



SAFETY OF REB A SCIENTIFIC SUMMARY

Rebaudioside A (Reb A) is an all natural zero calorie sweetener extracted from the *Stevia rebaudiana* (Bertoni) plant, which belongs to the chrysanthemum family and is native to Brazil and Paraguay. Reb A is the second most abundant glycoside in stevia and is structurally similar to other steviol glycosides, including stevioside. Two hundred times sweeter than sugar, Reb A is suitable for use to sweeten foods and beverages because of its solubility in water and its excellent taste profile.

An extensive and widely known safety database exists for Reb A, steviol glycosides and steviol which supports the safety of Reb A. The data cover the standard range of safety studies for determination of safety of food ingredients including acute, subchronic and chronic toxicity, genotoxicity, reproductive/developmental toxicity, and carcinogenicity, as well as absorption, distribution, metabolism and excretion (ADME). In addition, there have been a number of special studies in animals and humans that address issues related to the safety of steviol glycosides including potential effects on blood pressure and blood glucose control.

Acceptable Daily Intake (ADI)

An internationally recognized panel of experts (GRAS Panel) reviewed the safety and confirmed the safety of stevia for use in tabletop sweeteners, beverages, cereals and cereal bars in April, 2008 and concluded that the acceptable daily intake (ADI) was between 12-30 mg/kg body weight (bw)/day. (GRAS Panel, 2008)

Steviol glycosides, including Reb A have also been reviewed by other authoritative bodies, including the World Health Organization’s Joint Expert Committee on Food Additives (JECFA, 2008) and the Food Standards Australia and New Zealand (FSANZ, 2007). The US FDA is reviewed the Whole Earth Sweetener Company’s Reb A GRAS determination and issued a letter of no objection in December 2008. In 2008, JECFA established an ADI for steviol glycosides of 0-4 mg/kg bw/day. This ADI is expressed as steviol equivalents so that it can be applied to all the different steviosides by adjusting for their molecular weight. The corresponding ADI for Reb A (adjusting for the difference in molecular weight between steviol and Reb A) is 12 mg/kg bw/day. In 2007 FSANZ published an ADI for steviol glycosides of 4 mg/kg bw/day (expressed as steviol). Adjusting for molecular weight, the equivalent Reb A ADI is 12 mg/kg bw/day. Upon reviewing current data, the Reb A GRAS Panel concluded that the ADI is higher than 12 mg/kg. However, to assure international consistency, the Whole Earth Company is accepting the lower ADI of 12 mg/kg for its assessments of the safety of Reb A for use in its products.

Basis for ADI and Estimated Daily Intake

Three different groups have reviewed the safety data for Reb A and other steviol glycosides and determined an ADI. All three groups concluded that the ADI for Reb A was at least 12 mg/kg bw/day: This is equivalent to 30 packets of PureVia™ sweetener a day for a 150-pound human.

Basis of ADI	Expressed as Steviol (mg/kg bw/day)	Expressed as Reb A (mg/kg bw/day)
JECFA ADI	4	12
FSANZ	4	12
GRAS Expert Panel	7	30
Conclusion for Reb A	4-7	12

The estimated daily intake (EDI) of Reb A from the proposed uses including tabletop sweeteners, sweetened ready-to-drink iced teas, diet carbonated soft drinks, fruit juice drinks, energy drinks, flavored waters, cereal bars, oatmeal, and cold cereals (sweetened), was estimated using the maximum proposed use rates and data from the most recent National Health and Nutrition Examination Survey (2003-2004). The EDI on a per user basis is approximately 2 mg/kg bw/day at the mean and 5 mg/kg bw/day for consumers with large intakes of these foods (the 90th percentile consumer). The assumptions that were used in estimating consumer intakes from all proposed uses are conservative and, therefore, the estimates most certainly overestimate potential consumer intakes of Reb A. Even with these conservative assumptions, all calculated EDIs for the food categories of interest are well below the ADI (12 mg/kg bw/day).

Summary of Safety Database for Reb A

The absorption, distribution, metabolism and excretion (ADME) of Reb A and other steviol glycosides have been extensively studied and are key to understanding the relevance of the available toxicology data. Therefore, the ADME is discussed first; followed by summaries of the toxicology data related to safety evaluation. The studies have been grouped by period of exposure and/or type of study.

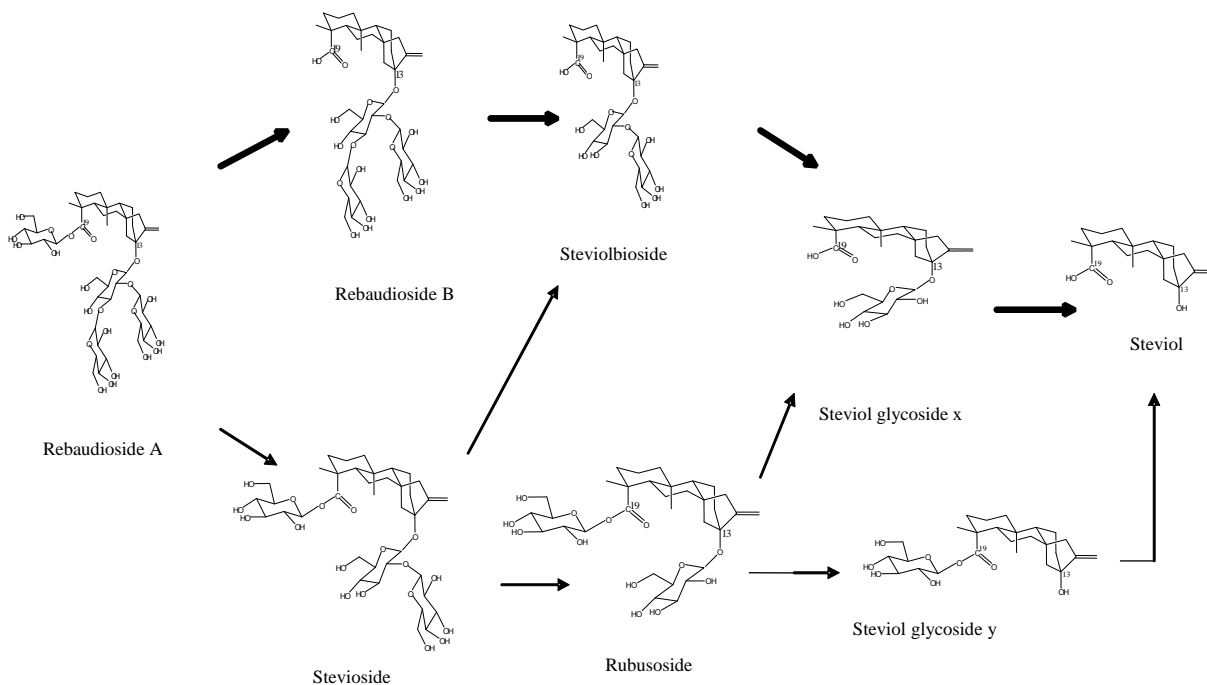
Absorption, Distribution, Metabolism and Excretion (ADME)

All steviol glycosides are metabolized via the same intermediates and same hydrolysis pathways; therefore, the safety data for all steviol glycosides are relevant regardless of which test substance, Reb A or steviol, was administered to test animals (Roberts and Renwick, 2008).

No absorption or structural modification of steviol glycosides in the stomach was found in steviol glycoside-dosed test animals or human volunteers. The principal steviol glycosides, Reb A and steviol glycoside are metabolized in experimental animals and humans by intestinal bacteria. The bacteria remove glucose molecules from the compound to create steviol (Renwick and Tarka 2008). Human fecal microflora was found to completely hydrolyze steviol glycoside and Reb A to steviol, in 10 and 24 hours, respectively. Based on the results

of all of the metabolism studies, ingested steviol glycosides are eliminated in the feces or hydrolyzed to steviol prior to absorption from the gut. Because of the common hydrolysis pathways (see figure below) of steviol glycosides and steviol, any study in which the test material was a steviol glycoside is relevant in evaluating safety of Reb A. However, some studies have been conducted on crude (and uncharacterized) extracts. Studies using such extracts may have observed effects that were due to other non-glycosidic substances that were present in the crude extract. The studies in which the test material was characterized as a high purity steviol glycoside are the most reliable (and relevant).

Common Hydrolysis Pathways of Reb A to the Aglycone, Steviol



Acute Toxicity (Single Dose)

Reb A and stevioside (purity >96%) were not acutely toxic to mice, rats, or hamsters at doses as high as 15,000 mg/kg bw. Similarly, steviol is not acutely toxic to rats and mice at doses as high as 14,000 mg/kg bw.

Subchronic Toxicity

Curry and Roberts (2008) administered Reb A (97% purity) via the diet to male and female rats for either four or 13 weeks. The authors concluded that there were no results that were of toxicological relevance. Reductions in body weight were observed in many of the groups which were attributable to the reduced caloric intake in the treated diet (Curry and Roberts, 2008). In another study, administration of Reb A via the diet to rats (Nikiforov and Eapen, 2008) for 90 days and, at doses as high as 2,000 mg/kg bw/day resulted in no adverse systemic toxicity. Lower body weight gains were reported only in the high-dose (2,000 mg/kg bw/day) group males. The study authors concluded that the lower body weights were due to the large proportion of the diet represented by the non-nutritive test material. Similar results have been reported with other intense sweeteners administered at high dietary concentrations and in that research it was concluded that body weight gain decreases are not an appropriate basis for a No-Observed-Adverse-Effect-Level (NOAEL) for intense sweeteners (Flamm *et al*, 2003); therefore, the NOAEL for the subchronic Reb A study was $\geq 2,000$ mg/kg bw/day.

Chronic Toxicity

Two chronic toxicity/carcinogenicity studies have been conducted with stevioside. Both studies were two-year studies that examined the effect of oral stevioside in rats. In the first study, stevioside administration in the diet showed no carcinogenic effects in the rat, and a NOAEL of 1.2% (600 mg/kg bw/day) in the diet was reported (Xili *et al.*, 1992). In a more recent study, stevioside administered in the diet was not carcinogenic in rats, and a NOAEL of 2.5% (970 mg/kg bw/day in males) was established (Toyoda *et al.*, 1997).

Genotoxicity

Steviol glycosides and the hydrolysis product, steviol, have been tested for potential mutagenicity using a broad range of *in vitro* and *in vivo* protocols. Brusick *et al* (2008) critically reviewed the literature and concluded:

- Steviol glycosides rebaudioside A and stevioside are not genotoxic *in vitro*.
- Steviol glycosides rebaudioside 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* appeared to be flawed 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 from very specific bacteria tests or purified plasmid DNA that lack DNA repair capabilities.
- Stevioside is not a carcinogen or cancer promoter in well-conducted rodent bioassays.

Reproductive/Developmental Toxicity

Eleven studies have been reported in the literature in which reproductive/developmental toxicity was evaluated. Studies have been conducted using Reb A, stevioside and steviol as the test material in hamsters and rats at doses up to 3,000 mg/kg bw/day. No treatment-related reproductive or developmental effects have been reported. The most recent study is summarized below.

In a two-generation dietary reproductive/developmental study, Reb A was administered via the diet to male and female rats for two generations (starting 10 weeks prior to mating for F0 group). No treatment-related effects of Reb A were observed in either the F0 or F1 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 F1 and F2 offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation, were not adversely affected by Reb A treatment. Food consumption was increased in the high dose groups in the F0 and F1 generation, as well as during lactation, but body weights of males and females in the high dose F1 and F2 groups were reduced. The authors attribute this to reduced caloric density of the diet and concomitant initial poor palatability of the diet (Curry *et al*, 2008).

Human Studies

Reb A was administered in a randomized placebo-controlled double-blind study in 100 healthy men and women with normal blood pressure at doses of four 250 mg capsules Reb A per day. Systolic and diastolic blood pressure and mean arterial pressure were measured. Systolic and diastolic blood pressure, mean arterial pressure and heart rate

in supine and standing positions pre- and post-meal were also measured. No significant effect on most blood pressure values measured was seen. Small (< 3 mm Hg), but statistically significant changes in diastolic blood pressure and mean arterial pressure between weeks 0 and 4 were noted in the supine and standing pre- and post-meal tests, but were not considered by the authors to be clinically meaningful from a safety perspective (Maki *et al*, 2008).

A second randomized, double-blind, placebo-controlled, parallel-design study administered 250 mg stevioside three times/day for three months stevioside to type 2 diabetics, type 1 diabetics and non-diabetics with normal to low normal blood pressure. No significant effects on either blood pressure or blood glucose in normal and diabetic individuals were reported (Barriocanal, *et al*, 2008).

Two older studies reported decreases in blood pressure in hypertensive individuals who were not on antihypertensive medication following intake of stevioside (Chan *et al*, 2000 and Hsieh *et al*, 2003). The Reb A Expert Panel concluded that a decrease in blood pressure was not clinically significant, though this effect would not be adverse either.

In summary, the human study results confirm that there are unlikely to be treatment-related effects of Reb A on blood glucose or blood pressure at doses that could result from its use as a sweetener in normal or diabetic individuals.