kg bw/day.

In an unpublished two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30/sex/group) were offered either basal diet or the test article, Reb A (purity 95.7%), continuously in the diet for at least 70 consecutive days prior to mating (Sloter, 2008). Reb A doses were 0, 500, 1000 and 2000 mg/kg bw/day for the F_0 and F_1 generations. F_0 animals were approximately 7 weeks of age at the initiation of test diet exposure. The test diet was offered to the offspring selected to become the F_1 generation following weaning beginning on postnatal day 21. The F_0 and F_1 males continued to receive Reb A throughout mating and continuing through the day of euthanasia. The F_0 and F_1 females continued to receive Reb A throughout mating, gestation and lactation, until day of euthanasia. The authors concluded that there were no effects on reproduction in males or females (estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints). A dose level ≥ 2000 mg/kg bw/day (highest dose administered) was assigned to be the NOAEL for parental systemic and reproductive toxicity.

4. Mutagenicity and Genotoxicity Studies

Reb A was evaluated for genotoxicity with a set of *in vitro* and *in vivo* assays covering mutation, chromosome damage and DNA strand breakage with consistent and uniformly negative results (Pezzuto et al, 1985; Nakajima, 2000a; Nakajima, 2000b; Sekihashi et al., 2002) as reviewed by Brusick (2008b).

An unpublished chromosome aberration assay of Reb A in cultured mammalian cells was submitted for JECFA review (Nakajima, 2000a). The JECFA review of this study indicated that no increases in chromosome aberrations were found. In their GRAS Notification, Merisant submitted three unpublished studies on Reb A including a bacterial mutagenicity study, a mouse lymphoma study and a mouse micronucleus study. All three studies indicated lack of mutagenic or genotoxic activity.

Table 9 summarizes these results.

5. Clinical Studies

A randomized, double-blind trial evaluated the hemodynamic effects of four weeks' consumption of 1000 mg/day Reb A (97% purity) versus placebo in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Maki et al., 2008a). Subjects were predominantly female (76%, Reb A and 82%, placebo) with a mean age of ~41 (range 18 to 73) years. At baseline, mean resting, seated SBP/DBP was 110.0/70.3 mm Hg and 110.7/71.2 mm Hg for the Reb A and placebo groups, respectively. Compared with placebo, Reb A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-hour ambulatory blood pressure responses. The authors concluded that these results indicated that consumption of as much as 1000 mg/day of Reb A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.

Table 9. Mutagenicity and Genotoxicity Studies on Rebaudioside A

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCEN-TRATION / DOSE	RESULT	REFERENCE
Bacterial Mutagenicity	5 Salmonella strains with and without exogenous metabolic activation system	Reb A	99.5%	1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate	No mutagenic response	Wagner and Van Dyke (2006)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence and presence of exogenous metabolic activation system	Reb A	99.5%	Cloning conc. of 500, 1000, 2000, 3000, 4000 and 5000 µg/mL	No mutagenic or clastogenic response	Clarke (2006)
Mouse Micronucleus	Micronucleus study consisted of 7 groups, each containing 5 male and 5 female ICR mice.	Reb A	99.5%	500, 1000 and 2000 mg/kg bw	No increase in micronuclei formation	Krsmanovic and Huston (2006)
DNA damage (comet assay)	*Male BDF1 mouse stomach, colon, liver*	Stevia extract	Stevioside, 52%; Reb A, 22%	250 - 2000 mg/kg bw	Negative[a]	Sekihashi et al. (2002)
Chromosomal aberration	*CHL/IU Chinese hamster lung fibroblasts*	Reb A	NS	1.2 - 55 mg/mL	Negative[b]	Nakajima (2000a)
Micronucleus formation	*BDF1 mouse bone marrow*	Reb A	NS	500-2000 mg/kg bw per day for 2 days	Negative[c]	Nakajima (2000b)
Forward mutation	*S. typhimurium* TM677	Reb A	NS	10 mg/plate	Negative[b]	Pezzuto et al. (1985)

NS = Not specified. [a] Sacrificed at 3 hours and 24 hours. [b] With or without metabolic activation (source not specified in original monograph).
[c] Sacrificed at 30 hours after 2nd administration.

Another randomized, double-blind, placebo-controlled clinical trial (conducted at six research sites in the US) evaluated the effects of 16 weeks of consumption of 1000 mg Reb A (97% purity, n= 60) compared to placebo (n = 62) in men and women (33-75 years of age) with type 2 diabetes mellitus (Maki et al., 2008b). Mean ± standard error changes in glycosylated hemoglobin levels did not differ significantly between the Reb A (0.11 ± 0.06%) and placebo (0.09 ± 0.05%; p = 0.355) groups. Changes from baseline for Reb A and placebo, respectively, in fasting glucose (7.5 ± 3.7 mg/dL and 11.2 ± 4.5 mg/dL), insulin (1.0 ± 0.64 µU/mL and 3.3 ± 1.5 µU/mL), and C-peptide (0.13 ± 0.09 ng/mL and 0.42 ± 0.14 ng/mL) did not differ significantly (p > 0.05 for all). Assessments of changes in blood pressure, body weight, and fasting lipids indicated no differences by treatment. Reb A was well tolerated, and records of hypoglycemic episodes showed no excess versus placebo. The authors suggested that chronic use of 1000 mg Reb A did not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

D. Studies on the Principal Metabolite: Steviol

A number of studies have been conducted on steviol, the principal mammalian metabolite of stevioside, and the results of these studies are provided below.

1. Acute Toxicity Studies

In male and female mice and rats given steviol (purity, 90%) orally, the LD_{50} was > 15 g/kg bw, and 1/15 animals died within 14 days of administration. The LD_{50} values in hamsters given steviol orally were 5.2 g/kg bw in males and 6.1 g/kg bw in females. Histopathological examination of the kidneys revealed severe degeneration of the proximal tubular cells, and these structural alterations were correlated with increased serum blood urea nitrogen and creatinine. The authors concluded that the cause of death was acute renal failure (Toskulkao et al., 1997).

2. Developmental Toxicity Studies

Groups of 20 pregnant golden hamsters were given steviol (purity, 90%) at doses of 0, 250, 500, 750, or 1000 mg/kg bw/day (only 12 animals at the highest dose) by gavage in corn oil on days 6 - 10 of gestation. A significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12) were observed at the three highest doses, and the number of live fetuses per litter and mean fetal weight decreased in parallel. Histopathological examination of the maternal kidneys showed a dose-dependent increase in the severity of effects on the convoluted tubules (dilatation, hyaline droplets). However, no dose-dependent teratogenic effects were seen. The NOEL was 250 mg/kg bw/day for both maternal and developmental toxicity (Wasuntarawat et al., 1998).

3. Mutagenicity and Genotoxicity Studies

Several mutagenicity and genotoxicity studies have been conducted on steviol. The studies reviewed by JECFA are summarized in Table 10.

Table 10. Mutagenicity and Genotoxicity Studies on Steviol

STUDY	*IN VIVO/IN VITRO*	SYSTEM	TEST SAMPLE PURITY	AUTHOR CONCLUSION	RESULTS AND REMARKS
Sekihashi et al., 2002[a]	*In Vivo/In Vitro*	Comet Assay	Not reported	Negative	In *in vitro* study, steviol at 62.5, 125, 250 and 500 µg/ml did not damage DNA of TK6 and WTK1 cells in presence or absence of S9 mix. In *in vivo* study, mice sacrificed 3 or 24 hours after one-time oral administration of 250, 500, 1000 or 2000 mg/kg of steviol. Stomach, colon, kidneys, testis and liver DNA not damaged. An identical *in vivo* experiment with stevia extract performed, which also gave negative results.
Oh et al., 1999[b]	*In Vivo?*	Cell Mutation and DNA damage	Not reported	Negative	Steviol gave negative results for cell mutation and DNA damage in cultured cells.
Matsui et al., 1996[c]	*In Vivo?*	Mutagenicity and Chromosome aberration (Chinese hamster lung fibroblasts)	Not reported	Positive	Gene mutation and chromosomal aberration found in Chinese hamster lung fibroblasts after metabolic activation of steviol. In hamsters, several metabolites of stevioside found that have not been found in rats or humans. Therefore, experimental relevance should be questioned when hamsters are used.
Terai et al., 2002[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Steviol found to be mutagenic in Aroclor induced rat liver S9 fraction. 15-oxo-steviol found to be mutagenic at 10% level of steviol. Specific mutagenicity of lactone derivative in presence of S9 mixture 10x lower than that of derivative without S9 mixture.
Temcharoen et al., 1998[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Mutagenic effects of steviol and/or metabolites found in *S.typhimurium* TM677 by tranversions, transitions, duplications, and deletions at the guanine phosphoribosyltransferase (gpt) gene. Magnitude of increase of these mutations over the control not reported.
Klongpanich-pak et al., 1997[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	Steviol and stevioside inactive in TA strains of *S. typhimurium, e. coli WP2, uvrA/PKM101* and rec assay using *B. subtilis* even when microsomal activated fraction present. Magnitude of increase of these mutations over the control not reported.
Matsui et al., 1996[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	Testing of Southern Blot technique with probe for gpt gene DNA of *E. coli.* The chromosomal DNA of TM677 and steviol-induced TM677 mutants digested by restriction enzymes and probed. No significant differences found in fragment length between wild-type and mutant DNA.
Matsui et al., 1996[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Both	Steviol weakly positive in umu test, either with or without metabolic activation. Steviol negative in reverse mutation and other bacterial assays even in presence of S9 activation.

STUDY	*IN VIVO/IN VITRO*	SYSTEM	TEST SAMPLE PURITY	AUTHOR CONCLUSION	RESULTS AND REMARKS
Procinska et al., 1991[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	The direct mutagenic activity of 15-oxo-steviol was refuted.
Compadre et al., 1988[a]	*In Vitro*	Bacterial Mutagenicity, Mass Spec	Not Reported	Positive	Mass spectral analysis of steviol and analogues under conditions known to produce a mutagenic response. 15-oxo-steviol, a product of the metabolite, 15-alpha-hydroxysteviol was found to be direct-acting mutagen. Magnitude of increase over control in assay not discussed.
Pezzuto et al., 1985[d]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Using *S. typhimurium* TM677 strain, steviol found to be highly mutagenic in presence of 9000 x g supernatant from livers of Aroclor 1254-pretreated rats. This mutagenicty dependent on pretreatment of rats with Aroclor and NADPH addition, as unmetabolized steviol was inactive. None of other metabolites tested was mutagenic. Authors concluded that structural features of requisite importance for the expression of mutagenic activity may include a hydroxy group at position 13 and an unsaturated bond joining the carbon atoms at positions 16 and 17.
Temacha-roen et al., 2000[c]	*In Vivo*	Micronucleus (rat)	90%	Negative	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.
Temacha-roen et al., 2000[c]	*In Vivo*	Micronucleus (mouse)	90%	Negative	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.
Matsui et al., 1996[a]	*In Vivo*	Micronucleus (mouse)	Not Reported	Negative	Steviol did not increase number of micronuclei observed in this study.
Temacha-roen et al., 2000[c]	*In Vivo*	Micronucleus (hamster)	90%	Negative	Very high doses (4 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.

[a] Abstract only. [b] As reported in JECFA, 2006. [c] As reviewed by Geuns, 2003. [d] Full article.

VI. DISCUSSION OF GRAS CRITERIA AND PANEL SAFETY FINDINGS

A. GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as:

> "...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance."[8]

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that:

> "...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food."
>
> "General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information."[9]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following component elements:[10]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA and the National Academy of Sciences.

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[8] See 21 CFR 170.3(i).
[9] See 21 CFR 170.30(a).
[10] See Footnote 1.

The apparent imprecision of the terms "appreciable", "at the time" and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety, in this or any other area (Lu 1988; Renwick 1990).

As noted below, this safety assessment to ascertain GRAS status for steviol glycosides for the defined food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

B. Panel Findings on Safety Studies of Steviol Glycoside

The WNB's high purity steviol glycosides product meets the JECFA specifications for purity in that the seven specific glycosides make up 95% or more of the dry weight. In addition, the product consists of roughly equal parts of stevioside and Reb A. Both of these steviol glycosides have been well studied in clinical, pharmacokinetic and toxicological studies. A substantial majority of these studies have been published in peer reviewed journals. The Panel has reviewed the data on both glycosides as well as the data on steviol, the principal metabolite, and the Panel has also addressed several reviews which have expressed some concerns on the safety of steviol glycosides.

1. Discussion of Safety Data on Stevioside and Stevia Extracts that are Predominantly Stevioside

Steviol glycosides are unique compounds in that they have viable uses as non-nutritive sweeteners in foods.[11] The series of reviews by JECFA over several years indicate the progression of knowledge on the toxicology and clinical effects of these compounds. The early toxicology studies were largely performed on crude extracts of stevia. Several early concerns were noted, including impairment of fertility and mutagenicity. As more studies were done on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, reproductive and chronic studies have been conducted in laboratory animals. The studies were, in general, adequately designed with appropriately high doses and adequate numbers of animals to maximize the probability of detection of important adverse effects. Notably, the reproductive toxicity studies with purified steviol glycosides refuted the concern over effects on fertility that were initially reported with stevia leaves or crude extracts.

As discussed in Section V.A, JECFA reasoned that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), on which to base a temporary ADI with an adequate margin of safety. The Committee was satisfied that the lack of carcinogenic response in these well-conducted studies justified their conclusion that the *in vitro* mutagenic activity of steviol, buttressed by the evidence of rapid biotransformation and

[11] It has also been reported that steviol glycosides can impart pharmacological properties which can be utilized in the treatment of certain disease conditions, such as hypertension and type 2 diabetes when administered at elevated levels.

elimination of aborbed steviol, did not present a risk of carcinogenic effects *in vivo*. In addition, they concluded that all common steviol glycosides share the same basic metabolic and excretory pathways. Therefore, JECFA has concluded that high purity preparations of various steviol glycosides are safe to use as a non-nutritive sweetener. The additional clinical data subsequently presented allowed JECFA to establish a permanent ADI of 0 - 4 mg/kg bw/day (based on steviol equivalents), which translates to 0 -10 mg/kg bw/day for stevioside. The estimated consumption levels for stevioside containing sweeteners summarized in Section IV are comfortably within the JECFA ADI.

The Panel agrees with this reasoning. It should be noted that in a recent study, DNA damage was seen in a variety of organs in a comet assay in rats maintained on drinking water containing 4 mg/mL steviol glycosides for up to 45 days (Nunes et al., 2007a). Several experts in the field have since questioned the methodology used in this study (Geuns, 2007; Williams, 2007; Brusick, 2008b). The Panel has reviewed the cited publications and concurs with the challenges to the methodology utilized by Nunes et al., 2007a, thereby discounting the validity and importance of this study.

Regarding pharmacological effects in humans, JECFA called for additional studies in individuals that are neither hypertensive nor diabetic (WHO, 2006) to corroborate the observations that the effects of steviol glycosides to reduce blood glucose or blood pressure (or both) only occur in patients who already have these diseases. As reviewed by FSANZ, new data presented to JECFA demonstrated the lack of pharmacological effects of steviol glycosides at 11 mg/kg bw/day in normal individuals (Barriocanal et al., 2008). This level of intake is only slightly more than 4 mg/kg bw/day on the basis of steviol equivalents. The Panel has reviewed the clinical studies and concludes that there should be no effects on reducing blood pressure or glucose metabolism in humans at the doses of stevioside expected from use in food as a non-nutritive sweetener.

2. Discussion of Safety Data on Rebaudioside A

In addition to the information on steviol glycosides discussed in the previous section, there are recent additional data specific to Reb A. Eleven papers describing the results of a comprehensive research program on Reb A have been published in July, 2008 (*Food and Chemical Toxicology*, Supplement 1, 2008). These studies formed the basis of the Cargill GRAS notification (GRN 253). Several other studies were sponsored by Merisant, and these were also then submitted with their GRAS notification (GRN 252). Previously, only a limited number of toxicology studies and clinical studies on Reb A were conducted and reported. As in the previous section on steviol glycosides, JECFA had concluded, even before these new studies were completed, that seven common steviol glycosides are safe for use as sweetener preparations when present in any combination at a combined purity of 95% or more.

The strategy of the new research on Reb A was to conduct a limited number of toxicology and clinical studies on the specific compound and to demonstrate in rats and in humans that it is pharmacokinetically similar to stevioside, which is the steviol glycoside on which

most previous pharmacokinetic research was conducted. These studies were also done to help justify using the JECFA-generated ADI (for steviol glycosides, expressed as steviol) without having to conduct a new chronic study in rats on Reb A. In addition, the Merisant group upgraded the mutagenicity and genotoxicity data available on Reb A with three assays that FDA reportedly believes are most predictive for carcinogenicity. The Cargill group also conducted two clinical studies to assure that Reb A does not have potentially adverse pharmacological effects on blood glucose and blood pressure as was previously demonstrated in some stevioside studies.

The most recent research on Reb A was summarized and combined with the body of knowledge on stevioside by Carakostas et al. (2008) and Brusick (2008b). These reviews summarized the findings of the Cargill research program as follows:

- Steviol glycosides, Reb A, and stevioside are not genotoxic *in vitro*.
- Steviol glycosides, Reb A, and stevioside have not been shown to be genotoxic *in vivo* in well-conducted assays.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes et al., 2007a) and was improperly interpreted as a positive response.
- Steviol genotoxicity in mammalian cells is limited to *in vitro* tests that may be affected by excessive concentrations of the compound.
- The primary evidence for steviol genotoxicity is derived from very specific bacterial tests or purified plasmid DNA that lack DNA repair capabilities.
- Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
- The pharmacokinetic similarity between Reb A and stevioside justifies the use of the ADI established by JECFA, that was determined on studies employing stevioside as the main component, as the ADI for Reb A.
- The dietary levels expected from consumption of Reb A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers.

Regarding possible pharmacological effects of Reb A in decreasing blood pressure and blood glucose, there is a recently published clinical data study on Reb A (Maki et al., 2008a, b). The Panel has reviewed these clinical studies and concludes that there should be no effects on blood pressure and glucose metabolism in humans at the doses of Reb A expected from use in food as a non-nutritive sweetener.

C. Discussion of Safety Concerns Expressed by Others

Over the last two decades, concerns have been voiced by FDA and selected others about the safety of sweeteners derived from stevia. The studies which showed possible adverse effects included several older toxcology studies using crude extracts of stevia or various *in vitro* mutagenicity studies employing high concentrations. Although no formal review was

ever published by FDA, similar concerns were expressed by Dr. Ryan J. Huxtable, a professor of pharmacology at the University of Arizona. More recently, a review of Reb A toxicology (sponsored by the Center for Science in the Public Interest) was issued by two UCLA scientists who questioned the safety of Reb A. The Panel's evaluation of their respective concerns is summarized below.

1. **Earlier FDA Concerns**

FDA has previously expressed concerns about the safety of consumption of stevia and stevia extracts, based on selected publications that addressed questions about steviol glycoside metabolism, effects on glucose metabolism, effects on fertility, mutagenicity, and renal effects in rats.

As part of its broader safety evaluation of high purity steviol glycosides, the Panel has reviewed the older studies cited by FDA and has made the following general observations about the studies taken as a group. All of the cited studies were published in or before 1996, and there has been significant research reported since that time in most of the areas of concern. In addition, a number of these earlier studies are not particularly applicable to newly anticipated human intakes of high purity steviol glycosides because they used very high doses, impure extracts or because the route of exposure was not oral. Finally, many of the studies exist only as abstracts without critical details needed to determine the validity of the results.

The Panel has concluded that the concerns covered by these studies can be dismissed based on several factors: (1) the favorable results observed in a number of studies conducted on the key toxicity and clinical endpoints subsequent to the safety concerns cited by FDA; (2) the careful, ongoing review and final evaluation by JECFA and other international regulatory bodies (through 2008) that steviol glycosides can be considered to be safe at the recommended ADI of 4 mg/kg bw/day (steviol basis); (3) FDA's own independent GRAS evaluations of the more recently developed safety information as contained in the Merisant and Cargill GRAS notifications; and (4) the Panel's own conclusions regarding the toxicity and clinical endpoints previously considered.

2. **Huxtable Concerns**

Huxtable published a non-peer reviewed book chapter (Huxtable, 2002) in which he expressed misgivings about the safety of stevioside glycosides, most notably due to effects on glucose metabolism and the metabolic conversion to steviol. Though Huxtable acknowledged that animal and human testing to that date had not shown any problems, he discussed concern with potential problems arising from these mechanistic research studies.

The Panel notes that no adverse health findings have been demonstrated in the improved animal or human testing which has taken place since his review. Huxtable's concerns, therefore, appear to be driven by the "potential" effects of high-dose *in vitro* and *in vivo*

testing, without the proper consideration given to the problems found when extrapolating these potential effects to the vastly lower dietary intakes by consumers. Consequently, the Panel concludes that Huxtable's expressed safety concerns can now be dismissed.

3. UCLA Researchers and the Center for Science in the Public Interest (CSPI) Concerns

In August 2008, two UCLA scientists published a criticism of the GRAS Assessment by Cargill (Kobylewski and Eckhert, 2008). These scientists were recruited for this task by CSPI, a public interest group which has historically commented on a number of issues related to food ingredient safety. The basic criticism of the UCLA evaluation can be summarized as follows:

- There is insufficient mutagenicity and genotoxicity data on Reb A as compared to stevioside to confirm that it is not likely to have carcinogenic properties.
- The metabolism of Reb A is too different from stevioside to rely on the rat chronic studies on stevioside to set an ADI for Reb A.
- The carcinogenic potential of both stevioside and Reb A should be examined in a second rodent species. They suggest that a mouse study is needed in accordance with FDA Redbook guidelines.

The Panel has reviewed the UCLA evaluation and the Reb A studies submitted by Merisant and Cargill. The Panel understands that it is easier to avoid making a safety decision while asking for more data. A good deal of scientific judgment is certainly needed to conclude that any data set is sufficient and that the ingredient is safe. The Panel notes that JECFA is composed of dozens of scientists who are expert in food ingredient safety and that JECFA expert panels have been establishing ADIs for food ingredients for over 40 years.

Both Merisant and Cargill took rigorous scientific approaches to demonstrate the safety of Reb A. The studies were as well conducted as those supporting stevioside safety. The safety profiles compiled by Merisant and Cargill differ somewhat, yet the results are complementary and are mutually reinforcing of Reb A safety. We also note that the UCLA evaluation did not review the Merisant studies.

The Cargill studies and evaluation provided significant insight into the pharmacokinetics of Reb A while demonstrating clinical safety of Reb A regarding lack of effects on blood pressure and glucose metabolism that could result from doses expected from use in food. The Merisant notification augmented genotoxicity data in three systems recognized by FDA as good predictors of carcinogenic potential. Two of these assays were conducted in mouse systems. Merisant also added a subchronic toxicity study in dogs and a teratology study in rats. Both Cargill and Merisant relied on the JECFA ADI for steviol glycosides as determined largely by published chronic studies in rats. Both groups justified the use of the ADI on pharmacokinetic arguments showing the similarity of stevioside and Reb A metabolism and excretion.

Therefore, the Panel disagrees with the conclusions of the UCLA/CSPI evaluation. The pharmacokinetic work shows that stevioside and Reb A are not absorbed *per se* but are converted to steviol in the GI tract. This occurs more slowly for Reb A due to the fact that it has a disaccharide side chain instead of stevioside's monosaccharide side chain. In both humans and rats, the steviol is rapidly converted to the glucuronide, and the glucuronide is not further metabolized but is efficiently excreted. In the rat, elimination occurs in the bile to the large intestine. In humans, elimination of the glucuronide occurs both in bile and urine. The UCLA evaluation indicates that this is a profound difference, which makes the rat a poor model for the extrapolation of an ADI to humans. The Panel disagrees with this concern. It is more important that the glucuronide is not expected to be toxic and is not further metabolized, but instead is efficiently eliminated. The route of elimination is different, but glucuronide elimination is a detoxification mechanism. The Panel believes that a mouse carcinogenicity study is not indicated because the rat is pharmacokinetically a model sufficiently similar to the human. Moreover, there are no data to indicate that the mouse is a better model than the rat. In addition, the Merisant mouse lymphoma and mouse micronucleus studies, as well as several mouse micronucleus studies conducted by others, do not indicate that the mouse is especially sensitive to Reb A, other steviol glycosides or steviol. These mouse studies give no indication that there are any undiscovered carcinogenic pathways that may operate in the mouse.

The Panel rejects the concerns contained in the UCLA evaluation and endorses instead the JECFA, FSANZ, Cargill and Merisant conclusion that sufficient data have been generated on steviol glycosides, partculary on test samples rich in stevioside and Reb A (as is the case with SweetLeaf® Stevia) to determine that these substances can be safely used at expected levels in food.

4. Panel's Overall Conclusions on These Concerns

Over the last several decades, there have been many *in vivo* and *in vitro* studies of a wide variety conducted on stevia extracts and steviol glycosides of varying purity. It is not surprising to find biological activity related to glucose metabolism since stevia products have been reported to be useful in the treatment of type 2 diabetes at doses higher than those projected to be used as a sweetener in foods. Moreover, there are three basic tenets of food ingredient safety methodology that need to be kept in mind. The first is that various effects observed *in vitro* can be refuted by well-conducted studies done *in vivo*. The second is that the higher concentrations used in *in vitro* studies and doses administered in *in vivo* studies (both animal and human) are very important to compare when assessing the significance of a potentially adverse *in vitro* finding. Oftentimes as noted in this evaluation, the *in vitro* doses can be orders of magnitude higher than the intakes that even high consumers of a food ingredient can be expected to ingest throughout their lifetimes. The third tenet is that primary evidence for human safety comes from studies designed to investigate safety *per se*. As evidenced by the JECFA review and evaluation and the Panel's findings in Section VI.B., the Panel agrees that there is an ample margin of safety demonstrated by the doses used in the *in vivo* studies that meet the accepted design criteria for safety studies; furthermore, the studies of concern cited by

FDA, the theoretical concerns raised by Huxtable, and the issues about Reb A described by UCLA/CSPI can be discounted based on sound use of safety and risk assessment principles.

It is the conclusion of the Panel that the safety concerns expressed above have been answered satisfactorily in the final JECFA opinion, the FSANZ review, the assessment by the Cargill and Merisant teams (as found acceptable by FDA) and in the Panel's own comprehensive, systematic review.

D. Common Knowledge Elements for a GRAS Determination

The first common knowledge element for a GRAS determination is that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The majority of the studies reviewed in this safety assessment have been published in the scientific literature as reported in Section V. Most of the literature relied upon by JECFA has also been published, most importantly the chronic rat studies on steviol glycosides. JECFA did make limited use of unpublished studies, and they were summarized in the two JECFA monographs. Moreover, JECFA publicly releases the results of their safety reviews, and their meeting summaries and monographs are readily available on their website. Thus, these studies become generally available to the scientific community. JECFA reviewed only a limited number of studies conducted specifically on Reb A. The collection of supporting data on Reb A has recently been enhanced by the publication of the 2008 studies. The newest clinical studies that address JECFA's concern on unwanted pharmacological effects with steviol glycosides (Barriocanal et al., 2008) and with Reb A (Maki et al., 2008 a, b) are now published in the scientific literature.

To be sure, the Panel recognizes that the safety of steviol glycoside in human foods has been the subject of interest for many years. In addition to the reported substantial history of consumption of stevia, especially in South America and Asia, many scientific studies have been conducted and published. Some of the studies have raised safety concerns, and the Panel has given careful attention to such concerns. The overriding evidence, particularly with high purity steviol glycosides, has certainly diminished the Panel's concerns based on better study designs, better study execution and new investigations that better reflect state-of-the-art toxicological and clinical principles and findings.

The remaining common knowledge element for a GRAS determination is that there must be a basis to conclude that there is consensus among qualified scientists about the safety of the substance for its intended use. The 2008 JECFA final opinion largely meets the common knowledge test on its own. The Panel is cognizant of the scientific rigor and broad base of scientific expertise that resides with the prestigious JECFA. JECFA is composed of expert scientists from various regulatory agencies around the world, as well as other scientists chosen because of their specific expertise on various classes of food ingredients. In addition, FDA participates in JECFA deliberations.

The JECFA conclusion has been reviewed and validated by other respected regulatory agencies, including FSANZ and the Switzerland Office of Public Health (FSANZ, 2008 and Switzerland Office of Public Health, 2008). A number of other well-respected scientists have indicated that steviol glycosides are safe for human consumption at doses in the range of the JECFA ADI (Xili et al., 1992; Toyoda et al., 1997; Geuns, 2003; Williams, 2007).

The common knowledge element has been recently embellished by the many well-respected scientists that participated in the new Cargill-sponsored research conducted on Reb A, most notably David Brusick, Nigel Brown and Andrew Renwick. An assertion of "general recognition of safety" was also made by Carakostas et al. (2008). In summary, there are many diverse groups of scientists from all corners of the globe that together provide strong fulfillment of the consensus requirement. Of particular significance from the perspective of establishing consensus for the safety of high purity steviol glycosides is the mid-December 2008 "no objection" determinations by FDA for the GRAS notifications for Reb A as submitted by Merisant and Cargill.

While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, the Panel believes that a wide consensus does exist in the scientific community to support a GRAS conclusion as outlined in this evaluation. The scientific community will likely conclude that concerns expressed by others over the years (Huxtable, 2002) are likely to be satisfied by newer data on more purified materials and the rigid specifications for purity published by JECFA for steviol glycosides, including Reb A. Most notably the concerns on effects of fertility with crude extract have been addressed with a number of reproductive effects in rats and hamsters with purer materials (Usami et al., 1995a; Yodyingyuad and Bunyawong, 1991; Mori et al., 1981). Several chronic rat studies with sufficiently high no effect levels (most notably the study by Toyoda et al., 1997) are available to set an acceptable ADI based on FDA tested review methodology. The recent clinical studies put to rest the concern that effects on blood pressure and blood glucose will be seen at the dietary levels expected (Barriocanal et al., 2008). There is also a wide consensus that the body of new research on Reb A is sufficient to establish safety, as opposed to the small group of scientists that argue that more studies need to be done before the sweetener is made available in the US.

VII. CONCLUSIONS[12]

Part of JECFA's review included anticipated dietary patterns and the use concentrations expected in various foods in order to calculate the EDI (WHO, 2003, 2006). For US consumption, based on the very conservative assumption of 100% substitution of steviol glycosides for all sugars, an EDI of 5 mg/kg bw/day steviol was calculated by JECFA, however JECFA concluded that the replacement estimates were highly conservative and that this calculated intake of steviol glycosides (as steviol) would more likely be 20 - 30% of these values. In addition, Renwick (2008) concluded that if only Reb A were used as a total sugar replacement, the levels would be below the JECFA ADI. The Panel concurs that an EDI of 5 mg/kg bw/day steviol very conservatively represents a potential high user of steviol glycosides, if this non-nutritive sweetener becomes widely available in food. As part of this GRAS evaluation, the Panel agrees with the JECFA EDI for application to SweetLeaf® Stevia.

In consideration of the aggregate safety information available, the Panel concludes that JECFA has conducted an expert evaluation and agrees with JECFA's conclusion. The per person ADI for steviol glycosides of adequate purity as defined by JECFA specifications has been properly determined to be 4 mg/kg bw/day (as steviol equivalents). The Panel calculates that this is equivalent to 8 mg/kg bw/day for stevioside and 12 mg/kg bw/day for Reb A on a weight basis. The Panel agrees that adverse pharmacological effects are not likely to occur at this level and that even high consumers of steviol glycosides are not likely to exceed this level. Therefore, the Panel agrees with the JECFA-derived ADI as a safe intake level of steviol glycosides and that food uses meeting the JECFA specifications, within the intake limits determined by JECFA, can be considered to be generally recognized as safe (GRAS) within the meaning of the Food, Drug, and Cosmetic Act.

High purity steviol glycosides, with the composition as established for SweetLeaf® Stevia, that is produced in accordance with FDA Good Manufacturing Practices requirements and which meets at a minimum the JECFA purity specifications for steviol glycosides is Generally Recognized As Safe when consumed as a non-nutritive sweetener within the JECFA ADI of 4 mg/kg bw/day on steviol equivalent basis. In order to remain within the designated ADI, it is important to observe good manufacturing practices principles in that the quantity of a substance added to food should not exceed the amount reasonably required to accomplish its intended technical effect.

[12] The educational and professional credentials for the individuals serving on the Expert Panel are summarized in Appendix C. Each has extensive technical background in the evaluation of food ingredient safety.

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This declaration has been made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

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DATE: February 26, 2009

VIII. REFERENCES

Akashi, H., Yokoyama, Y., 1975. Security of dried-leaf extracts of Stevia. Toxicological tests. Food Industry 18, 34-43.

Al-Achi, A., Greenwood, R., 2000. Stevia: a plant for Sweetness? US Pharmacist 25, 12.

Anonymous, 2004a. Evaluation of the ingestion of stevioside, orally, in humans through a randomized clinical study of the type blind double. Subproject 1: Investigation of the hypolipidemic and hepatotoxic potential of the stevioside using doses usually consumed of the stevioside as sweetener. Unpublished report of a study conducted by the State University of Maringá and the Academical Hospital of Maringá. Submitted to WHO by State University of Campinas, Brazil.

Anonymous, 2004b. Evaluation of the ingestion of stevioside, orally, in humans through a randomized clinical study of the type blind double. Subproject 2: Investigation of the antihypertensive potential, insulintropic, hypolipidemic and toxic (hepatotoxic potential, nephrotoxic and of interference in the endocrine system) of the stevioside using doses above the usually consumed, but previously respecting values used in humans. Unpublished report of a study conducted by the State University of Maringá and the Academical Hospital of Maringá. Submitted to WHO by State University of Campinas, Brazil.

Aze, Y., Toyoda K., Imaida, K., Hayashi, S., Imazawa, T., Hayashi, Y., Takahashi, M., 1991. Subchronic oral toxicity study of stevioside in F344 rats. Bull. Natl. Inst. Hyg., 48-54 (in Japanese).

Barriocanal, L.A., Palacios, M., Benitez, G., Benitez, S., Jimenez, J.T., Jimenez, N., Rojas, V., 2008. Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans. A pilot study of repeated exposures in some normotensive and hypotensive individuals and in Type 1 and Type 2 diabetics. Regul. Toxicol. Pharmacol. 51, 37-41.

Brandle, J.E., Starratt, A.N., Gijzen, M., 1998. Stevia rebaudiana: its agricultural, biological and chemical properties. Can. J. of Plant Sci. 78, 527-536.

Bridel, M., Lavielle, R., 1931. Le principe a' saveur sucre'e du Kaa'-he'-e' (Stevia rebaudiana) Bertoni. Bull. Soc. Chim. Biol., 13, 636-655.

Brusick, D.J., 2008a. Postscript. Food Chem.Toxicol. doi:10.1016/j.fct.2008.05.010.

Brusick, D.J., 2008b. A critical review of the genetic toxicity of steviol and steviol glycosides. Food Chem. Toxicol. doi:10.1016/j.fct.2008.05.002.

Carakostas, M.C., Curry, L.L., Boileau, A.C., Brusick, D.J., 2008. Overview: the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. Food Chem. Toxicol. doi:10.1016/j.fct.2008.05.003.

Cargill GRAS Notification for Rebaudioside A, 2008, Submitted to the US Food and Drug Administration, Washington, DC and identified as GRAS Notification 253; see FDA website at http://www.cfsan.fda.gov/~rdb/opa-grsn.html.

Chan, P., Tomlinson, B., Chen, Y., Liu, J., Hsieh, M., Cheng, J., 2000. A double-blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. Br. J. Clin. Pharmacol., 50, 215–220.

Chang, S.S., Cook, J.M., 1983. Stability studies of stevioside and rebaudioside A in carbonated beverages. J. Agric. Food Chem. 31, 409-414.

Clarke, J.J., 2006. *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay) [with Rebaudioside A]. BioReliance, Rockville, MD. Unpublished Report (Study Number AB21TG.704.BTL).

Compadre, C.M., Hussain, R.A., Nanayakkara, N.P., Pezzuto, J.M., Kinghorn, A.D., 1988. Mass spectral analysis of some derivatives and *in vitro* metabolites of steviol, the aglycone of the natural sweeteners, stevioside, rebaudioside A, and rubusoside. Biomed. Environ. Mass Spectrom., 15, 211-222.

Curi, R., Alvarez, M., Bazotte, R.B., Botion, L.M., Godoy, J.L., Bracht, A., 1986. Effect of Stevia rebaudiana on glucose tolerance in normal adult humans. Braz.J. Med. Biol. Res.19, 771-774 (In Portugese, English abstract only).

Curry, L.L., Roberts, A., 2008. Subchronic toxicity of rebaudioside A. Food Chem. Toxicol. doi:10.1016/j.fct.2008.04.042.

Curry, L.L., Roberts, A., Brown, N., 2008. Rebaudioside A: two-generation reproductive toxicity study in rats. Food Chem. Toxicol. doi:10.1016/j.fct.2008.05.005.

Eapen, A.K., 2007. A 90-Day Oral (Dietary) Toxicity Study of Rebaudioside A in Rats. WIL Research Laboratories, LLC. Unpublished Report (Study Number WIL-568002).

Eapen, A.K. 2008. A 6-Month Oral (Dietary) Toxicity Study of Chrysanta® 99-P in Beagle Dogs. WIL Research Laboratories, LLC. Unpublished Report (Study Number WIL-568011).Krsmanovic, L., Huston, T., 2006. Rebaudioside A: Mammalian Erythrocyte Micronucleus Test. BioReliance, Rockville, MD. Unpublished Report (Study Number AB21TG.123.BTL).

European Commission, 1999a. Opinion on Stevia Rebaudiana Bertoni plants and leaves. Scientific Committee on Food (CS/NF/STEV/3 Final, 17 June 1999).

European Commission, 1999b. Opinion on stevioside as a sweetener. Scientific Committee on Food (CS/ADD/EDUL/167Final, 17 June 1999).

European Food Safety Authority, 2008. Steviol glycosides (New submission), EFSA Question Number EFSA-Q-2008-041). EFSA in Focus – Food, Issue 02, December, p. 12.

FAO (Food and Agriculture Organization), 2007a. Steviol Glycosides. FAO JECFA Monographs 4.

FAO (Food and Agriculture Organization), 2007b. Chemical and Technical Assessment: Steviol Glycosides. Revised by Paul M. Kuznesof, PhD for the 68th JECFA Meeting.

FDA, (Food and Drug Administration), 1993. Clinical Evaluation of Foods and Food Additives In: Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food Redbook II Draft Guidance. US Food and Drug Administration, Bureau of Foods, Washington, DC. Available: http://www.cfsan.fda.gov/~acrobat/red-via.pdf.

FDA, (Food and Drug Administration), 2006. Toxicological Principles for the Safety of Food Ingredients. Redbook, US Food and Drug Administration, Bureau of Foods, Washington, DC. Available: http://www.cfsan.fda.gov/~redbook/red-toca.html.

Frankos, V., Rodricks, J., 2001. In: Gad, S. (Ed.), Regulatory Toxicology, Food Additives and Nutrition Supplements. Taylor and Francis, New York, 133 -166.

FSANZ, (Food Standards Australia New Zealand), 2008. Final Assessment Report, Application A540, Steviol Glycosides as Intense Sweeteners.

Gardana, C., Simonetti, P., Canzi, E., Zanchi, R., Pieta, P., 2003. Metabolism of stevioside and rebaudioside A from Stevia rebaudiana extracts by human microflora. J. Agri. Food Chem., 51, 6618–6622.

Geuns, J.M.C., 2003. Molecules of interest stevioside. Phytochemistry 64, 913-921.

Geuns, J.M.C., Augustijns, P., Mols, R., Buyse, J.G., Driessen, B., 2003a. Metabolism of stevioside in pigs and intestinal absorption characteristics of stevioside, rebaudioside A and steviol. Food Chem. Toxicol., 41, 1599-1607.

Geuns, J.M.C., Malheiros, R.D., Moraes, V.M.B., Decuypere, E.M.P., Compernolle, F. Buyse, J.G., 2003b. Metabolism of stevioside by chickens. J. Agri. Food Chem. 51, 1095-1101.

Geuns, J.M., Buyse, J., Vankeirsbilck, A., Temme, E.H., Compernolle, F., Toppet, S., 2006. Identification of steviol glucuronide in human urine. J. Agric. Food Chem. 5: 2794-2798.

Geuns, J.M.C., 2007. Letter to the Editor: Comments to the paper by Nunes et al. (2007), Analysis of genotoxic potentiality of stevioside by comet assay, Food and Chemical Toxicology 45 (2007) 662-666.

Gregersen, S., Jeppensen, P.B., Holst, J.J., Hermansen, K., 2004. Antihyperglycemic effects of stevioside in type 2 diabetic subjects. Metabolism, 53, 73–76.

Hanson, J.R., De Oliveira, B.H., 1993. Stevioside and related sweetditerpenoid glycosides. Nat. Prod. Rep.,10, 301-309.

Hawke, J., 2003. The Bittersweet Story of the Stevia Herb. Nexus Magazine, Vol.10, No. 2. Available at: http://www.nexusmagazine.com/articles/stevia.html.

Hong Kong Government, 2002. Updated July 2002. [Online] Risk in Brief, issue no. 10, "Stevioside in Foods", Hong Kong Government, Risk Assessment Section at: http://www.info.gov.hk/fehd/textmode/safefood/report/stevioside/stevioside.html

Hsieh, M., Chan, P., Sue, Y., Liu, J., Liang, T., Huang, T., Tomlinson, B., Chow, M.S., Kao, P., Chen, Y., 2003. Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: A two-year, randomized, placebo-controlled study. Clin. Therap. 25, 2797–2808.

Hübler, M.O., Bracht, A., Kelmer-Bracht, A.M. , 1994. Influence of stevioside on hepatic glycogen levels in fasted rats. Res. Commun. Chem. Pathol. Pharmacol., 84, 111-118.

Hutapea, A.M., Tolskulkao, C., Wilairat, P., Buddhasukh, D., 1999. High-performance liquid chromatographic separation and quantitation of stevioside and its metabolites. J. Liq.Chromatogr. & Rel. Technol. 22, 1161–1170.

Huxtable, R.J., 2002. Pharmacology and toxicology of stevioside, rabaudioside A, and steviol. In: Kinghorn, A.D. (Ed.) Stevia: The Genus of Stevia, Taylor and Francis Inc., New York.

Ishidate, M., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., Matsuoka, A., 1984. Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol. 22, 623–636.

Ishii, E.L., Schwab, A.J., Bracht, A., 1987. Inhibition of monosaccharide transport in the intact rat liver by stevioside. Biochem. Pharmacol. 36, 1417-1433.

Kelmer-Bracht, A.M., Alvarez, M., Bracht, A., 1985. Effects of Stevia rebaudiana natural products on rat liver mitochondria. Biochem. Pharmacol. 34, 873-882.

Kennelly, E.J., 2002. Sweet and non-sweet constituents of Stevia rebaudiana (Bertoni). In: Kinghorn, A.D. (Ed.), Stevia, The Genus Stevia. Medicinal and Aromatic Plants – Industrial Profiles, Vol. 19. Taylor and Francis Inc., London and New York, pp. 68-85.

Kerr, W.E., Mello, M.L.S., Bonadio, E., 1983. Mutagenicity tests on the stevioside from Stevia rebaudiana (Bert.) Bertoni. Brazil. J. Genetics 1, 173–176.

Kinghorn, A.D., 2002. Overview. In: Kinghorn, A.D. (Ed.), Stevia, the Genus Stevia. Medicinal and Aromatic Plants—Industrial Profies, Vol. 19. Taylor and Francis, London and NY, pp. 1-17.

Klonpanichpak, S., Temcharoen, P., Toskulkao C., Apibal, S., Glinsukon, T., 1997. Lack of mutagenicity of stevioside and steviol in Salmonella typhimurium TA 98 and TA 100. J. Med. Assoc. Thai 80 (suppl. 1), S121-S128.

Kobayashi, M., Horikawa, S., Degrandi, I. H., Ueno, J. and Mitsuhashi, H. 1977. Dulcosides A and B, new diterpene glycosides from Stevia rebaudiana. Phytochemistry 16: 1405-1408.

Kobylewski, S., Eckhert, C.D., 2008. Toxicology of Rebaudioside A: A Review. University of California at Los Angeles, unpublished but can be viewed at www.cspinet.org/new/200808281.html.

Kokoski, C.J., Henry, S.H., Lin, C.S., Ekelman, K.B., 1990. In: Branen, A.L., Davidson, P.M., Salimen, S. (Eds.), Methods Used in Safety Evaluation. Food Additives, Marcel Dekker, New York, pp. 579-616.

Koyama, E., Sakai, N., Ohori, Y., Kitazawa, K., Izawa, O., Kakegawa, K., Fujino, A., Ui, M., 2003a. Absorption and metabolism of glycosidic sweeteners of *Stevia* mixture and their aglycone, steviol in rats and humans. Food Chem. Toxicol. 41, 875–883.

Koyama, E., Ohori, Y., Kitazawa, K., Izawa, O., Kakegawa, K., Fujino, A., Ui, M. 2003b. *In vitro* metabolism of the glycosidic sweeteners, *Stevia* mixture and enzymically modified *Stevia* in human intestinal microflora. Food Chem. Toxicol. 41, 359–374.

Krsmanovic, L. and Huston, T., 2006. Rebaudioside A: Mammalian Erythrocyte Micronucleus Test. BioReliance, Rockville, MD. Unpublished Report (Study Number AB21TG.123.BTL).

Lester, T., 1999. *Stevia rebaudiana*. The Australian New Crops Newsletter, Issue 11, January 1999. Available: www.newcrops.uq.edu.au/newslett/ncn11161.htm.

Lu, F.C., 1988. Acceptable daily intake: inception, evolution and application. Regul. Toxicol. Pharmacol. 8, 45-60.

Maki, K.C., Curry, L.L., Carakostas, M.C., Tarka, S.M., Reeves, M.S., Farmer, M.V., McKenney, J.M., Toth, P.D., Schwartz, S.L., Lubin, B.C., Dicklin, M.R., Boileau, A.C., Bisognano, J.D., 2008a. The hemodynamic effects of rebaudioside A in healthy adults with normal and low-normal blood pressure. Food Chem. Toxicol. doi:10.1016/j.fct.2008.04.040.

Maki, K.C., Curry, L.L., Reeves, M.S., Toth, P.D., McKenney, J.M., Farmer, M.V., Schwartz, S.L., Lubin, B.C., Boileau, A.C., Dicklin, M.R., Carakostas, M.C., Tarka, S.M., 2008b. Chronic consumption of rebaudioside A, a steviol glycoside, in men and women with type 2 diabetes mellitus. Food Chem. Toxicol. doi:10.1016/j.fct.2008.05.007.

Matsui, M., Matsui, K., Nohmi, T., Mizusawa, H., Ishidate, M., 1989. Mutagenicity of steviol: An analytical approach using a Southern blotting system. Mutat. Res., 203, 377.

Matsui, M., Matsui, K., Kawasaki, Y., Oda, Y., Noguchi, T., Kitagawa, Y., Sawada, M., Hayashi, M., Nohmi, T., Yoshihira, K., Ishidate, M. & Sofuni, T., 1996. Evaluation of the genotoxicity of stevioside and steviol using six *in vitro* and one *in vivo* mutagenicity assays. Mutagenesis 11, 573–579.

Medon, P.J., Pezzuto, J.M., Hovanec-Brown, J.M., Nanayakkara, N.P., Soejarto, D.D., Kamath, S.K., Kinghorn, A.D., 1982. Safety assessment of some Stevia rebaudiana sweet principles. Fed. Proc. 41, 1568.

Merisant GRAS Notification for Rebaudioside A, 2008, Submitted to the US Food and Drug Administration, Washington, DC under the name Whole Earth Sweetener Company LLC and identified as GRAS Notification 252; see FDA website at http://www.cfsan.fda.gov/~rdb/opa-grsn.html.

Melis et al., 1986. Effects of two concentrations of stevioside on renal function and mean arterial pressure in rats, IRCS Medical Science 14, 973. (This reference and full citation is unavailable).

Mitsuhashi, H., 1976. Safety of Stevioside. In: Tama Biochemical Co. Ltd. Report on Safety of Stevia, pp. 9-10.

Mori, N., Sakanoue, M., Takeuchi, M., Shimpo, K., Tanabe, T., 1981. Effect of stevioside on fertility in rats. J. Food Hyg. Soc. Jpn. 22, 409-414 (in Japanese).

Nakajima, (initials unknown), 2000a. Chromosome aberration assay of rebaudioside A in cultured mammalian cells. Test number 5001 (079–085). Unpublished report of a study conducted at the Biosafety Research Center, Japan. Submitted to WHO by Ministry of Health and Welfare, Japan.

Nakajima, (initials unknown), 2000b. Micronucleus test of rebaudioside A in mice. Test numer 5002 (079-086). Unpublished report of a study conducted at the Biosafety Research Center, Japan. Submitted to WHO by Ministry of Health and Welfare, Japan.

Nakayama, K., Kasahara, D.,Yamamoto, F., 1986. Absorption, distribution, metabolism and excretion of stevioside in rats. J. Food Hyg. Soc. Jpn., 27, 1-8.

Nanayakkara, N.P., Klocke, J.A., Compadre, C.M., Hussain, R.A., Pezzuto, J.M., Kinghorn, A.D., 1987. Characterization and feeding deterrent effects on the aphid, Schizaphis graminum, of some derivatives of the sweet compounds, stevioside and rebaudioside A. J. Nat. Prod. 50, 434-441.

Nikiforov, A.I. and Eaton, A.K., 2008. A 90-day (oral) toxicity study of rebaudioside A in Sprague-Dawley rats. Int. J. Toxicol. 27, 65-80.

Nunes, P. and Pereira, N.A., 1988. The effect of *Stevia rebaudiana* on the fertility of experimental animals. Revista Brasileira de Farmacia 69, 46-50.

Nunes, A.P., Ferreira-Machado, S.C., Nunes, R.M., Dantas, F.J., De Mattos, J.C., Caldiera de Araujo, A., 2007a. Analysis of genotoxic potentiality of stevioside by comet assay. Food Chem. Toxicol. 45, 662-666.

Nunes, A.P., Ferreira-Machado, S.C., Nunes, R.M., Dantas, F.J., De Mattos, J.C., Caldiera de Araujo, A., 2007b. Response. Food Chem. Toxicol. 45, 2599-2600.

Nunes, A.P., Ferreira-Machado, S.C., Nunes, R.M., Dantas, F.J., De Mattos, J.C., Caldiera de Araujo, A., 2007c. Response. Food Chem. Toxicol. 45, 2603-2604.

NZFSA (New Zealand Food Safety Authority), 2006. Letter posted to unnamed applicant. Available: http://www.nzfsa.govt.nz/labelling-composition/publications/submissions-to-fsanz/2006/a540.htm.

Ogawa, T., Nozaki, M., Matsui, M., 1980. Total synthesis of stevioside. Tetrahedron 36, 2641-2648.

Oh, H., Han, E., Choi, D., Kim, J., Eom, M., Kang, I., Kang, H., Ha, K., 1999. *In vitro* and *in vivo* evaluation of genotoxicity of stevioside and steviol, natural sweetener. J. Pharm. Soc. Korea 43, 614–622.

Oliveira-Filho, R.M., Uehara, O.A., Minett, C.A.S.A., Valle, L.B.S., 1989. Chronic administration of aqueous extract of Stevia rebaudiana (Bert.) Bertoni in rats: endocrine effects. Gen. Pharmac. 20, 187-191.

Oviedo, C.A., Fronciani, G., Moreno, R., Maas, L.C., 1970. Hypoglycemic action of *Stevia Rebaudiana* Bertoni. Seventh Congress of the International Diabetes Federation, August 23-28, Buenos Aires, Argentina. Excerpta Medica Foundation, Internation Congress Series No. 209, pp. 92-93.

Pezzuto, J.M., Compadre, C.M., Swanson, S.M., Nanayakkara, D., Kinghorn, A.D.,1985. Metabolically activated steviol, the aglycone of stevioside, is mutagenic. Proc. Natl. Acad. Sci. USA 82, 2478–2482.

Planas, G. M., Kuc, J., 1968. Contraceptive properties of Stevia rebaudiana. Science 162, 1007.

Procinska, E., Bridges, B.A., Hanson, J.R., 1991. Interpretation of results with the 8-azaguanine resistance system in Salmonella typhimurium: No evidence for direct acting mutagenesis by 15-oxosteviol, a possible metabolite of steviol. Mutagenesis 6, 165-167.

Renwick A.D., 1990. Acceptable daily intake and the regulation of intense sweeteners. Food Addit Contam. 7, 463-75.

Renwick, A.G., 2008. The use of a sweetener substitution method to predict dietary exposures for the intense sweetener rebaudioside A. Food Chem. Toxicol. doi:10.1016/j.fct.2008.05.009.

Roberts, A., Renwick, A.G., 2008. Comparative toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol in rats. Food Chem. Toxicol. doi:10.1016/j.fct.2008.05.006.

Schvartzman, J.B., Krimer, D.B., Moreno-Azorero, R., 1977. Cytological effects of some medicinal plants used in the control of fertility. Experientia 33, 663-665.

Sekihashi, K., Saitoh, H., Sasaki, Y., 2002. Genotoxicity studies of Stevia extract and steviol by the comet assay. J. Toxicol. Sci. 27 (suppl. 1), 1-8.

Sasaki, Y.F., Kawaguchi, S., Kamaya, A., Ohshita, M., Kabasawa, K., Iwama, K., Taniguchi, K., Tsuda, S., 2002. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat. Res. 519, 103–119.

Sloter, E.D., 2008. A Dietary Two-Generation Reproductive Toxicity Study of Chrysanta® 99-P in Rats. WIL Research Laboratories, LLC. Unpublished Report (Study Number WIL-568006).

Soejarto, D.D., Kinghorn, A.D., Farnsworth, N.R., 1982. Potential sweetening agents of plant origin. III. Organoleptic evaluation of stevia leaf herbarium samples for sweetness. J. Nat. Prod., 45, 590-599.

Sung, L.H., 2002. Report on pharmacokinetic (PK) studies of T100 sunstevia 95% stevioside in rats. Unpublished report from Sunlabel Pte Ltd, Singapore. Submitted to WHO by the Ministry of Health and Welfare, Japan.

Suttajit, M., Vinitketkaumnuen, U., Meevatee U., Buddhasukh, D., 1993. Mutagenicity and human chromosomal effect of stevioside, a sweetener from Stevia rebaudiana Bertoni. Environ. Health Perspect.101, 53–56.

Suzuki, H., Kasai, T., Sumihara, M., Sugisawa, H., 1977. Influence of oral administration of stevioside on levels of blood glucose and liver glycogen of intact rats. J. Agr. Chem. Soc. Japan 51, 45-47.

Switzerland Office of Public Health, 2008, http.www.bag.admin.ch/themen/lebensmittel/04861/04972/index.html?lang=fr.

Temcharoen, P., Pimbua, J., Glinsukon, T., Rojanapo, W., Apibal, S., 1998. Mutagenic activity of steviol to Salmonella typhimurium TM 677: Comparison of the activity of S9 liver fractions from five laboratory animal species. Bull. Health Sci. & Tech. 1, 38–45.

Temcharoen, P., Klopanichpah, S., Glinsukon, T., Suwannatrai, M., Apibal, S.,Toskulkao, C., (2000). Evaluation of the effect of steviol on chromosomal damage using micronucleus test in three laboratory animal species. J. Med. Assoc. Thai., 83, s101–s108.

Terai, T., Ren, H., Mori, G., Yamaguchi, Y., Hayashi, T., 2002. Mutagenicity of steviol and its oxidative derivatives in Salmonella typhimurium TM677. Chem. Pharm. Bull., 1007–1010.

The Food Institute Report, 2006. FDA News May 15, 2006. From Newsday, May 2, 2006.

Toskulkao, C., Chaturat, L., Temcharoen, P., Glinsukon, T., 1997. Acute toxicity of stevioside, a natural sweetener, and its metabolite, steviol, in several animal species. Drug Chem.Toxicol. 20, 31-44.

Toyoda, K., Matsui, H., Shoda, T., Uneyama, C., Takahashi, M., 1997. Assessment of the carcinogenicity of stevioside in F344 rats. Food Chem. Toxicol. 35, 597–603.

Usami, M., Sakemi, K., Kawashima, K., Tsuda, M., Ohno, Y., 1995. Teratogenicity study of stevioside in rats. Bull. Natl Inst. Hyg. Sci. 113, 31-35 (in Japanese).

von Schmeling, G.A., De Carvelho, F.V., Espinosa, A.D., 1977. *Stevia rebaudiana* Bertoni; Evaluation of the hypoglycemic effect on alloxanized rabbits. Ciencia e Cultura 29, 599-601.

Wagner, V.O. and Van Dyke, M.R., 2006. Bacterial Reverse Mutation Assay of Rebaudioside A. BioReliance, Rockville, MD. Study Number AB21TG.503.BTL.

Wang, L.Z., Goh, B.C., Fan, L., Lee, H.S., 2004. Sensitive high-performance liquid chromatography/ mass spectrometry method for determination of steviol in rat plasma. Rapid Commun. Mass Spectrom. 18, 83-86.

Wasuntarawat, C., Temcharoen, P., Toskulkao, C., Mungkornkarn, P., Suttajit, M., Glinsukon, T., 1998. Developmental toxicity of steviol, a metabolite of stevioside, in the hamster. Drug Chem.Toxicol. 21, 207-222.

Wheeler, A., Boileau, A.C., Winkler, P.C., Compton, J.C., Prakash, I., Jiang, X., Mandarino, D.A., 2008. Pharmacokinetics of rebaudioside A and stevioside after single oral doses in healthy men. Food Chem.Toxicol. doi:10.1016/j.fct.2008.04.041.

WHO, 2000. Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additive Series; 42. Safety evaluation of certain food additives. Stevioside.

WHO, 2003. GEMS/Food regional diets (regional per capita consumption of raw and semi processed agricultural commodities). Geneva: Global Environment Monitoring System 144 steviol glycosides K2 Food Contamination Monitoring and Assesssment Programme and Food Safety Department, World Health Organization.

WHO, 2006. Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additive Series; 54. Safety evaluation of certain food additives, Steviol Glycosides, pp.117-144.

WHO, 2007. Joint FAO/WHO Expert Committee on Food Additves. Sixty-eighth meeting, Summary and Conclusions, Steviol Glycosides. Issued July 12, 2007.

WHO, 2008. Joint FAO/WHO Expert Committee on Food Additves. Sixty-ninth meeting, Summary and Conclusions, Steviol Glycosides. Issued July 4, 2008.

Williams, G. M., 2007. Letter to the Editor, Food Chem. Toxicol. 45 (2007) 2597-2598.

Wingard, R.E., Brown, J.P., Enderlin, F.E., Dale, J.A., Hale, R.L., Seitz, C.T., 1980. Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A. Experientia 36, 519-520.

Xili, L., Chengjiany, B., Eryi, X., Reiming, S., Yuengming, W., Haodong, S., Zhiyian, H., 1992. Chronic oral toxicity and carcinogenicity study of stevioside in rats. Food Chem. Toxicol. 30, 957-965.

Yamada, A., Ohgaki, S., Noda, T., Shimizu, M., 1985. Chronic toxicity of Dietary Stevia Extracts. Shokuin Eiseigaku Zasshi. 2, 169-183.

Yamamoto, N.S., Kelmer-Bracht, A.M., Ishii, E.L., Kemmelmeier, F.S., Alvarez, M., Bracht, A., 1985. Effect of steviol and its structural analogues on glucose production and oxygen uptake in rat renal tubules. Experientia, 41, 55-57.

Yodyingyuad, V., Bunyawong, S., 1991. Effect of stevioside on growth and reproduction. Hum. Reprod. 6, 158-165.

APPENDIX A

JECFA Steviol Glycosides Specifications & Analytical Method

STEVIOL GLYCOSIDES

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding tentative specifications prepared at the 63rd JECFA (2004), in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A temporary ADI of 0-2 mg/kg bw (expressed as steviol) was established at the 63rd JECFA (2004).

SYNONYMS INS no. 960

DEFINITION The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with methanol to release the glycosides and product is recrystallized with methanol. Ionexchange resins may be used in the purification process. The final product may be spray-dried.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property Associated glycosides include rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

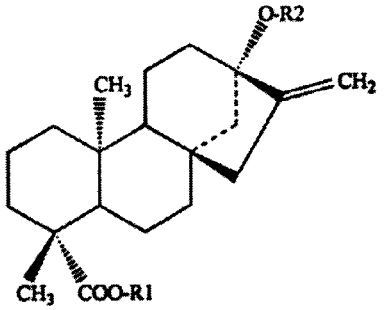
Chemical name *Stevioside*: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-6-en-8-oic acid, β-D-glucopyranosyl ester

C.A.S. number Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula Stevioside: $C_{38}H_{60}O_{18}$
Rebaudioside A: $C_{44}H_{70}O_{23}$

Structural formula The seven named steviol glycosides:



Compound name	*R1*	*R2*
Stevioside	β-Glc	β-Glc-β-Glc(2→1)
Rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside C	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
Dulcoside A	β-Glc	β-Glc-α-Rha(2→1)
Rubusoside	β-Glc	β-Glc
Steviolbioside	H	β-Glc-β-Glc(2→1)
Rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides.
Glc and Rha represent, respectively, glucose and rhamnose sugar moieties.

Formula weight Stevioside: 804.88
Rebaudioside A: 967.03

Assay Not less than 95% of the total of the seven named steviol glycosides, on the dried basis.

DESCRIPTION White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water and in ethanol
Stevioside and rebaudioside A	The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.
pH (Vol. 4)	Between 4.5 and 7.0 (1 in 100 solution)

PURITY

Total ash (Vol. 4)	Not more than 1%
Loss on drying (Vol. 4)	Not more than 6% (105°, 2h)
Residual solvents (Vol. 4)	Not more than 200 mg/kg methanol (Method I in Vol. 4, General Methods, Organic Components, Residual Solvents)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under "General Methods, Metallic Impurities).

METHOD OF ASSAY Determine the percentages of the individual steviol glycosides by high pressure liquid chromatography (Volume 4).

Standards
Stevioside, >99.0% purity and rebaudioside A, >97% purity (available from Wako pure Chemical Industries, Ltd. Japan).

Mobile phase
Mix HPLC-grade acetonitrile and water (80:20). Adjust the pH to 3.0 with phosphoric acid (85% reagent grade). Filter through 0.22 µm Millipore filter or equivalent.

Standard solutions
(a) Accurately weigh 50 mg of dried (105°, 2 h) stevioside standard into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase.
(b) Repeat with previously dried rebaudioside A standard.

Sample solution
Accurately weigh 60-120 mg of dried (105°, 2 h) sample into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with the mobile phase.

Chromatography Conditions

Column: Supelcosil LC-NH2 or equivalent (length: 15-30 cm; inner diameter: 3.9-4.6 mm)

Mobile phase: A 80:20 mixture of acetonitrile and water (see above)
Flow rate: Adjust so that the retention time of rebaudioside A is about 21 min.

Injection volume: 5-10 µl

Detector: UV at 210 nm

Column temperature: 40°

Procedure

Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the sample solution and of the standard solutions.

The retention times relative to rebaudioside A (1.00) are:

0.45-0.48 for stevioside	0.12-0.16 for rubusoside
0.25-0.30 for dulcoside A	0.35-0.41 for steviolbioside
0.63-0.69 for rebaudioside C	0.73-0.79 for rebaudioside B

Measure the peak areas for the seven steviol glycosides from the sample solution (the minor components might not be detected). Measure the peak area for stevioside for the standard solution.

Calculate the percentage of each of the seven steviol glycosides, *X*, in the sample from the formula:

$$\%X = [W_S/W] \times [f_X A_X/A_S] \times 100$$

where

W_S is the amount (mg) of stevioside in the standard solution
W is the amount (mg) of sample in the sample solution
A_S is the peak area for stevioside from the standard solution
A_X is the peak area of *X* for the sample solution
f_X is the ratio of the formula weight of *X* to the formula weight of stevioside: 1.00 (stevioside), 0.98 (dulcoside A), 1.20 (rebaudioside A), 1.18 (rebaudioside C), 0.80 (rubusoside), 0.80 (steviolbioside), and 1.00 (rebaudioside B).

Calculate the percentage of total steviol glycosides (sum the seven percentages).

APPENDIX B

Determination Methods & Specific Analyses of Multiple Production Lots for SweetLeaf® Stevia

Specific Analysis Performed to Selected Lots

1) Steviol Glycosides Determination

High Performance Liquid Chromatography (HPLC) is used for glycoside determination both for the raw materials (leaves) and the final product (free flowing white powder). The qualitative and quantitative analysis of total steviol glycosides (main 7 glycosides) in stevia extract is performed using HPLC (High Performance Liquid Chromatography) and a NH2 column (normal phase mode), in the following manner.

About 60 to 120 mg of the sample is accurately weighed and dissolved in 80% vol/vol acetonitrile to make 100 ml of a test solution. About 50 mg each of stevioside and rebaudiosido A, dried previously for 2 hours at 105° C are accurately weighted, and the standard solutions of both compounds and prepare in the same fashion at the test solution. The test solution and standard solutions are injected into the HPLC in the following conditions of flow rate and retention times:

HPLC Method

- Mobile Phase: 80 % v/v of HPLC Acetonitrile (Merck, KGaA. Darmstadt, Germany) and 20 % v/v of HPLC water (Merck, KGaA. Darmstadt, Germany)
- Flow: 2 mL/min
- Column: Kromasil 100-5NH2. Column Amino. 250 * 4.6 mm (Akzo Nobel)
- Wavelenght:210 nm
- Injection Volume:20 uL
- Dilution: Within 2 – 4 g/L. Samples are analyzed for weight injection correction by drying in stove at 105°C for 5 hours, both for standards (Stevioside and Rebaudioside A) and sample.
- Equipment: HPLC Shimadzu , Detector SCL 10AVP, Controller SPD- 10A, manual Injector Rheodyne, Authomatic Injector SIL 10A and Pump LC-10ADVP. Software Peak Simple 3.29
- Chromatogram

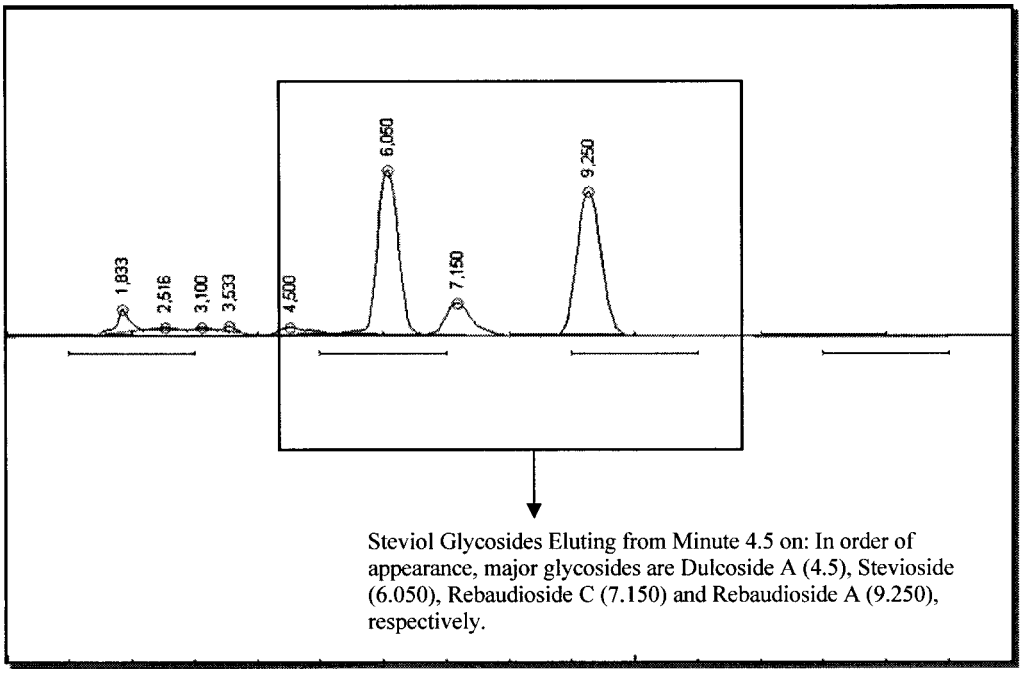


Figure 1: Peaks of major steviol glycosides. Peak eluting at minute 1,833 is a solvent peak, while peaks at 2,5; 3,1 and 3.5 are not the main steviol glycosides so not considered for glycoside purity determination.

From the chromatogram reading, the first eluting peak (1,833) is a solvent peak, while minor impurities with high absorbance profiles elute before the peak at minute 4.5, as in accordance to the typical purified profile of a stevia extract in Stevia (Kinghorn, 2002).

After the obtention of the chromatogram, the calculations for glycoside content is performed by considering the areas in the glycoside eluting zone and comparing them to standard measurements. Peak areas of major glycosides in the chromatogram of the test solution and those of the standard Stevioside, Rebaudioside A and a standard commercial lot of a purity above 97% are measured and quantified for all major peaks eluting, depending of the steviol glycoside profile. These are normally Stevioside, Rebaudioside A, Rebaudioside C and Dulcoside A.

Glycoside contents are determined as follows:

- Stevioside (%): (WS/WT) x (TS/SS) x 100
- Dulcoside A (%): (WS/WT) x (TD/SS) x 100
- Rebaudioside A (%): (WR/WT)x(TRA/SR) x 100
- Rebaudioside C (%): (WR/WT)x(TRC/SR) x 100

Where W: Weight (mg) of Stevioside Standard; WR: Weight (mg) of Rebaudioside A standard, WT: weight (mg) of the test sample; TS: Peak Area of Stevioside in the sample chromatogram; TD: Peak area of dulcoside A in the sample chromatogram; TRA; Peak Area of Reb A in the test sample chromatogram; TRC; Peak area of the Reb A in the test sample chromatogram; SS; Peak area of the stevioside in the standard chromatogram; SR: Peak area of Rebaudioside A in the standard chromatogram. Total amount of steviol glycosides is obtained as the sum of previous percentages.

Elution times change from equipment to equipment, column and injection date. Therefore, the use of external standards and a production standard lot ensures the correct use of the method.

- **References**

Japan Food Additive Association. Stevia extract. In Voluntary Specifications of Non-Chemically Synthesized Food Additives (2nd Ed.), Japan Food Additive Association (Ed.), Tokyo, pp. 119-124. 1993.
N. Kolb, J. L. Herrera, D. J. Ferreyra, and R. F. Uliana. Analysis of Sweet Diterpene Glycosides from *Stevia rebaudiana*: Improved HPLC Method. J. Agric. Food Chem., 49 (10), 4538 -4541, 2001.

Mizutani, K and Tanaka, O. Use of *Stevia rebaudiana* sweeteners in Japan. In Stevia. The genus Stevia. Edited by Kinghorn, Douglas. A, Departament Medicinal Chemistry and Pharmacognosy. University of Illinois at Chicago. USA. Taylor and Francis Group. New York, 2002.

Analyzed Lots:

a) **Lot 172** (Injected July 5, 2008)

Lot 172				Standards				
		(1)	(2)		(3)	(4)	(5)	(6) = ((1) x (4))/((3) x (2)) x (5)
Component	Retention	Area	Weight (g/Kg)	Retention	Area	Weight (g/Kg)		%
	1,783	241,34						
	2,633	37,37						
	3,533	182,59						
Dulc A *	4,6	49,97	2,21		3862,93	3,45		2,02
Stevioside (a)	6,083	1181,56	2,21	6,05	3862,93	3,45	99,8	47,72
Reb C *	7,183	194,44	2,21		3954,67	3,62		8,04
Reb A (b)	9,283	978,87	2,21	9,25	3954,67	3,62	99,8	40,46

Total Purity Steviol Glycosides 98,24

Note: * Dulcoside A calculated by using Stevioside standard and Rebaudioside C calculated by using Rebaudioside A standard, as in accordance to Mizutani et al in Stevia (Kinghorn, 2002).

a) Standard Obtained from EKA Chemicals (Akzo Nobel) , Sweden, 2008..
b) Standard Obtained from Soolite, 2007.

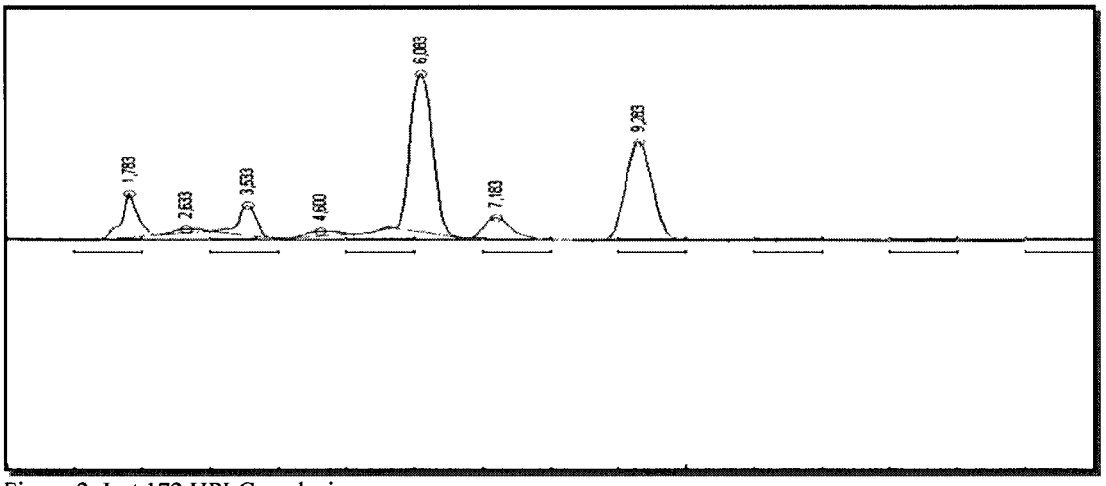


Figure 2: Lot 172 HPLC analysis.

Note: For purity calculation of glycoside content is considering the sum of major peaks eluting from minute 4.5 on, as in Mizutani et al, 2002. Other glycosides do not give any significant eluting peaks, so they were conservatively not integrated to determine purity. They account for less than 1% of additional purity. Elution times were adjusted to define major peaks and integration did not considered minor peaks in the steviol glycoside elution timeframe.

b) Lot 174

Lot 174				Standard				
		(1)	(2)		(3)	(4)	(5)	(6) = ((1) x (4))/((3) x (2)) x (5)
Component	Retention	Area	Weight (g/Kg)	Retention	Area	Weight (g/Kg)	St Purity *	%
	1,783	243,215						
	2,6	41,1265						
	3,4	285,785						
Dulc A *	4,566	38,782	2,5		3956,87	2,43		0,95
Stevioside (a)	6,15	1939,293	2,5	6,133	3956,87	2,43	99,8	47,54
Reb C *	7,216	248,1095	2,5		4057,99	3,65		8,91
Reb A (b)	9,383	1129,728	2,5	9,316	4057,9885	3,65	99,8	40,56
								97,97

Note: * In accordance to Mizutani et al in Stevia (Kinghorn, 2002).
a) Standard Obtained from EKA Chemicals (Akzo Nobel) , Sweden, 2008..
b) Standard Obtained from Soolite, 2007.

- Chromatogram Lot 174 (injected 08-02-08)

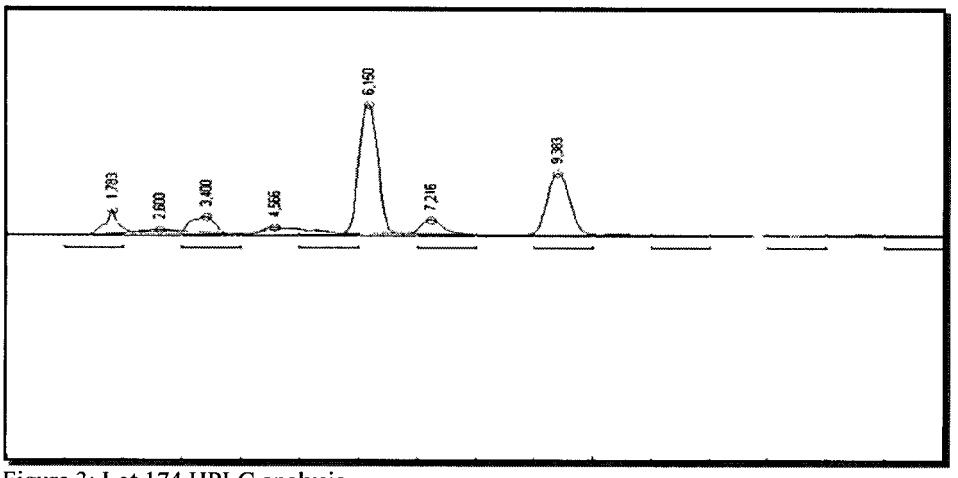


Figure 3: Lot 174 HPLC analysis.

c) **Lot 177** (Injected September 2, 2008)

Lot 177				Standard				
		(1)	(2)		(3)	(4)	(5)	(6) = ((1) x (4))/((3) x (2)) x (5)
Component	Retention	Area	Weight (g/Kg)	Retention	Area	Weight (g/Kg)	St Purity *	%
	1,916	341,792						
	2,783	41,3145						
	3,733	192,466						
Dulc A *	4,683	123,554	2,4					3,14
Stevioside (a)	6,116	1776,192	2,4	6,133	3960,23	2,42	99,8	45,13
Reb C *	7,133	202,162	2,4					7,54
Reb A (b)	9,166	1106,946	2,4	9,200	4011,98	3,6	99,8	41,30
Total Purity Steviol Glycosides								97,12

Note: Dulcoside A calculated by using Stevioside Standard and Rebaudioside C calculated by using Rebaudioside A Standard, as in accordance to Mizutani et al in Stevia (Kinghorn, 2002).

c) Standard Obtained from EKA Chemicals (Akzo Nobel) , Sweden, 2008.

d) Standard Obtained from Soolite, 2007.

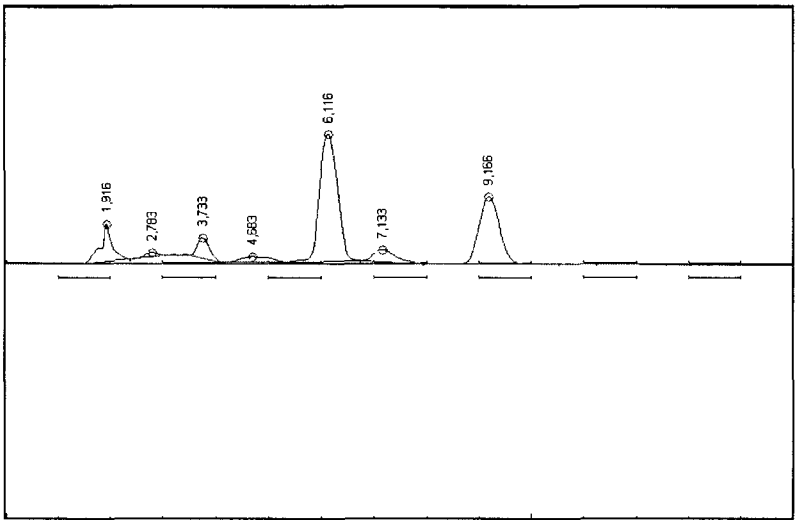


Figure 4: Lot 177 HPLC analysis.

d) **Lot 178** (Injected September 10, 2008)

Lot 178								
		(1)	(2)		(3)	(4)	(5)	(6) = ((1) x (4))/((3) x (2)) x (5)
Component	Retention	Area	Weight (g/Kg)	Retention	Area	Weight (g/Kg)	St Purity *	%
	2	288,74						
	3,85	74,96						
Dulc A *	4,816	135,39	2,3					3,56
Stevioside (a)	6,333	1650,22	2,3	6,300	3990,28	2,42	99,8	43,43
Reb C *	7,383	263,21	2,3					10,10
Reb A (b)	9,483	1050,24	2,3	9,433	4070	3,6	99,8	40,31
								97,40

Note: Dulcoside A calculated by using Stevioside Standard and Rebaudioside C calculated by using Rebaudioside A Standard, as in accordance to Mizutani et al in Stevia (Kinghorn, 2002).

a) Standard Obtained from EKA Chemicals (Akzo Nobel) , Sweden, 2008.

b) Standard Obtained from Soolite, 2007.

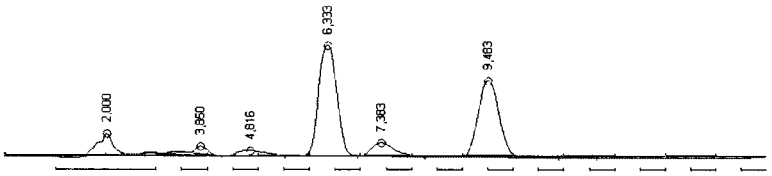


Figure 5: Lot 178 HPLC analysis.

e) **Lot 179** (Injected September 17, 2008)

Lot 179								
		(1)	(2)		(3)	(4)	(5)	(6) = ((1) x (4))/((3) x (2)) x (5)
Component	Retention	Area	Weight (g/Kg)	Retention	Area	Weight (g/Kg)	St Purity *	%
	2,300	338,8						
	3,566	42,34						
	4,133	111,65						
Dulc A *	4,833	140,96	2,3					3,58
Stevioside (a)	6,083	1783,65	2,3	6,100	4070,46	2,38	99,8	45,25
Reb C *	6,950	217,56	2,3					8,43
Reb A (b)	8,550	1045,45	2,3	8,566	4063,35	3,63	99,8	40,53
								97,79

Note: Dulcoside A calculated by using Stevioside Standard and Rebaudioside C calculated by using Rebaudioside A Standard, as in accordance to Mizutani et al in Stevia (Kinghorn, 2002).

a) Standard Obtained from EKA Chemicals (Akzo Nobel) , Sweden, 2008.

b) Standard Obtained from Soolite, 2007.

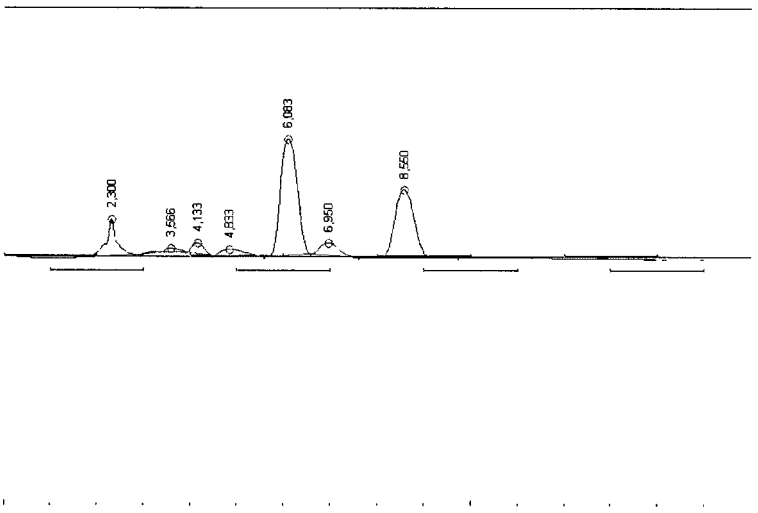


Figure 6: Lot 179 HPLC analysis.

2) Determination of Total Phenols

Phenolic compounds are found commonly in fruits, coffee, green tea, chocolate and wine. During the process of purification of steviol glycosides, most phenolics are permeated out of the extract. The remaining phenolics can be quantified precisely by the Folin Cicalteau method (referenced and attached below) and commonly used for determining the finest quality of wines. As an example, phenolic maturity is a critical indicator of good wine quality.

The Folin Cicalteau reagent method is as follows for total phenolics measurements:

1) Get aliquots of extract in test tubes (between 0.02 and 0.1 ml of extract), filling to 0,5 ml with distilled water, 0,25 ml of Folin Cicalteau reagent and 1,25 ml of a sodium carbonate solution.
2) Measure absorbance after 40 min at 725 nm and record value.
3) Calculate phenolics as total tannic acid equivalents from the calibration table described in the method.

Phenolic levels in lots 172 , 174, 177, 178 and 179 are below 1% w/w, as our process is validated to produce lots with phenolic contents below a 1%, when getting purities above 95%. All of our lots achieving the steviol glycosides purities fulfill this requirement. During the process validation all residual phenolic contents were determined in final products, and examples of this are the following lots:

Figure 7: Summary of Total Phenolic Determination for Finished Product

Lot	m	Dry Weight (g/L)	Absorbance (uA)	Dilution	V1 (uL)	% Total Phenols	% Total Phenols
lote 177	22,75	2,74	0,058 0,042	1 1	300 300	0,16 0,12	0,14
lote 172	22,75	2,75	0,064 0,066	1 1	300 300	0,18 0,18	0,18
lote174	22,75	2,82	0,058 0,046	1 1	300 300	0,16 0,12	0,14
lote178	22,75	2,94	0,060 0,061	1 1	300 300	0,16 0,16	0,16
lote179	22,75	2,66	0,048 0,052	1 1	300 300	0,14 0,15	0,15

- **Reference Specific Phenolic Method: Gallic Acid, Catechins, Epicatechins, Caffeic Acid, Quercetin, within others.**

Donovan, J. L., McCauley, J.C., Tobella Nieto, N., Waterhouse, A. Effects of Small-scale finning on the phenolic composition and antioxidant activity of merlot wine. In Chemistry of Wine Flavor. Edited by Waterhouse, Andrew and Ebeler, Susan. American Chemical Society. Washington DC, 1998.

4) Determination of Product Moisture

Moisture determination for finished product is done at 105°C for 3 hours A.O.A.C. 1995 925.10. The results for the selected lots are the following:

BEST ORIGINAL COPY

Figure 8: Summary of Moisture determinations for Stevia finished products.

Lot	% Total Moisture
lote 177	3,5
lote 172	4,0
lote174	3,6
lote178	3,2
lote179	3,4

3) Microbiological and Heavy Metals Determinations (by Analab /CONDECAL, Chile)

INFORME DE ENSAYO ANALAB

SAB –51680

Solicitante : Productora Alysa Ltda. Orden de Trabajo: 347.994
Atención Sra. : Susana Morales Fecha de Emisión: 28.10.2008

ANTECEDENTES

Se recibió en nuestros laboratorios una (01) muestra de Endulzante Natural en Polvo para ser sometidas a Análisis Químico y Bacteriológico debidamente identificadas según Carta del 10 de Octubre de 2008.

FECHA DE RECEPCIÓN DE MUESTRAS : 14.10.2008 Hora: 10:55

FECHA DE INICIO ANÁLISIS : 14.10.2008 Hora: 11:15

FECHA DE OBTENCIÓN RESULTADOS : 24.10.2008 Hora: 18:00

RESULTADOS

Efectuados los análisis, los resultados son los siguientes:

1.- Análisis Microbiológicos

Análisis	Endulzante Natural en Polvo Lote: 177	Metodologías
Recuento Mesófilos Aerobios, u.f.c. / g	$6,1 \times 10^{2}$	NCh 2659 Of. 2002
Coliformes Totales NMP / g	< 3	NCh 2635/1 Of. 2001
Coliformes Fecales NMP / g	< 3	
Escherichia coli, NMP / g	< 3	NCh 2636 Of. 2001
Salmonella Presencia/Ausencia, 25 g	Ausencia	NCh 2675 Of. 2002
Recuento Hongos u.f.c. / g	20	NCh 2734 Of. 2002
Recuento Levaduras u.f.c. / g	< 10	

SAB-51680
Fecha Emisión Informe: 28.10.2008

2.- Químicos

Análisis	Endulzante Natural en Polvo Lote: 177	Metodologías
Cenizas g/100g	< 0,5	NCh 515 Of. 1980
Plomo, mg/kg	< 0,5	NCh 2751 Of. 2002
Arsénico, mg/kg	< 0,5	AOAC 986, 15

NOTA: Los resultados son válidos sólo para la muestra analizada, la cual fue proporcionada por el solicitante.

Montserrat Mendizabal E.
Supervisor
Div. Química y Alimentos

Analab

Report N° 211714
This report consists of 1 sample
Date of issue: 06/11/2007

Page 1 of 1
Laboratory Area Chemistry

Client's identification

Name : PRODALYSA LTDA.
Attention : Mrs. SUSANA MORALES
Address : PARQUE INDUSTRIAL GUIMUE LOTE 13 CAMINO INTERNACIONAL
City : CONCON V REGION CHILE
Phone N° : (5632)-2819652

Sample N° : 439547 Sample integrity: Apt for specific analysis
Code : STEVIA PURE LOT : 46 DATE : 31 – 08-07
Type : VEGETAL
Sampled by : The client Sampling date : —

Requested Analysis		Results
MERCURY	(mg/Kg)	0,23
CADMIUM	(mg/Kg)	< 0.5
LEAD	(mg/Kg)	0,8
ARSENIC	(mg/Kg)	0,20

REF: Atomic Absorption Spectrometry

Waldo Jaña Morales
Chemist
Chemistry Area Supervisor

Jorge Espinoza Munita
Laboratory Manager

Results are only valid for the analyzed sample.

EZEQUIEL FERNANDEZ 3062 - MAGUL - FONO (56-2) 7131500 -CASILLA 919 - CORREO 11 - SANTIAGO

Analab

Analab - Report Nr. 211714

This report consists of 1 sample
Client ID : 76667160-8
Date of issue: 26/Oct/2007

Laboratory Area : PESTI

Client's identification

Name	: *PRODALYSA LTD..*
Attention	: *Mrs.SUSANA MORALES*
Address	: *PARQUE INDUSTRIAL GULMUE. Lote 13. Camino Internaional. Concon.*
City	: *CONCON . CHILE*
Phone N°	: *09 – 4893426* Fax N° : ----

Sample: 439547

Code	: *STEVIA PURE.Lote:46. Date: 31.08.07*
Type	: *Vegetal*
Sub-Type	:
Sampled by	: *THE CLIENT*
Sampling date	: ----

L.O.D.	Requested Analysis	Results
---ORGANOCHLORINES PIRETHROIDS AND OTHERS		mg/Kg *(ppm)*
0.05	ALACHLOR	*Not Detected*
0.01	ALDRIN	*Not Detected*
0.01	ALPHA-BHC	*Not Detected*
0.01	ALPHA-CHLORDANE	*Not Detected*
0.01	BETA BHC	*Not Detected*
0.01	BIFENTHRIN	*Not Detected*
0.01	BROMOPROPYLAT	*Not Detected*
0.02	CAPTAN	*Not Detected*
0.01	CYANAZYN	*Not Detected*
0.02	CYFLUTHRIN	*Not Detected*
0.03	CYPERMETRHIN	*Not Detected*
0.01	pp – DDD	*Not Detected*
0.01	op – DDD	*Not Detected*
0.01	op – DDE	*Not Detected*
0.01	pp – DDE	*Not Detected*
0.02	op – DDT	*Not Detected*
0.02	pp – DDT	*Not Detected*
0.03	DELTAMETHRIN	*Not Detected*
0.03	DICHLOFLUANID	*Not Detected*
0.02	DICLORAN	*Not Detected*
0.05	DICOFOL	*Not Detected*
0.01	DIELDRIN	*Not Detected*
0.02	ENDOSULFAN I	*Not Detected*
0.02	ENDOSULFAN II	*Not Detected*
0.01	ENDRIN	*Not Detected*
0.03	ESFENVALERATE	*Not Detected*
0.03	FENARIMOL	*Not Detected*

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Analab

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Laboratory Area :

0.03	FENVALERATE	*Not Detected*
0.01	FIPRONIL	*Not Detected*
0.01	FLUAZINAM	*Not Detected*
0.02	FLUVALINATO	*Not Detected*
0.07	FOLPET	*Not Detected*
0.01	GAMMA CLHORDANE	*Not Detected*
0.01	HEXACHLOROBENXENE	*Not Detected*
0.01	HEPTACHLOR	*Not Detected*
0.01	HEPTACHLOR EPOXIDE	*Not Detected*
0.02	IPRODIONE	*Not Detected*
0.01	KRESOXYM-METHYL	*Not Detected*
0.01	LAMBDA-CYHALOTHRIN	*Not Detected*
0.01	LINDANE	*Not Detected*
0.02	METOXICLORO	*Not Detected*
0.02	PERMETHRIN	*Not Detected*
0.03	PROCYMIDONE	*Not Detected*
0.03	PYRIDABEN	*Not Detected*
0.02	CHINOMETIONATE	*Not Detected*
0.01	QUINTOZENE	*Not Detected*
0.01	SPIRODICLOFEN	*Not Detected*
0.03	TETRADIFON	*Not Detected*
0.02	VINCLOZOLIN	*Not Detected*

---ORGANOPHOSPHORUS ORGANONITROGEN AND OTHERS mg/Kg (ppm)

OP

0.01	ACEPHATE	*Not Detected*
0.02	ATRAZINE	*Not Detected*
0.03	AZINPHOS-ETHYL	*Not Detected*
0.01	AZINPHOS-METHYL	*Not Detected*
0.01	BITERTANOL	*Not Detected*
0.01	BROMOPHOS METHYL	*Not Detected*
0.01	BROMOPHOS ETHYL	*Not Detected*
0.01	BUPROFEZIN	*Not Detected*
0.01	BUPIRIMATE	*Not Detected*
0.01	CADUSAFOS	*Not Detected*
0.01	CARBOPHENOTHION	*Not Detected*
0.02	CHLORFENVINPHOS	*Not Detected*
0.01	CHLORPYRIFOS-ETHYL	*Not Detected*
0.01	CHLORPYRIFOS-METHYL	*Not Detected*

0.03	DIAZINON	*Not Detected*
0.01	DICHLORVOS	*Not Detected*

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Laboratory Area :

0.01	DIPHENYLAMINE	*Not Detected*
0.01	DIMETHOATE	*Not Detected*
0.01	DISULFOTON	*Not Detected*
0.01	ETHION	*Not Detected*
0.03	ETHOPROPHOS	*Not Detected*
0.03	FENAMIPHOS	*Not Detected*
0.01	FENBUCONAZOLE	*Not Detected*
0.01	FENCHLORFOS	*Not Detected*
0.01	FENITROTHION	*Not Detected*
0.01	FENTHION	*Not Detected*
0.01	FLUSILAZOLE	*Not Detected*
0.01	PHOSALONE	*Not Detected*
0.01	PHOSMET	*Not Detected*
0.01	HEXACONAZOLE	*Not Detected*
0.02	HEXAZINONE	*Not Detected*
0.01	MALATHION	*Not Detected*
0.02	METALAXYL	*Not Detected*
0.01	METHAMIDOPHOS	*Not Detected*
0.03	METHIDATHION	*Not Detected*
0.01	METRIBUZINE	*Not Detected*
0.01	MEVINPHOS	*Not Detected*
0.01	MYCLOBUTANIL	*Not Detected*
0.03	MONOCROTOPHOS	*Not Detected*
0.03	OMETHOATE	*Not Detected*
0.01	PACLOBUTRAZOL	*Not Detected*
0.01	PARATHION-ETHYL	*Not Detected*
0.02	PARATHION-METHYL	*Not Detected*
0.01	PENDIMETHALIN	*Not Detected*
0.01	PYRIMETHANIL	*Not Detected*
0.02	PIRIMICARB	*Not Detected*
0.01	PIRIMPHOS-ETHYL	*Not Detected*
0.01	PIRIMPHOS-METHYL	*Not Detected*
0.02	PYRIPROXYFEN	*Not Detected*
0.01	PROFENOFOS	*Not Detected*
0.01	QUINALPHOS	*Not Detected*
0.01	SIMAZINE	*Not Detected*
0.02	TEBUCONAZOLE	*Not Detected*
0.01	TERBUTRYNE	*Not Detected*
0.02	TERBUTHYLAZINE	*Not Detected*
0.01	TOLYLFLUANID	*Not Detected*
0.02	TRIADIMEFON	*Not Detected*

0.02	TRIADIMENOL	*Not Detected*
0.01	TRIAZOPHOS	*Not Detected*
0.01	TRICHLORFON	*Not Detected*
0.02	TRIFLUMIZOLE	*Not Detected*
0.01	TRIFLURALINA	*Not Detected*
0.03	VAMIDOTHION	*Not Detected*

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Analab

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This report consists of 1 sample

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Laboratory Area :

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Date of issue: 26/Oct/2007

---CARBAMATES		*mg/Kg (ppm)*
0.03	CARBOFURAN	*Not Detected*
0.02	CARBARYL	*Not Detected*
0.10	THIABENDAZOLE	*Not Detected*
0.05	METHOMYL	*Not Detected*
0.10	THIOPHANATE-METHYL	*Not Detected*
0.02	OXAMYL	*Not Detected*
0.05	BENOMYL	*Not Detected*
0.03	PROPOXUR	*Not Detected*
0.06	ALDICARB SULPHOXIDE	*Not Detected*
0.03	ALDICARB SULPHONE	*Not Detected*
0.03	ALDICARB	*Not Detected*
0.04	METHIOCARB	*Not Detected*

OBSERVATIONS:

METHOD: P – 002 / LUKE

Pesticide Analytical Manual (PAM) Volume I : Multiresidues Methods. 302 - E1 Luke, M.A. et al.
3rd Edition, 1999.
Food and Drug Administration (FDA) U.S.A.

L.O.D: LIMIT OF DETECTION (mg /Kg).

Results are only valid for the analysed sample.

OFICIAL S.A.G. Analab CHILE

ALEJANDRO MIRANDA L.
CHEMIST
AREA SUPERVISOR

JORGE ESPINOZA MUNITA.
LICENCIADO EN QUIMICA
LABORATORY MANAGE

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APPENDIX C

Credentials of Expert Panel Members

Richard C. Kraska, Ph.D., DABT

Robert S. McQuate, Ph.D.

James R. Coughlin, Ph.D.

Robin C. Guy, M.S., DABT

Michael C. Falk, Ph.D.

Walter H. Glinsmann, M.D.

Robert J. Nicolosi, Ph.D.

Richard C. Kraska
Chief Operating Officer and Co Founder
GRAS Associates, LLC
12068 Via Cercina Dr.
Bonita Springs, FL 34135

Curriculum Vitae

EDUCATION B.S., Chemistry; Providence College
Ph.D., Pharmacology;

PROFESSIONAL CERTIFICATION Diplomate,

EXPERIENCE year in toxicology and regulatory affairs for industry and government in broad aspects of the chemical industry including food additives, foods, food contact materials, cosmetics, lubricants and fuels, coatings, defoamers, anti-microbial pesticides and pharmaceuticals.

GRAS ASSOCIATES, LLC

Chief Operating Officer and Co Founder

- Serve as Lead Scientist and Panel Chair for GRAS determinations.
- Coordinate drafting and report review by chemists, toxicologists and scientists of other disciplines as needed.
- Ingredients reviewed include natural antioxidants, novel sources of dietary fiber, fats and oils and extracts from exotic fruit.

KRASKA CONSULTANTS, INC.

Vice President and Principal

- Toxicology and Regulatory Consultant for a variety of lubricant, chemical, food processing companies and trade associations
- Offer services in Toxicology and Product Safety including FDCA, TSCA and FIFRA regulations and filings, International Hazard Communication Support, Product Stewardship, Expert Witness and Litigation Support
- Founder and Technical Consultant for the Defoamer Industry Trade Association
- Toxicology Consultant for the Independent Lubricant Manufacturers Association

THE LUBRIZOL CORPORATION

MANAGER OF SPECIAL TOXICOLOGY AND REGULATORY PROJECTS (2001 to 2004)

- Toxicology and regulatory consultant for organic growth initiatives and new acquisitions.
- Coordinating inhalation toxicology program on engines emissions with a novel diesel fuel formulation for registration with EPA under the Clean Air Act.
- Coordinating world wide implementation of compliance with revised European hazard communication regulations
- Consultant to Lubrizol defoamer, coating, process chemical, metalworking and lubricant businesses on regulations and toxicology
- Team member studying and planning implementation of sustainable development at Lubrizol.

MANAGER OF TOXICOLOGY AND RISK ASSESSMENT)

- Provided leadership and management for corporate toxicologists and product safety specialists.
- Direct responsibility for toxicology testing and evaluation of all Company specialty chemicals and products.
- Manage annual toxicology and environmental testing budget for regulatory approvals and product stewardship.
- Lead consultant for business units on novel regulatory approvals, product stewardship and risk evaluation.
- Developed and institutionalized product risk assessment process for all Lubrizol businesses.
- Provide leadership role representing Company on trade association task groups involved in legislative and regulatory advocacy.
- Co-team leader for development and implementation of award -winning expert system for writing MSDSs from a product safety database.

BP AMERICA INC (formerly THE STANDARD OIL CO)
)

MANAGER OF PRODUCT SAFETY AND REGULATORY COMPLIANCE

- Assumed responsibility for assuring all Company products complied with federal regulations (TSCA, FIFRA, FDCA, USDA).
- Coordinated and expedited all regulatory submissions for premarket approval, reporting rules and rulemaking comment.
- Conscientiously developed Company Product Safety Policies and Manual.
- Critically evaluated Corporate Hazard Communication Program in a decentralizing company.
- Successfully initiated labeling program to comply with OSHA Hazard Communication Standard.

AMERICAN CYANAMID COMPANY, CHEMICALS GROUP

MANAGER OF TOXICOLOGY PROGRAMS

- Wide range of responsibility for recommending, contracting, monitoring and evaluating mammalian, genetic and aquatic toxicology studies for chemical products.
- Responsible for) total contract value for testing, quality assurance and consultants.
- Effectively guided regulatory staff in strategy and data requirements for premarket approvals.
- Successfully orchestrated targeted research programs for mechanistic studies on key chemicals for aquatic and mammalian toxicity.
- Actively represented Company in a wide spectrum of trade association activities.

FOOD AND DRUG ADMINISTRATION

GRAS Review Branch
Division of Food and Color Additives
SUPERVISORY CONSUMER SAFETY OFFICER (1981-1983)

- Successfully managed group of 3-4 professionals in regulatory program to implement expert panel reviews of GRAS list food ingredients.
- Projects of responsibility included salt, caffeine, BHA, BHT, cellulose, enzymes, rapeseed oil, vitamins, iron, manganese and zinc salts.
- Co-directed agency expertise on toxicology, chemistry, law and policy to propose regulatory action on food uses of DSS. Negotiated consistency with Bureau of Drugs proposal on OTC and Rx uses.
- Advised Branch Chief in matters of policy, consistency and personnel.
- Interacted with industry regarding regulatory opinions and new product approvals.

Petitions Control Branch
Division of Food and Color Additives
CONSUMER SAFETY OFFICER (1977-1981)

- Coordinated scientific review and regulatory response to review food additive petitions submitted by industry for direct additives and food packaging materials.
- Scientific and historical expert for General Counsel, U. S. Attorney and Department of Justice for legal proceedings on cyclamate.
- Expert on food/drug interface of vitamins and dietary supplements.
- Analyzed quality of critical studies on aspartame and served on GLP review committee
- Served as Bureau representative in Interagency Regulatory Liaison Group on phthalate plasticizers.
- Assistant to Bureau Director on advocacy activities on behalf of U.S. industry for WHO programs

PUBLICATIONS

Reed, MD, Blair LF, Burling K, Daly I, Gigliotti AP, Gudi R, Mercieca MD. McDonald JD, O'callaghan JP, Seilkop, SK, Ronsko NL, Wagner VO, Kraska RC Health effects of subchronic exposure to diesel-water-methanol emulsion emissions Toxicology & Industrial Health Vol 22 In Press.

Reed, MD, Blair LF, Burling K, Daly I, Gigliotti AP, Gudi R, Mercieca MD. McDonald JD, Naas DJ, O'callaghan JP, Seilkop, SK, Ronsko NL, Wagner VO, Kraska RC Health effects of subchronic exposure to diesel-water emulsion emissions. Inhal Toxicol 17: 851-70 (2005)

Kraska, RC , Industrial Chemicals. Regulation of new and existing chemicals. In: Gad S.C. editor. *Regulatory Toxicology.* Taylor and Francis Ltd. London 2001.

Kraska, RC . and Hooper DH, Industrial Chemicals. Hazard Communication, exposure limits, labeling and other workplace and transportation requirements under OSHA, DOT, and similar authorities around the world. In: Gad S.C. editor. *Regulatory Toxicology.* Taylor and Francis Ltd. London 2001.

Strother, DE, Mast RW, Kraska RC, Frankos V Acrylonitrile as a carcinogen. Research needs for better risk assessment. Ann NY Acad Sci 534:169-78 (1988)

Petersen DW, Kleinow KM, Kraska RC, Lech JJ Uptake, disposition and elimination of acrylamide in rainbow trout Toxicol Appl Pharmacol 80: 58-65 (1985)

Mast RW, Jeffcoat AR, Sadler BM, Kraska RC and Friedman MA Metabolism, disposition and excretion of [C14] melamine in male Fischer 344 rats. Food Chem Toxicol 21: 807-810 (1983)

SPEAKER

Talks given on following topics at national meetings, seminars and workshops
- GRAS Criteria
- REACH and GHS Regulations
- HPV Toxicology Testing
- Risk Assessment and Risk Management
- Lubricant Additive Safety
- Trade Association Environmental Activism
- Product Deselection Lists
- MSDS Expert Systems
- Confidential Business Information under TSCA
- TSCA Section 12(b) Compliance

TRAINING COURSES

Training courses given to business, research and legal groups at Lubrizol
- General Regulatory Overview
- TSCA New Chemicals
- FDA Food Additive Requirements
- Product Regulatory Law Course (TSCA, FDCA, OSHA)

Trainer, Toxicology Module, Metalworking Fluids Certificate Course (2005-2006)

TRADE ASSOCIATION ACTIVITIES

Chemical Reporting Task Group (1983-1998)
Chemical Manufacturers Association
Chairperson (1997-1998)
Safety, Health, Environmental and Regulatory Affairs Committee, Independent Lubricant Manufacturers Association (1997 to present)
- Vice chairperson (2001-2002)
- Chairperson (2003-2004)
- Toxicology consultant (2006)

Oversight Committee, Metalworking Fluid Product Stewardship Group, Independent Lubricant Manufacturers Association (1997-2004)
Health Environmental and Regulatory Task Group, Petroleum Additives Panel (1997-2002)
- Chairperson, Sensitization Work Group (1999 to 2002)

Biocides Panel, AEATF II Protocol Committee and Technical Committee (2003-2006) Team Leader for Metalworking Study (2005-2006)
Defoamer Industry Trade Association, Founder and Technical Consultant (2005-2006)

PROFESSIONAL SOCIETY MEMBERSHIPS

Society of Toxicology (SOT)
American Standards and Testing Methods (ASTM)
Society of Tribology and Lubrication Engineers (STLE)
Regulatory Affairs Professionals Society (RAPS)
Roundtable of Toxicology Consultants (RTC)

ROBERT S. MCQUATE, Ph.D.

Phone:
Emails:

WORK HISTORY

CEO, and Co-Founder, GRAS Associates, LLC, Bend, OR
President & CEO, R. S. McQuate & Associates, Inc., Bend, OR
Chemistry Instructor, Truckee Meadows Community College, Reno, NV
Senior Vice President, Scientific & Regulatory Affairs, AminoPath Labs, LLC, Portland, OR
Board Member & Business Consultant, National Institute of Standards & Technology, Advanced Technology Program, Gaithersburg, MD
Executive Director, Advanced Science & Technology Institute, Eugene & Corvallis, OR
Adjunct Professor, Food Science & Technology, Oregon State University, Corvallis, OR
Science Director, National Soft Drink Association, Washington, DC
Senior Regulatory Scientist and Group Leader of Regulatory and Nutrition, The Dial Company, Inc., Scottsdale, AZ
Consumer Safety Officer, Food and Drug Administration, Center for Food Safety & Applied Nutrition, Division of Food and Color Additives, Washington, DC
Assistant Professor of Chemistry, Willamette University, Salem, OR

EDUCATION

Postdoctoral Research Fellow with Professor R. G. Wilkins, New Mexico State University, Las Cruces, NM
Ph.D. in Chemistry, The Ohio State University, Columbus, OH
B.S. in Chemistry with Honors, Lebanon Valley College, Annville, PA

PROFESSIONAL EXPERIENCE

CONSULTING SERVICES

CEO, GRAS Associates, LLC; President & CEO, R. S. McQuate & Associates, Inc.

- Provide food ingredient safety evaluations, focusing on independent GRAS evaluations.
- Provide broad-based business consulting services to universities & companies involved in technology commercialization.
- Rapid assimilation of technical and business background for use in formulating commercialization strategies.
- Critically evaluate new technologies and business plans compared to competitive firms and products for economic potential.
- Implement marketing activities to establish strategic alliances and/or licensing agreements.
- Facilitate start-up ventures, including drafting of business plans.
- Utilize negotiation skills to achieve successful execution of deals.

UNIVERSITY EXPERIENCE

Executive Director, Advanced Science & Technology Institute

- Managed industry-university interface program on behalf of University of Oregon, Oregon State University, Oregon Health Sciences University and Portland State University.
- Strategic planning and program implementation; managed staff of up to 8.
- Facilitated linkages between university research community and private sector, working with over 500 faculty members to yield consulting contracts, industrial research sponsorship, technology licensing and business start-ups.
- Aggressively marketed faculty expertise, universities' technologies, and research capabilities through network of contacts, Internet, publications, and conferences.
- Represented universities in broad-based statewide and regional economic development initiatives.

Faculty, Willamette University, Oregon State University, & Truckee Meadows Community College

- Presented introductory and upper level chemistry lecture and laboratory courses.
- Conducted independent research in molecular biology, enzymology, and metal ion catalysis.
- Generated external grant funding to support six research students & acquire equipment.

- Provided food safety guidance to industry.
- Various scientific and chemical education publications.

PRIVATE SECTOR EXPERIENCE

Technical Management, The Dial Company & National Soft Drink Association

- Managed 5-person technical regulatory group with corporate responsibility for compliance with FDA, USDA, EPA, FTC, OSHA, CPSC, and NRC.
- Creatively interpreted regulations to favorably impact company revenues by over annually.
- Special focus on product and ingredient safety; formulated regulatory strategies in anticipation of and in response to agency positions; applied quantitative risk analysis to product safety considerations.
- Provided regulatory support and training to Manufacturing and QA on Good Manufacturing Practices requirements.
- Teamed with Marketing by evaluating advertising, product claims, and labeling for compliance.
- Assessed university research proposals in response to industry solicitations for funding.
- Served as liaison for industry interests on food ingredient safety before FDA officials.
- Served as industry spokesperson with media on technical topics such as NutraSweet addition to soft drinks.

GOVERNMENT EXPERIENCE

Staff, Food & Drug Administration

- FDA representative with regulated food industry officials.
- Managed safety evaluations of food and color additives and GRAS ingredients among FDA scientific divisions and with legal staff.
- Generated food safety notices, proposals, and regulations.
- Evaluated complex net weight food labeling and compliance issues and formulated agency position for Commissioner.
- Participated on special FDA Food Labeling Task Force to develop total food label requirements.
- Formulated recommended agency policy on iron bioavailability nutritional concerns.

PROFESSIONAL AFFILIATIONS

American Chemical Society
Institute of Food Technologists
Licensing Executives Society
Regulatory Affairs Professional Society

BOARD AND COMMITTEE MEMBERSHIPS

External Evaluator, Kansas Technology Enterprise Corporation, Higuchi Biosciences Center (2001)
Judge, Ohio State University Business Plan Competition (2001)
Board of Directors - Universal Pulping, Inc. (1996 - 2004)
Scientific Advisory Committee - Bainbridge Technology Group, Ltd. (1991 - 2000)
Board of Directors - Regional Council of Project SBIR West (1994 - 1996)
Board of Directors - Oregon Environmental Technology Association (1994 - 1995)
Co-Director – Oregon Governor's Task Force on Technology Transfer (1991 - 1992)
Board of Directors - LEAP, Inc. (1988 - 1994)
Board of Directors - Oregon Biosciences Association (1991 - 1993)
Board of Directors - BioForum (1988 - 1991)
Oregon Governor's Biotechnology Industry Advisory Council (1988)

HONORS AWARDS AND FELLOWSHIPS

Governor Barbara Roberts Certificate of Appreciation - Task Force on Technology Transfer (1993)
Governor Neil Goldschmidt Letter of Commendation - Biotechnology Industry Advisory Council (1988)
FDA Award of Merit from FDA Commission Jere Goyan (1980)
Letter of Commendation from FDA Commissioner Donald Kennedy (1979)
Seven Research Grants Awarded as Faculty Member at Willamette University (1974 - 1977)
National Science Foundation - Graduate Research Fellowship, The Ohio State University (1971 - 1973)
Graduated with Honors, Lebanon Valley College (1969)
Petroleum Research Fund - Undergraduate Research Fellowship, Lebanon Valley College (1967 and 1968)
Dean's List Student, Lebanon Valley College (1966 - 1969)
Salutatorian, South Lebanon High School, Lebanon, PA (1965)

JAMES R. COUGHLIN, Ph.D.
COUGHLIN & ASSOCIATES

Tel. 17 Fax

EDUCATION

9 Postdoctoral Toxicologist, Department of Environmental Toxicology, University of California, Davis
Ph.D., Agricultural and Environmental Chemistry, University of California, Davis
M.S., Food Science and Technology, University of California, Davis
B.S., Chemistry, Siena College, Loudonville, New York

AREAS OF EXPERTISE

Over 30 years experience in the area of food, chemical and environmental toxicology/safety. Particular emphasis on U.S. and international regulatory, nutritional and toxicologic evaluation of food/environmental chemicals, food and personal care products, coffee and meat safety, drinking water, functional foods/ingredients and food bioterrorism threats. Extensive assessment, communication, and management of risks of foods, chemicals, consumer products. Twenty years experience with California Proposition 65 issues management. Consultant to industry on strategic health, safety, and regulatory issues management, including crisis communications counseling.

OCCUPATIONAL EXPERIENCE

Coughlin & Associates (1992-present)

- President, Consultants in Food/Chemical/Environmental Toxicology & Safety, Laguna Niguel, California.

ENVIRON International Corp. (1991-1992)

- Principal, Counsel in Health and Environmental Science, Irvine, California.

Kraft General Foods Inc. (1981-1991)

- Director, International Scientific Relations, KGF International, Tarrytown, N.Y. (1990-91).
- Director, Product Safety & External Technical Affairs, KGF International and General Foods Worldwide, Rye Brook, N.Y. (1988-90).
- Manager, Toxicological Affairs, Office of Scientific Affairs, General Foods Corp., N.Y. (1981-87).

Armour & Co. (1979-1981)

- Manager, Food Safety & Regulatory Affairs, Armour Research Center, Scottsdale, Arizona.

University of California, Davis (1972-1979)

- Research Assistant, Department of Food Science and Technology. Developed methods to modify lactose in cheese whey to reduce environmental waste disposal burden (M.S. thesis). Performed chemical and toxicological studies on previously unreported, nonvolatile N-nitroso compounds formed from nitrite and Maillard compounds resulting from food nonenzymatic browning reactions (Ph.D. dissertation).

Albany Medical College (Summers, 1964-1968)

- Biochemical Research Technician, Department of Medicine.

HONORS

2003	FDA/CFSAN Director's Special Citation, IFT Bioterrorism Threat Assessment Group
2000	Industry Advancement Award for Sodium Nitrite Safety Defense, American Meat Institute.
1991	Medal of Honor, Association Scientifique Internationale du Café, Paris.

PROFESSIONAL SOCIETY MEMBERSHIPS AND ELECTED POSITIONS

- President, Association for Science and Information on Coffee, 1990-1992; re-elected Board Member for 2004-2008; Member since 1989, Paris.
- Vice President, International Society for Trace Element Research in Humans, 1998-2000.
- Member, Toxicology Forum, 1992-present, Washington, D.C.
- Member, Society for Risk Analysis, 1998-2005, McLean, Virginia.
- Member, Institute of Food Technologists, 1972-present, Chicago, Illinois.
  IFT Expert Panel on Food Bioterrorism, 2002-present (three Task Order contracts since 2002 on chemical and microbial threat assessment for the U.S. FDA's Center for Food Safety and Applied Nutrition).

Toxicology and Safety Evaluation Division, Chairman-Elect, 2006, Chairman, 1996-97 and 1986-87; Executive Committee, 1983-86; Member, 1981-present.
IFT Senior Food Officials Group, 2004-present.
Global Policy and Regulations Committee, member 2004-2005.
Codex Subject Expert on Food Additives and Contaminants, 2005-present.
Food Laws and Regulations Division, 1996-present.
Nutrition Division, 1997-present.
International Division, 1990-1995.
International Relations Committee, 1989-1992.
Southern California Section, 1992-present.

- Member, American Environmental Institute, Editorial Advisory Board, *Prop 65 News*, San Francisco.
- Member, Prop 65 Clearinghouse, Advisory Board, 2004-present.
- Advisor, 1982-89, and Member, 1990-91, Physiological Effects of Coffee Committee, Paris.
- Member, National Coffee Association of U.S.A., New York City.
  Chairman, Standards Regulations Committee, 1990-91.
  Chairman, 1985-88, and Member, 1981-91, Scientific Advisory Group.
  Public Relations Subcommittee, 1981-91.
- Member, National Association of Chewing Gum Manufacturers, Washington, D.C.
  Technical Committee, 1987-91.
  Vice Chairman, EC Additives Working Group, 1989-91.
- Member, International Life Sciences Institute/Nutrition Foundation, Washington, D.C.
  International Food Biotechnology Committee, 1998-present.
  Antioxidant Technical Committee, Chairman of Toxicology Task Force, 1984-91.
  Caffeine Technical Committee, 1982-91.
  Risk Assessment Subcommittee, 1982-86.
  Residue Subcommittee, 1984-87.
  Proposition 65 Committee, 1987-91.

SELECTED PUBLICATIONS (complete list available upon request)

Coughlin, J.R. 2003. "Acrylamide: What we have learned so far." Food Technology 57(2): 100.

Rainey, C.J., L.A. Nyquist, J.R. Coughlin, and R.G. Downing. 2002. "Dietary boron intake in the United States: CSFII 1994-96." J. Food Comp. Anal. J. Food Comp. Anal. 15: 237-250.

Coughlin, J.R., and F.H. Nielsen. 1999. "Advances in boron essentiality research: Symposium summary." In *New Aspects of Trace Element Research.* Eds., M. Abdulla, M. Bost, S. Gamon, P. Arnaud, and G. Chazot, Smith-Gordon, London, pp. 33-41.

Coughlin, J.R. 1999. "Advances in Boron Essentiality Research. " Guest Editor. *Proceedings of the Vth International Conference of the International Society for Trace Element Research in Humans,* Lyon, France, September 1997, J. Tr. Elem. Exp. Med. 12(3): 171-284.

Rainey, C.J., L.A. Nyquist, R.E. Christensen, P.L. Strong, B.D. Culver, and J.R. Coughlin. 1999. "Daily boron intake from the American diet." J. Am. Diet. Assn. 99(3): 335-340.

Coughlin, J.R. 1998. "Sources of human exposure: Overview of water supplies as sources of boron." Biol. Trace Elem. Res. 66: 87-100.

Coughlin, J.R. 1997. "Essentiality vs. toxicity of essential trace elements: A nutritional toxicologist looks at the Upper Safe Level. " In *Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals - TEMA-9*, National Research Council of Canada Press, 399-400.

Coughlin, J.R. 1997. "Role of risk assessment in the interpretation of studies." In *Proceedings of the Ceres Forum on Nitrite as a Food Additive: State of the Science; Chapter 8: Forum Summary and Industry Overview,* Georgetown University's Center for Food and Nutrition Policy, December 10-11, 103-111.

SYMPOSIA CHAIRMAN AND ORGANIZER

International Society for Trace Element Research in Humans.

- Vice President and Organizing Committee for Sixth International Congress, Quebec City, September, 2001.
- Fifth International Congress. *Advances in Boron Essentiality Research.* Lyon, France, September, 1998.
- Fourth International Congress. *Essentiality vs. Toxicity of Trace Elements: New Approaches to Risk Assessment—Boron as a Case Study.* Taormina, Sicily, September, 1995.

Trace Elements in Man and Animals (TEMA).

- 11th International Symposium on Trace Elements in Man and Animals (TEMA-11). Member of Local Organizing Committee and Organizer of session on *Toxicity and Risk Assessment of Trace Elements,* Berkeley, Calif., June, 2002.
- 10th International Symposium on Trace Elements in Man and Animals (TEMA-10). Organizer and Co-Chair of Symposium on *"Nutritional Essentiality of Boron."* Evian, France, May, 1999.

Joint Institute for Food Safety and Nutrition (JIFSAN / FDA).

- 2002 Acrylamide in Food Workshop - Scientific Issues, Uncertainties, and Research Strategies, October 28-30, Chicago, Ad hoc Planning Committee.
- 2004 Acrylamide in Food Workshop: Update - Scientific Issues, Uncertainties, and Research Strategies, April 13-15, Chicago, Risk Characterization Working Group and Ad hoc Planning Committee.

Toxicology Forum.

- *Risk Assessment of Nutrients.* Aspen, Colorado, July, 1999.
- *Risk Assessment of Trace Mineral Elements: Implications for Standard Setting.* Aspen, Colorado, July, 1995.
- *Issues in Reproductive and Developmental Risk Assessment: Boron Toxicity as a Test Case.* Washington, D.C., February, 1994.

Association Scientifique Internationale du Café (ASIC). President of ASIC (1989-1991); Chairman of the Organizing Committee, *14th International Conference on Coffee Science*, San Francisco, July, 1991; Member of the Board, 2004-2008.

Institute of Food Technologists.

- *Recent Advances in the Safety Assessment of Sodium Nitrite and Cured Meats.* Annual Meeting, New Orleans, June, 2001.
- *Establishing Upper Reference Levels for Nutrients in North America: Scientific Basis and Future Directions.* Eastern Food Science Conference X, IFT Regional Meeting, Newport, Rhode Island, November, 1997.
- *Risk Assessment in Establishing Upper Reference Levels of Nutrients.* Annual Meeting, Orlando, June, 1997; jointly sponsored by the NAS/IOM Food and Nutrition Board.
- *International Differences in Food Safety Standards and Effects on Health and Trade.* Annual Meeting, New Orleans, June, 1992.
- *Risk Communication: Problems and Future Needs with Food Safety Issues.* Annual Meeting, Chicago, June, 1989.
- *Pesticides: Scientific and Regulatory Issues.* Annual Meeting, Dallas, June, 1986.
- *Caffeine: Biological Effects.* Annual Meeting, June, 1983.

Southern California Institute of Food Technologists Section. *Symposium on Water Safety and Waste Water Management: A Look at Comparative Risks in Food Production and Processing,* Southern California Food Industry Conference on "Emerging Issues in Food Science, Nutrition and Technology 1998," California State Polytechnic University, Pomona, Calif., January, 1998.

Society for Risk Analysis. 10th Annual Symposium on Health Risk Assessment: Current Issues. *Issues Encountered in the Assessment of Health Risks of Essential Elements.* Monterey, Calif., October, 1997.

INVITED PRESENTATIONS (available upon request)

Robin C. Guy, M.S., DABT

FDA Experience

- Assisted with aspartame defense and re-affirmation while at the NutraSweet Company
- Participated in GRAS review of several ingredients
- Managed self-GRAS justifications for food ingredients, including defoaming agents
- Participated in the product development and preparation of food additive petitions for Neotame
- Interact with CDER on pharmaceutical products and impurities for clients
- Consult on GRAS status
- Conduct Good Laboratory Practice audits of laboratories for compliance with GLP regulations
- Manage preclinical packages for submission to the FDA
- Monitor nonclinical studies for FDA submission
- Prepared dozens of INDs and participated in writing some NDAs for FDA approvals

Employment History

- Toxicology Consultant, Robin Guy Consulting, LLC, Lake Forest, Illinois. August 2000 to present.
- Senior Toxicologist, Worldwide Regulatory Affairs, Nutrition and Consumer Products Sector, Monsanto (formerly The NutraSweet Company), Skokie, Illinois. January 1990 to October 2000.
- Research Investigator, Department of Toxicology, Product Safety & Metabolism, G. D. Searle & Co., Skokie, Illinois. August 1987 to January 1990.
- Senior Research Toxicologist (Research Associate), Product Safety Assessment, G. D. Searle & Co., Skokie, Illinois. August 1985 to August 1987.
- Research Toxicologist, Product Safety Assessment, G. D. Searle & Co., Skokie, Illinois. March 1982 to August 1985.
- Graduate Research Assistant, Department of Animal Science, University of Illinois, Urbana-Champaign 1979-1981.

Professional Experience

Robin Guy Consulting, LLC - Toxicology and Product Safety Assessment

- Provide clients in the pharmaceutical, food additive/ingredient and dietary supplement industries with toxicology and preclinical safety assessment guidance potentially leading to product commercialization. Perform safety/risk assessments on main products, excipients, degradents and metabolites. Consult with clients in the evaluation and interpretation of the safety of existing and potential products. Serve on GRAS Expert Panels.
- Effectively design, initiate, monitor and report GLP and range-finding preclinical studies to determine safety utilizing a reliable worldwide network of contract research laboratories. Outsourcing experience includes negotiation of pricing, study design and timelines. Utilize vast experience in monitoring preclinical animal studies including general (acute to repeated dose), carcinogenicity (with or without *in utero* phase), reproduction and teratology, and genetic toxicology (*in vitro* and *in vivo*) in addition to safety pharmacology. Coordinate and facilitate activities and communications between laboratories, vendors and clients. Synchronize events when more than one laboratory was utilized for a single project. In addition, highly successful in the management of multiple projects for multiple clients. Perform study and facility GLP audits at laboratories, in addition to qualification of data for Japanese submissions and due diligence evaluations. Provides GLP training to trainers and technical staff.
- Prepare toxicology summaries of potential products to be used as part of a clinical protocol submitted to an Institutional Review Board, for inclusion in Investigator's Brochures, and for food additive petitions, GRAS, IND and NDA submissions. Food industry projects included b-glucans, fat substitutes, artificial sweeteners, antifoaming and microbial control agents. IND Section 8s have been prepared for the relief of symptoms of various treatments including Alzheimer's disease, arthritis, cancer, diabetes, obesity, endometriosis, hormone replacement, incontinence, insomnia, neuropathy, irritable bowel syndrome, chronic functional vomiting and sepsis. Generation of other documents including SOPs, white papers and those submitted to management, investors and regulatory agencies. Participate in FDA meetings (CFSAN and CDER).

Monsanto - Food Industry Safety Assessment

- Provided a leadership role on Project Management Teams in evaluating and interpreting the safety of existing and potential products, made safety determinations, and formulated and presented the data to management of cross functional groups to facilitate critical decisions for the development and commercialization of potential products.

- Evaluated the safety and provided safety guidance for excipients, new food additives, dietary supplements, and GRAS affirmation petitions for US and ex-US approval. Successfully designed, initiated, monitored and reported preclinical studies to establish safety. Ran the toxicology program for stanols and worked on other projects including DHA, Neotame and other artificial sweeteners.
- Monitored general, reproductive and genetic toxicology (GLP and range-finding) studies conducted at contract laboratories worldwide for support leading to product regulatory approval. Animal studies ranged from genetic toxicology assays to single dose *in vivo* assays to reproduction/teratology and carcinogenicity studies. Studies monitored included most animal studies for Neotame, from its inception through submission to the US FDA. Study Monitor responsibilities included identifying contract laboratories, negotiating activities and timelines, assuring that protocols and contracts are in place, conducting quality control audits, assuring that studies are conducted in a highly scientific manner, coordination of internal reviews and release of reports.
- Prepared toxicology summaries of potential products to be used as part of a clinical protocol submitted to an Institutional Review Board and for inclusion in Investigator's Brochures.
- Additional responsibilities included: Participation in and preparation of management summaries for major presentations to regulatory agencies for approval of the Company's products. Research and preparation of numerous reviews and other scientific papers on a variety of toxicological issues that required evaluation. Presentation of study data to peers and upper management. Generation of miscellaneous documents including white papers and those submitted to regulatory agencies. Evaluate the safety of proposed changes in manufacturing processes for aspartame. Preparation of toxicology section for Material Safety Data Sheets (MSDSs) food ingredients and degradants. Preparation of manuscripts and abstracts for publication. Assist study monitors with bioanalytical and metabolism studies. Assist in budget preparation.

G.D. Searle & Co. - Pharmaceutical Safety Assessment

- Study Director of GLP and non-GLP Toxicology studies that consisted of repeated dose (up to 13 weeks), acute, and irritation studies in the dog, monkey, rat, and rabbit.
- Assisted Study Directors on longer-term studies. Responsible for the design and conduct of numerous toxicology studies including preparation of protocols, interpretation of results, and the publication/presentation of the report.
- Additional responsibilities included: Identify and research new methodology and procedures to improve the conduct of safety studies. Update Material Safety Data Sheets (MSDSs). Position included supervisory responsibilities for up to five Toxicologists. Ensured facility, equipment, personnel, and supply readiness for day-to-day study operations. Assist in budget and resource allocations.

Academic Employment - Nutritional Studies

- Performed nutritional and biological assays in the dog, chicken, rat, and pig. Formulated and mixed purified diets. Diets included deficient and toxic doses of amino acids.

Education

- Master of Science in Animal Science, University of Illinois, Urbana-Champaign, May, 1981; Major: Animal Science, Non-ruminant Nutrition.
- Bachelor of Science in Agriculture, University of Illinois, Urbana-Champaign, January, 1980; Major: Companion Animal Science.

Certification

- Diplomate, American Board of Toxicology (DABT); 1993
- Recertification, 1998, 2003.
- Laboratory Animal Technologist (LATG), American Association for Laboratory Animal
- Science; 1984

Memberships/Appointments

- Society of Toxicology, 1991 to present
- American College of Toxicology, 1987 to present
- Elected positions including: Long Range Planning Committee (2005-8), Membership Committee (2001-2004), Nominating Committee (2004-2005, 2000-2001), Secretary (1998-2000), Program Committee Member (1997-2000), Councilor (1994-1997), Animals in Research Committee Member (appointed, 1988-1992).
- Midwest Regional Chapter of the Society of Toxicology, 1982 to present

- Elected and appointed positions including: President (2006-2007), President-elect (2005-2006), Councilor (2002-2004, 1992-1994), Program Committee (1996-2004), Membership Committee Chairperson (2003-2004, 1993-1994), Secretary (1994-1996), Audit Committee Chairperson (1993-1994), Nominating Committee Chairperson (1992-1993), Nominating Committee Member (1990-1992), Audit Committee Member (1987-1988).

Roundtable of Toxicology Consultants

- Elected President-elect (2005-7), Secretary (2003-2005)
- Society of Toxicology Canada, 2000 to present
- Regulatory Affairs Professional Society, 2000 to present
- Institute of Food Technologists, 2003 to present
- Appointment to the Editorial Board of the International Journal of Toxicology, 2004-2007

Presentations/ Publications

- Good Laboratory Practice Regulations, A Primer. Robin Guy. Pre-Clinical Network of BC Chapter Meeting, QLT Inc., Vancouver, BC., October 20, 2005.
- Good Laboratory Practice Regulations, A Primer. Robin Guy. Regulatory Affairs Professional Society Chicago Chapter Meeting, January 11, 2005.
- The Regulatory Aspects of Food Contact Substances Used in Packaging. InDuStry, Inc (IDSPackaging.com) Web Conference. Food and Beverage Packaging Sector. January 19, 2004.
- INDs, Do You Have the Guts? Robin Guy. Regulatory Affairs Professional Society Chicago Chapter Meeting, November 20, 2003.
- Preclinical Testing Critical for Obtaining FDA Approval for Clinical Trials. Robin Guy. Regulatory Affairs Professional Society Chicago Chapter Meeting, May 13, 2003.
- The Genotoxicity of Benzamide. R.C. Guy, J.L. Allen, T.E. Lawlor, R.R. Young, and J.J. Hjelle. Society of Toxicology 30th Annual Meeting, February, 1991.
- Sources of Information on Animal Research Legislation and Animal Welfare. Robin C. Guy. American College of Toxicology 11th annual meeting. October 29 - October 31 1990. Orlando, FL. (symposia speaker).
- The Effect of Overnight Water Deprivation on Pharmacokinetic and Clinical Chemistry and Hematology Parameters in the Dog. Robin C. Guy. American College of Toxicology 10th annual meeting. October 30 - November 1, 1989. Williamsburg, VA.
- Two Week Oral Toxicity Study of Ibuprofen in the Dog. R.C. Guy, Z. Ruben, D.S. Siwik, and R. Leonard. American College of Toxicology 9th annual meeting. October 31 - November 2, 1988. Baltimore, MD.
- Evaluation of the Acute Intramuscular Irritation Potential of SC-34871, Morphine, and Haloperidol in the Male Rabbit. Gillian Haggerty, Robin Guy, and Stuart Levin. Society of Toxicology 27th Annual Meeting, February, 1988.
- Muscle Damage in the Rabbit Induced by an Enkephalin-type Peptide, SC-34871. Robin C. Guy, Christopher L. Durack, and James L. Allen. Society of Toxicology 26th Annual Meeting, February, 1987.
- The Electrocardiogram of the Unsedated versus Ketamine-sedated Chair Restrained Macaca fascicularis. Deborah A. Siwik, Robin C. Guy, and Christopher L. Durack. American Association for Laboratory Animal Science 1987 Annual Meeting, November, 1987.
- Guy, Robin (2006, in press). Methods for the Analysis of Gastrointestinal Function. In: Toxicology of the
- Gastrointestinal Tract, edited by Shayne C. Gad, Taylor and Francis, Philadelphia.
- Guy, Robin (2006, in press). Impurities and Degradants and their Safety Qualification. In: Preclinical Safety Handbook, edited by Shayne C. Gad, John Wiley & Sons, Inc., New York.
- Guy, Robin (2005). Ames. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). Aspartame. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). Delany Clause. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). Ginger Jake. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). Good Laboratory Practice Regulations. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). International Conference on Harmonisation. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.

- Guy, Robin (2005). Micronucleus Assay. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). Mouse Lymphoma Assay. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). Mutagenicity. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). Red Tide. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). Redbook. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). Saccharine. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). Sensitization. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (2005). Methylnitrosurea. In the Encyclopedia of Toxicology, Second Edition, edited by Philip Wexler, Elsevier, New York.
- Guy, Robin (1998). Hexachlorobutadiene. In the Encyclopedia of Toxicology, edited by Philip Wexler, Academic Press, New York, Volume 2, pages 81-82.
- Guy, Robin (1998). Hexachoraphene. In the Encyclopedia of Toxicology, edited by Philip Wexler, Academic Press, New York, Volume 2, pages 83-85.
- Guy, Robin (1998). Methoxychlor. In the Encyclopedia of Toxicology, edited by Philip Wexler, Academic Press, New York, Volume 2, pages 299-301.
- Guy, Robin (1998). Methylnitrosurea. In the Encyclopedia of Toxicology, edited by Philip Wexler, Academic Press, New York, Volume 2, pages 316-317.
- Guy, Robin (1998). Nitrites. In the Encyclopedia of Toxicology, edited by Philip Wexler, Academic Press, New York, Volume 2, pages 420-421.
- Guy, Robin (1998). Nitrobenzene. In the Encyclopedia of Toxicology, edited by Philip Wexler, Academic Press, New York, Volume 2, pages 421-423.
- Guy, Robin (1998). 2,4,5-T. In the Encyclopedia of Toxicology, edited by Philip Wexler, Academic Press, New York, Volume 3, pages 211-213.
- Guy, R.C, J.L. Allen, T.E. Lawlor, R.R. Young, and J.J. Hjelle (1991). The Genotoxicity of Benzamide. The Toxicologist 11(1):251 (meeting abstract).
- Guy, Robin C. and Curtis D. Port (1990). Oral Pyramiding Dose Study of Oxagrelate in Beagle Dogs. Acute Toxicity Data (Journal of the American College of Toxicology Part B) 1(1):52.
- Guy, Robin C. (1990). Oral Pyramiding Dose Study of SC-32561 in Beagle Dogs. Acute Toxicity Data (Journal of the American College of Toxicology Part B) 1(1):53.
- Guy, Robin C. (1990). Intravenous Toxicity of Propylene Glycol Ethanolic Solutions in Beagle Dogs. Acute Toxicity Data (Journal of the American College of Toxicology Part B) 1(1):53-54.
- Guy, Robin C. and Stuart Levin (1990). Oral Pyramiding Dose Study of SC-37111 in Beagle Dogs. Acute Toxicity Data (Journal of the American College of Toxicology Part B) 1(1):54.
- Guy, Robin C. and Curtis D. Port (1990). Acute Intravenous Toxicity of SC-38902B in Beagle Dogs. Acute Toxicity Data (Journal of the American College of Toxicology Part B) 1(1):55.
- Guy, Robin C. and Curtis D. Port (1990). Intravenous Pyramiding Dose Study of Actisomide in Beagle Dogs. Acute Toxicity Data (Journal of the American College of Toxicology Part B) 1(1):55-56.
- Robin C. Guy (1989). The Effect of Overnight Water Deprivation on Pharmacokinetic and Clinical Chemistry and Hematology Parameters in the Dog. Journal of the American College of
- Toxicology 8(8): 1213 (meeting abstract).
- R.C. Guy, Z. Ruben, D.S. Siwik, and R. Leonard (1988). Two Week Oral Toxicity Study of Ibuprofen in the Dog. Journal of the American College of Toxicology 7(7):1049 (meeting abstract).
- G. Haggerty, R. Guy, and S. Levin (1988). Evaluation of the Acute Intramuscular Irritation Potential of SC-34871, Morphine, and Haloperidol in the Male Rabbit. The Toxicologist 8(1):255 (meeting abstract).
- Robin C. Guy, Christopher L. Durack, and James L. Allen (1987). Muscle Damage in the Rabbit Induced by an Enkephalin-type Peptide, SC-34871. The Toxicologist 7(1):239 (meeting abstract).
- Protein-Amino Acid Evaluation of Steamed-Processed Feather Meal. Poultry Science. 1981. 60(8): 1865-1872

NAME Michael C. Falk	POSITION TITLE Executive Director, Life Sciences Research Office, Inc.
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Bates College, Lewiston, ME	B.S.		Chemistry
Cornell University, Ithaca, NY	Ph.D.		Biochemistry
Harvard Medical School Boston, MA			Biochemistry

RESEARCH AND PROFESSIONAL EXPERIENCE

Principal Investigator, Naval Medical Research Institute, Bethesda, MD
Division Head, Naval Medical Research Institute, Bethesda, MD
Director, Wound Repair Program, Naval Medical Research Institute, Bethesda, MD
Director, Biochemistry and Cell Biology, Naval Medical Research Institute, Bethesda, MD
Director, Septic Shock Research Program, Naval Medical Research Institute, Bethesda, MD
Acting Director, Resuscitative Medicine Program, Naval Medical Research Institute, Bethesda, MD

- present MCF Sciences Consultants, Rockville, MD
- present Executive Director, Life Sciences Research Office, Inc., Bethesda, MD

5 Outstanding Performance Awards
NMRI Most Significant Scientific Publication

PUBLICATIONS:

1. A. Brownawell, **Michael C. Falk**, K. Lenghaus, K. Lewis, P. Nixon, and C. St. Hilaire. **Evaluating the Scientific Evidence for Potential Reduced-Risk Tobacco Products**. Poster presentation at the Tobacco Carcinogenesis, Division of Chemical Toxicology, 230th American Chemical Society National Meeting, Washington, DC. (October 2005)
2. K.D. Lewis, A.M. Brownawell, **Michael C. Falk**, K. Lenghaus, P.M. Nixon, C. St. Hilaire. **Evaluating the Science Based Potential Reduced Exposure Products**. Abstract presented at the Frontiers in Aerosol Dosimetry Research. (October 2005).
3. C. Klein, D. Prough, B. Boucher, K. French, **Michael C. Falk**, **Novel Fluids for Resuscitation of Hemorrhagic Shock on the Battlefield**. Shock 23 (Suppl 3):27. 28th Annual Meeting, Shock Society, Marco Island, FL, June 4-8, 2005 (Abstr.)
4. C.J. Klein, **Michael C. Falk**, **Novel Fluids for Resuscitation of Hemorrhagic Shock on the Battlefield**. Xth International Symposium on Blood Substitutes, Providence, RI. (June 12-15, 2005) (Abstr.)
5. D.M. Byrd, A.M. Brownawell, **Michael C. Falk**, R. Feldman, K. Lewis, and P. Nixon. **Scientific Criteria to Test Ingredients Added to Cigarettes**. Poster presentation at the 44th Annual meeting of the Society of Toxicology. (March 6-10, 2005)
6. **Michael C. Falk,** Bruce M. Chassy, Susan K. Harlander, Thomas J. Hoban, IV, Martina N. McGloughlin, and Amin R. Akhlaghi. **Food Biotchnology: Benefits and Concerns**. J. Nutrition; (2002) 132, 1384-1390.

7. Michael C. Falk. **Model for a Third-Party Review of the Evidence Substantiating Food and Dietary Supplement Claims**. J. Nutrition; (2001) 131, 2219-2223.
8. Cindy Salkowski, Gregory Detore, Alice Franks, Michael C. Falk and Stefanie N. Vogel. **Pulmonary and Hepatic Gene Expression following Cecal Ligation and Puncture: Monophosphoryl Lipid A Prophylaxis Attenuates Sepsis-Induced Cytokine and Chemokine Expression and Neutrophil Infiltration**. Infection and Immunity; (1998) 6, 3569-3578.
9. Junkui Zhang, Timothy K. Morrison, Michael C. Falk, Yuan-Hsu Kang, and Che-Hung Lee. **Characterization of the Binding of Soluble CD14 to Human Endothelial Cells and Mechanism for CD14-dependent Cell Activation by LPS**. J. Endotoxin Research; (1996) 3, 307-315.
10. Michael C. Falk, Mark A. Fletcher, Taffy J. Williams, Wei-Mei Ching, Y. Wu and Timothy Morrison. **Aggregation of Serum Proteins with Lipopolysaccharide (LPS): Characterization of the Precipitable LPS-protein Complex**. J. Endotoxin Research; (1996) 3, 87-100.
11. Milton J. Axley, Robert G. Keefe, Michael C. Falk, and Andrea L. Harabin. **FMN Cofactor Dissociation from the Soluble Hydrogenase of Alcaligenes eutrophus H16**. Biofactors; (1996) 5, 87-92.
12. Yuan-Hsu Kang, Michael C Falk, Timothy Bentley, and Che-Hung Lee. **Distribution and Role of Lipopolysaccharide in the Pathogenesis of Acute Renal Proximal Tubule Injury**. Shock; (1995) 4, 441-449.
13. S. William Whitson, Marcia A. Whitson, Daniel Ed. Bowers, Jr., and Michael C. Falk. **Factors Influencing Synthesis and Mineralization of Bone Matrix: Fetal Bovine Cells Grown In Vitro**. J. Bone Mineral Research; (1992) 7, 727-741.
14. David P. Franklin, David C. Laux, Taffy J. Williams, Michael C. Falk and Paul S. Cohen. **Growth of Salmonella typhimurium SL5219 and Escherichia coli F-18 in Mouse Cecal Mucus: Role of Peptides and Iron**. FEMS Microbiology Ecology; (1990) 74, 229-239.
15. Lorrita P. Watson, Y. H. Kang, and Michael C. Falk. **Cytochemical Properties of Osteoblast Cell Membrane Domains**. J. Histochem. Cytochem; (1989) 37,1253-1246.
16. Mark S. Lamkin, Taffy J. Williams, and Michael C. Falk. **Excitation Energy Transfer Study of the Spatial Relationship between the Carbonyl and Metal Cofactors in Pig Plasma Amine Oxidase**. Arch. Biochem. Biophys; (1988) 261, 72-79.
17. Elliot Jacob, Linda C. Durham, Michael C. Falk, Taffy J. Williams, and Lawrence J. Wheat. **Antibody Response to Teichoic Acid and Peptidoglycan in Staphylococcus aureus Osteomyelitis**. J. Clin. Microbiology; (1987) 25, 122-127.
18. Taffy, J. Williams and Michael C. Falk. **Spatial Relationship between the Cofactors in the Active Site of Pig Plasma Amine Oxidase**. J. Biol. Chem.; (1986) 261, 15949-15954.
19. S. J. Schaberg, A. R. Liboff, and Michael C. Falk. **Wire Induced Osteogenesis in Marrow. J. Biomed**. Materials Res.; (1985) 19, 673-687.
20. Elliot Jacob, Douglas M. Arendt, Itzhak Brook, Linda C. Durham, Michael C. Falk, and Siegfried J. Schaberg. **Enzyme-Linked Immunosorbent Assay for Detection of Antibodies to Staphylococcus aureus Cell Walls in Experimental Osteomyelitis**. J. Clin. Microbiology; (1985) 22, 547-552.
21. Michael C. Falk, Annie J. Staton, and Taffy J. Williams. **The Heterogeneity of Pig Plasma Amine Oxidase: Molecular and Catalytic Properties of Chromatographically Isolated Forms**. Biochemistry; (1983) 22, 3746-3751.
22. Michael C. Falk. **Stoichiometry of Phenylhydrazine Inactivation of Plasma Amine Oxidase**. Biochemistry; (1983) 22, 3740-3745.
23. Michael C. Falk, J. L. Bethune, and Bert L. Vallee. **Formamide-induced Dissociation and Inactivation of Escherichia coli Alkaline Phosphatase. Metal Dependent Reassociation and Restoration of Activity from Isolated Subunits**. Biochemistry; (1982) 21, 1471-1478.

24. Jan Chlebowski, Susan Mabrey, and **Michael C. Falk**. **Calorimetry of Alkaline Phosphatase. Stability of the Monomer and Effect of Metal Ion and Phosphatase Binding on Dimer Stability**. J. Biol. Chem.; (1979) 254, 5745-5753.
25. William F. Bosron, Richard A. Anderson, **Michael C. Falk**, F. Scott Kennedy, and Bert L. Vallee. **Effect of Magnesium on the Properties of Zinc Alkaline Phosphatase**. Biochemistry; (1977) 16, 610-614.
26. Helmut Sigel, Christoph F. Naumann, Bernhard Prijs, Donald B. McCormick, and **Michael C. Falk**. **Ternary Complexes in Solution. 25. Influence of Alkyl Side Chains with Hydroxy or Thioether Groups on the Stability of Binary and Ternary Copper (II) - Dipeptide Complexes**. Inorganic Chemistry; (1977) 16, 790-796.
27. **Michael C. Falk**, Paul G. Johnson, and Donald B. McCormick. **Flavinyl Peptides Related to the Active Site of Mitochondrial Monoamine Oxidase. I. Chemical and Spectral Properties**. Biochemistry; (1976) 15, 639-645.
28. **Michael C. Falk** and Donald B. McCormick. **Synthetic Flavinyl Peptides Related to the Active Site of Mitochondrial Monoamine Oxidase. II. Fluorescence Properties**. Biochemistry; (1976) 15, 646-653.
29. Kiyoshi Shiga, Gordon Tollin, **Michael C. Falk**, and Donald B. McCormick. **Binding and Oxidation-Reduction of Monoamine Oxidase-Type 8a-(S-Peptidyl) Flavins with Azotobacter(Shethna) Flavodoxin**. Biochem. Biophys. Res. Commun; (1975) 66, 227-234.
30. Donald B. McCormick, **Michael C. Falk**, Frank Rizzuto, and Gordon Tollin. **Inter- and Intramolecular Effects of Tyrosyl Residues on Flavin Triplets and Radicals as Investigated by Flash Photolysis**. Photochemistry and Photobiology; (1975) 22, 175-181.

PROFESSIONAL AND OPEN MEETINGS

2006 Tech Council of Maryland – Bioalliance Meeting (spoke)
Washington, DC
2007 Williamsburg Conference (presented)
Williamsburg, VA
2007 TABEXPO (presented)
Paris, France
2008 GOED Webinar (presented)
Washington, DC
2008 ISSFAL (presented)
2008 TSRC (presented)

WALTER H. GLINSMANN, M.D.

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Internet:

EDUCATION:

B.A. - Columbia College, New York, NY
M.D. - Columbia College of Physicians and Surgeons, New York, NY
Internship and Residency in Medicine - New York Hospital (Cornell Medical School), New York, NY
Service and Training in Endocrinology and Metabolism at Walter Reed Army Medical Center, Department of Metabolism, Army Medical Center and Army Institute of Research, Washington, DC

MEDICAL LICENSE:

New York State Physician, No.

EMPLOYMENT HISTORY:

Principal activities are as President, Glinsmann, Inc.

As below, with change in academic affiliation to Fellow, Center for Food Safety and Nutrition Policy, re-located to Virginia Polytechnic Institute and State University (Virginia Tech), Alexandria, VA.
(Completed term as Scientific Advisor to Technical Committee, International Life Sciences Institute.)
Appointed as Fellow, American Society for Nutrition.

President, Glinsmann, Inc. Evaluations of safety and health effects of foods, the development of nutritional products, and food-related claims.
Fellow and Adjunct Professor, Georgetown Center for Food and Nutrition Policy, Medical College and Graduate School of Public Policy, Georgetown University, Washington, DC
Member, Expert Panel on Nutrition and Electrolytes, United States Pharmacopeial Convention, Inc.
Scientific Advisor to Technical Committee on Food Components for Health Promotion ("Functional Foods" or "Nutraceuticals"), International Life Sciences Institute, North America; to the International Food Information Council; and to U.S. and International Food Companies.

President, Glinsmann, Inc. Fellow and Adjunct Professor, Georgetown Center for Food and Nutrition Policy, Graduate School of Public Policy and Medical School, Georgetown University.
Advisor to the Committee on Nutrition and Electrolytes, United States Pharmacopeial Convention.
Scientific Advisor to the Technical Committee on Food Components for Health Promotion, International Life Sciences Institute, North America.
Expert Consultant, Office of the Deputy Director for Programs, Center for Food Safety and Applied Nutrition (CFSAN or the Center), U.S. Food and Drug Administration (FDA).

Expert in Nutrition, Office of Disease Prevention and Health Promotion, Office of the Assistant Secretary for Health, Department of Health and Human Services (DHHS). Provided management/technical support and coordination for nutrition and dietary guidance-related activities for DHHS among U.S. government agencies, e.g., USDA and National Institute of Health Components.

Visiting Fellow, Georgetown Center for Food and Nutrition Policy and Ceres Forum®, Georgetown Medical School.
Director, Nutrition Policy Staff, Office of Disease Prevention and Health Promotion, Office of the Assistant Secretary for Health, Public Health Service (PHS), DHHS. Provided leadership and coordination among DHHS agencies for Nutrition Policy Board functions; implementation of year 2000 nutrition objectives; development of dietary guidance with U.S. Department of Agriculture; and initiatives in food labeling, safety, and fortification: included program coordination for nutrition monitoring and related research; DHHS representative at international nutrition conferences and committees on interagency nutrition research initiatives; preparation of nutrition and health reports and briefing materials for Congress.

Associate Director for Clinical Nutrition, Division of Nutrition, CFSAN, FDA. Provided program direction for human clinical and population-based research on: food safety; special dietary use and medical food products; novel food ingredients; health impacts of dietary behaviors of select populations; nutrient imbalances that relate to human morbidity and mortality; and techniques for assessing and monitoring nutritional status and adverse reactions to foods, food additives, and contaminants. Chaired the Center's Health Hazard Evaluation Board and Task Force for developing Guidelines for Clinical Testing for Food Additives and served as Senior Center Medical Officer for developing Medical Foods Regulations. Represented Center or Agency interests and positions and provided guidance on human food safety evaluations to industry.

Chief, Clinical Nutrition Branch, Division of Nutrition, CFSAN, FDA. Developed and managed clinical nutrition activities, human food safety assessment programs, and supervised the revision of Nutrition Monitoring Programs. Special assignments included: FDA Research in Human Subjects Committee; CFSAN Health Hazards Evaluation Board; primary responsibility for Center programs dealing with Clinical Nutrition, Clinical Investigations, Foods for Special Dietary Use, and Health-and Injury-Related Surveillance; Chairperson, Task Forces on Medical Foods Regulation and Evaluation of Health Aspects of Sugars Contained in Carbohydrate

Sweeteners; and member Task Forces on Research Priorities in Biotechnology, Food Monitoring, Nutrition, and Toxicology.

Chief, Experimental Nutritional Branch and Director of Research, Division of Nutrition, CFSAN, FDA. Developed, managed, and conducted research based on CFSAN priorities; Special assignments included: FDA Research Involving Human Subjects Committee; Center/FDA Science Liaison; Center Health Hazards Evaluation Committee; and Task Forces on Nitrates-Nitrosamines, Research and Research Facilities Plans, Medical Foods Regulations, and the Coordinated Use of Databases to Estimate Exposures.

Chief, Section on Physiological Controls, Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development (NICHHD), National Institutes of Health (NIH), Bethesda, Maryland. Developed and managed research; Chief Intramural Research Contracts Officer (NICHHD); Institute Safety Officer; Member/Chair of Clinical Research Review and Grants and Contracts Review Committees.

Senior Research Investigator, Laboratory of Biomedical Sciences, NICHHD, NIH.

Guest Investigator, Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, NIH.

Medical Officer, Research Planning and Program Development, Growth and Development Program, NICHHD, NIH.

Assistant Chief, Department of Metabolism and Attending Physician in Medicine, Walter Reed Army Hospital, and Senior Investigator, Walter Reed Army Institute of Research, and Institute of Pathology, Washington, DC.

PROFESSIONAL MEMBERSHIPS

American Institute of Nutrition, renamed American Society for Nutritional Sciences (Membership Committee, 1986-1991, Fellow, 2002)
American Society for Clinical Nutrition (Membership Committee, 1986-1994: Chair, Publications Management Committee, American Journal of Clinical Nutrition, 1994-1996)
American Physiological Society
Institute of Food Technologists
American Association for the Advancement of Science
American Diabetes Association
New York Academy of Sciences

AWARDS, PUBLICATIONS & PRESENTATIONS ARE AVAILABLE UPON REQUEST

NAME	POSITION TITLE
Robert J. Nicolosi	Director - Center for Health & Disease Research Professor - Department of Clinical Laboratory and Nutritional Sciences
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
St. Anselm's College, New Hampshire	B.A.		Biology
University of New Hampshire, Durham, NH	M.S.		Zoology
University of New Hampshire, Durham, NH	Ph.D.		Zoology-Biochemistry

RESEARCH AND PROFESSIONAL EXPERIENCE:

Post Doctoral Research Fellow, Masonic Medical Research Laboratory, Utica, NY
Research Fellow in Nutrition, Harvard School of Public Health, Boston, MA
Lecturer, Northeastern University of Boston, MA
Research Associate in Nutrition, Harvard School of Public Health, Boston, MA
Lecturer in Nutrition, Harvard School of Public Health, Boston, M
Assistant Professor Comparative Pathology, Harvard Medical School, New England Regional Primate Research Center, Southborough, MA
Chairman, Division of Nutrition, Harvard Medical School, New England Regional Primate Research Center, Southborough, MA
Visiting Professor, Tufts University School of Nutrition, Medford, MA
Professor, Department of Clinical Sciences, University of Massachusetts-Lowell, Lowell, MA
Research Professor of Medicine, University of Massachusetts Medical School, Worcester, MA
Adjunct Professor, University of Massachusetts Medical School, Worcester, MA
Director, Center for Chronic Disease Control, University of Massachusetts-Lowell, Lowell, MA
Coordinator, Office of Collaborative Research, University of Massachusetts Lowell, Lowell, MA
Co-Director, Public Health Engineering & Policy Initiative, University of Massachusetts, Lowell, Lowell, MA
Special Assistant to the Provost, University of Massachusetts Lowell, Lowell, MA
Graduate Faculty, University of Massachusetts Amherst, Amherst, MA

Publications: (*Selected from 175 + Publications*)

1. **Nicolosi RJ**, Wilson TA, Handelman G, Foxall T, Keaney JF, Vita JA. **Decreased Aortic Early Atherosclerosis in Hypercholesterolemic Hamsters Fed Oil-Rich TriSun Oil Compared to Linoleic Acid-Rich Sunflower Oil**. J Nutr Biochem; (2002) 13:392-402.
2. Reece S, **Nicolosi RJ**, Holcroft C, Quattrocchi N, Faul MC. **Asthma in a University Setting: Severity, Impact and Quality of Asthma Care.** Nurse Practitioner (2002) 27: 35-42
3. Shea TB, Ekini FJ, Ortiz D, Dawn-Linsley M., Wilson T, **Nicolosi RJ**. **Efficacy of Vitamin E, Phosphatidyl Choline, and Pyruvate on Buffering Neuronal Degeneration and Oxidative Stress in Cultured Cortical Neurons and in Central Nervous Tissue of Apolipoprotein E-deficient mice.** Free Rad. Bio Med, (2003) 33: 276-282.
4. Shea TB, Rogers EJ, Ashline D, Ortiz D, Duarte N, Wilson TA, **Nicolosi RJ**. **Vitamin E Deficiency Does Not Induce Compensatory Antioxidant Increases in Central Nervous System Tissue of Apoliprotein E-deficient Mice.** J Alzheimer's Dis (2003) 5:9-14.

5. Shea TB, Ekinci FJ, Ortiz D, Wilson TA, Nicolosi RJ. **Efficacy of Vitamin E Phosphatidyl Choline and Pyruvate on ABETA Neurotoxicity in Culture.** J Nutr, Health Aging (2003) 7:252-255
6. Wilson TA, Idreis HM, Taylor R, Nicolosi RJ. **Whole Fatted Bran Reduces the Development of Early Aortic Atherosclerosis in Hypercholesterolemic Hamsters Compared with Wheat Bran.** Nutr. Res. (2002) 22: 1319-1332.
7. Wilson TA, Foxall T, Nicolosi RJ. **Doxazosin, a α-1 Antagonist, Prevents the further progression of the advanced atherosclerotic lesion in Hypercholesterolemic hamsters.** Metabolism (2003) 52:1240-1245.
8. Delaney B, Nicolosi RJ, Wilson TA, Carlson T, Frazer S, Zheng GH, Hess R, Ostergren K, Haworth J, Knudson N. **Beta-Glucan Fractions from Barley and Oats are Similarily Antiatherogenic in Hypercholesterolemic Syrian Golden Hamsters.** J Nutr. (2003) 133:468-495.
9. Yoganathan S, Nicolosi RJ, Wilson TA, Handelman G, Scollin P, Tao R, Binford P, Orthefor F. **Antagonism of Croton Oil Inflammation by Topical Emu Oil in CD-1 Mice**. Lipids (2003) 38:603 – 607.
10. Hancock AL, Nakuci E, Nicolosi RJ, Shea TB. **An Antioxidant Formulation That Induces Differentiation of Neuroblastoma in Culture.** Neuroscience Research Communications (2003) 33:73-76
11. Kritchevsky D, Tepper S, Wright S, Czarnecki SK, Wilson TA, Nicolosi RJ. **Cholesterol Vehicle in Experimental Atherosclerosis 24: Avocado Oil.** J Am. Coll. Nutr, (2003) 22: 52-55
12. Nicolosi RJ, Wilson TA, Romano CA, Kritchevsky D. **Dietary Cholesterol is Less Atherogenic Than Saturated Fat in Hamsters with Low Plasma NonHDL- Cholesterol, but More Atherogenic When Plasma NonHDL-Cholesterol is High.** Nutrition Research (2003) 23: 299-315.
13 Volicer BJ, Quattrocchi N, Nicolosi RJ. **Health and Weight Perceptions of Obese Students.** Nurse Practitioner (2003) 28: 13-14.
14. Hancock AL, Nakuci E, Nicolosi RJ, Shea T. **An Antitoxidant Formulation that Induces Differentiation of Neuroblastoma in Culture.** Neuroscience Res. Comm. (2003) 33: 73-76.
15. Yoganathan S, Nicolosi RJ, Wilson TA, Handelman RJ, Scollin P, Tao R, Binford P, Orthoefer F. **Anti-inflammatory Properties of Emu oil in CD-1 Mice**. Lipids (2003) 38: 603-607.
16. Shea TB, Rogers E, Ashline D, Ortiz D, Durante N, Wilson TA, Nicolosi RJ, Sheu MS. **Vitamin E Deficiency Does Not Induce Compenssatory Antioxidant Increases in Central Nervous System Tissue of Apolipoprotein E-Deficiency Mice**. J Alzheimers Dis (2003) 5: 9-14.
17. Nicolosi RJ, Wilson T.A., and Delaney B. **Anti-Atherogenic Properties of Beta-Glucans from Barley and Oats in Hypercholesterolemic Syrian Golden Hamsters**. *FASEB J.* (2003) Abstract 204.10
18. Wilson TA, Nicolosi RJ, Handelmann G, Kotyla T, Orthoefer F, Binford P. **Comparative Effects of EMU and Olive Oil on Early Atherosclerosis and Associated Risk Factors in Hypercholesterolemic Hamsters**. Nutrition Research. (2004) 24: 395-406
19. Binkoski A.E., Kris-Etherton P.M., Nicolosi RJ, Wilson T.A. and Blough M.L. **Lipid-Lowering Effects of a Mid-Oleic Sunflower Oil in Moderately Hypercholesterolemic Men and Women.** *(J.ADA. In Press 2004).*
20. Alexaki A, Wilson TA, Alallah MT, Handelman G, Nicolosi RJ. **Hamsters Fed Diets High in Saturated Fat have Increased Cholesterol Accumulation and Cytokine Production in the Aortic Arch Compared with Cholesterol-Fed Hamsters with Moderately Elevated Plasma Non-HDL Cholesterol Concentrations.** J Nutr. (2004) 134 (2): 410-415
21. Ockene IS, Chiriboga DE, Stanek EJ, Harmatz MG, Nicolosi RJ, Sapiera G, Well AD, Freedson P, Merriam PA, Reed G, Ma Y, Matthews CE, Hebert JR. **Seasonal Variations in Serum Cholesterol: Teatment Implications and Possible Mechanisms.** Arch of Internal Medicine (2004) 164:871-879

22. Nicolosi RJ, Woolfrey B, Wilson TA, Scollin P, Handelman P, Fisher R. **Decreased Aortic Early Atherosclerosis and Associated Risk Factors in Hypercholesterolemic Hamsters Fed a Diet High or Mid-oleic Acid Oil Compared to High-Linoleic Acid Oil.** J. Nutr. Biochem (2004) 15:540-547
23. Wilson TA, Nicolosi RJ, Handelmann G, Kotyla T, Orthoefer F, Binford P. **Comparative Effects of EMU and Olive Oil on Early Atherosclerosis and Associated Risk Factors in Hypercholesterolemic Hamsters.** Nutrition Research (2004) 24: 395-406
24. Wilson TA, Nicolosi RJ, Delaney B, Chadwell K, Moolchandani V, Kotyla T, Ponduru S, Zheng G-H, Hess R, Knutson N, Curry L, Kolberg L, Goulson M and Ostergren K. **Comparative Effects of Reduced and High Molecular Weight Barley β-Glucans on Early Atherosclerosis Risk Factors and Aortic Cholesterol Ester Accumulation in Hypercholesterolemic Syrian Golden Hamsters.** Journal of Nutrition. (Oct. 2004) 134: 2617-2622
25. Kumar R, Chen M, Parmar VS, Samuelson L, Kumar J, Nicolosi RJ, Yoganathan S, Watterson AC. **Supramolecular Assemblies Based on Copolymers of PEG600 and Functionalized Aromatic Diesters for Drug Delivery Applications.** J. Am. Chem. Soc. (2004) 126:10640-10644
26. Kritchevisky D, Tepper SA, Wright S, Czarnecki SK, Wilson T, Nicolosi RJ. **Conjugated Linoleic Acid (CLA) Isomer Effects in Atherosclerosis: Growth and Regression of Lesions.** Lipids July (2004) 39: 311-611
27. Goodrow EF, Wilson TA, Crocker Houde S, Vishwanathan R, Scollin P, Handelman G, **Nicolosi R. Consumption of One Egg per Day Increases Serum Lutein and Zeaxanthin Concentrations in Older Adults without Altering Serum Lipid and Lipoprotein Cholesterol Concentrations.** J Nutr (2006) 136:2519-2526.
28. Wenzel AJ, Gerweck C, Barbato D, **Nicolosi RJ,** Handelman G, Curran-Celentano J. **A 12-WK Egg Intervention Increases Serum Zeaxanthin and Macular Pigment Optical Density in Women.** J Nutr (2006) 136: 2568-2573.
29 Fongshu K, Kotyla T, Wilson T, Kifle L, Ortiz D, Panagiotou T, Gruverman I, Tagne, JB, Shea T, **Nicolosi R. A Nanoemulsion Encapsulated Anti-Oxidant Synergy Formulation Reduces Neuroblastoma-Induced Tumors in Mice**. J. Exp. Theraputics & Oncology (2007) 6,2:129-135.
30. Wilson T, **Nicolosi R**, Woolfrey B, Kritchevsky D. **Rice Bran Oil and Oryzanol Reduce Plasma Lipid and Lipoprotein Cholesterol**. J Nutr Bio (2007)18,105-112.
31. Wilson T, Orthoefer F, Nicolosi RJ. **Soy Protein Concentrate Lowers Both Serum Total Cholesterol and HDL-Cholesterol Concentrations Compared To Casein in Post-Menopausal-Induced Ovariectomized Rats**. Nutr. Res (In Press 2008).
32. Wilson TA, **Nicolosi RJ**, Kotyla T, Fleckingher B. **Soy Protein Without Isoflavins Reduces Aortic Total and Cholesterol Ester Concentrations Greater Than Soy Protein with Isoflavones Compare to Casein in Hypercholerolemic Hamsters.** Nutr. Res. (In Press 2008)
33. Fongshu K, Kotyla T, Wilson T, Kifle L, Ortiz D, Panagiotou T, Gruverman I, Tagne, JB, Shea T, **Nicolosi RJ. A Nanoemulsion Encapsulated Anti-Oxidant Synergy Formulation Reduces Neuroblastoma-Induced Tumors in Mice**. J. Exp. Theraputics & Oncology (2007) 6, 2 :129-135.
33. Kotyla T, Kuo F, Moolchandani V, Wilson T, **Nicolosi RJ. Increased Bioavailability of a Transdermal Application of a Nanosized Emulsion Preparation.** International J of Pharm (2008) 347, 144-148.
34. Subramanian B, Kuo F, Ada E, Wilson T, Kotyla T, **Nicolosi RJ. A Nanoemulsion Preparation of Aspirin Enhances its Anti-inflammatory Properties in Mice.** Pharm Research (In press, 2008).
35. Tagne JB, Kakumanu S, Ortiz D, Shea T, **Nicolosi RJ. A Nanoemulsion Formulation of Tamoxifen Increases Its Efficacy as it Relates to breast Cancer Treatment: In Vitro and In Vivi Experiments in a Xenograft Mouse Model.** Molecular Pharmaceutics (In Press 2008).
36. Fongshu K, Karthick B, Kotyla T, Wilson T, **Nicolosi R. A Nanoemulsion of Antioxidant Formulation Enhances Its Effect on CD-1 Inflammatory mice Model.** International J of Pharm (In press 2008).

SUBMISSION END