Use of *In vitro* Metabolism Assays to Substantiate the Safety of Novel Steviol Glycosides

Alex Eapen, PhD, DABT

Principal Scientist

Scientific & Regulatory Affairs Cargill

www.cargill.com

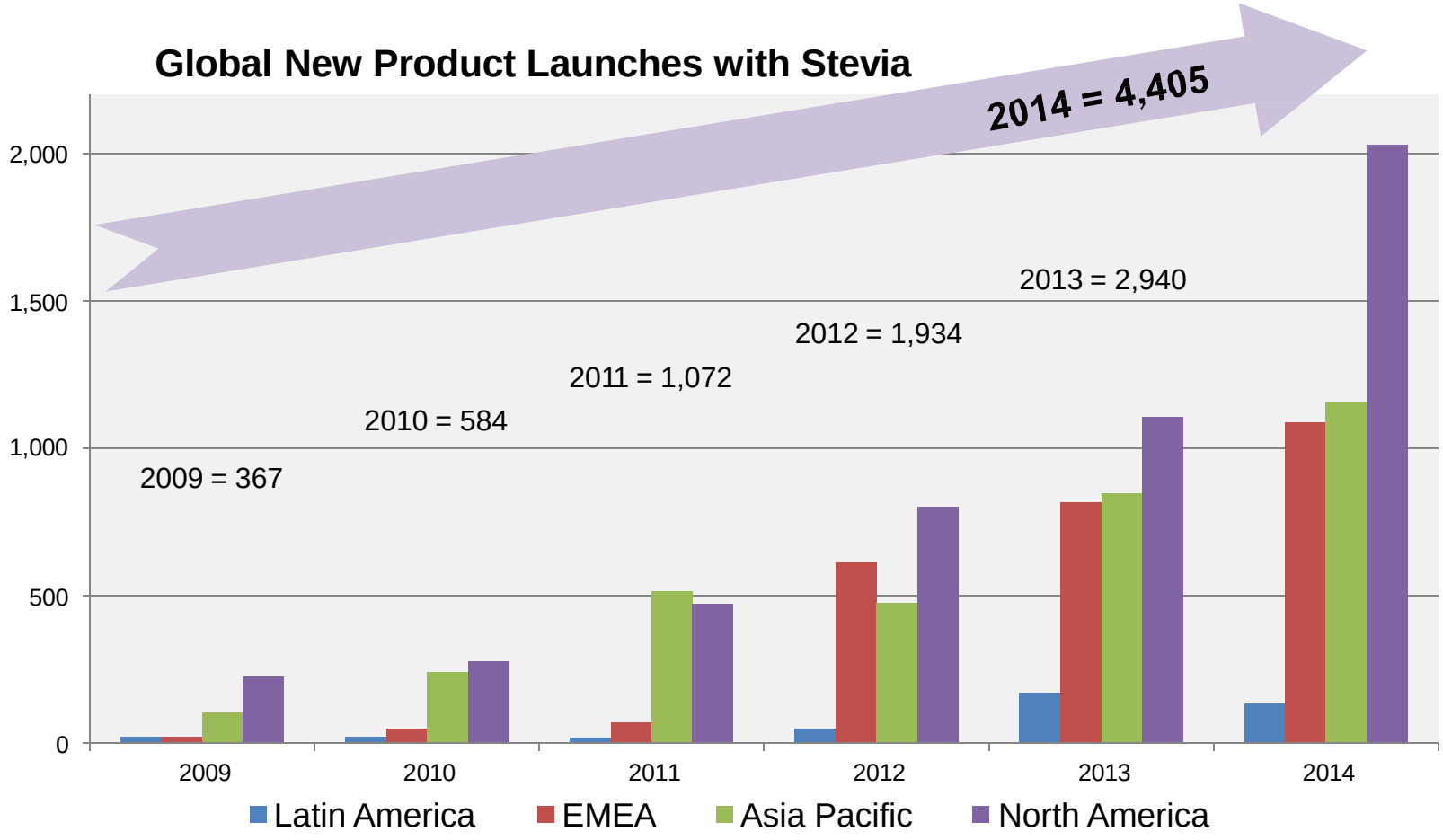
Overview

- **Introduction to Stevia**
- **Safety Studies Conducted – Steviol Glycosides**
- **Common Metabolic Pathway**
- ***In Vitro* Metabolism Assays**

Stevia: unique plant, unique story

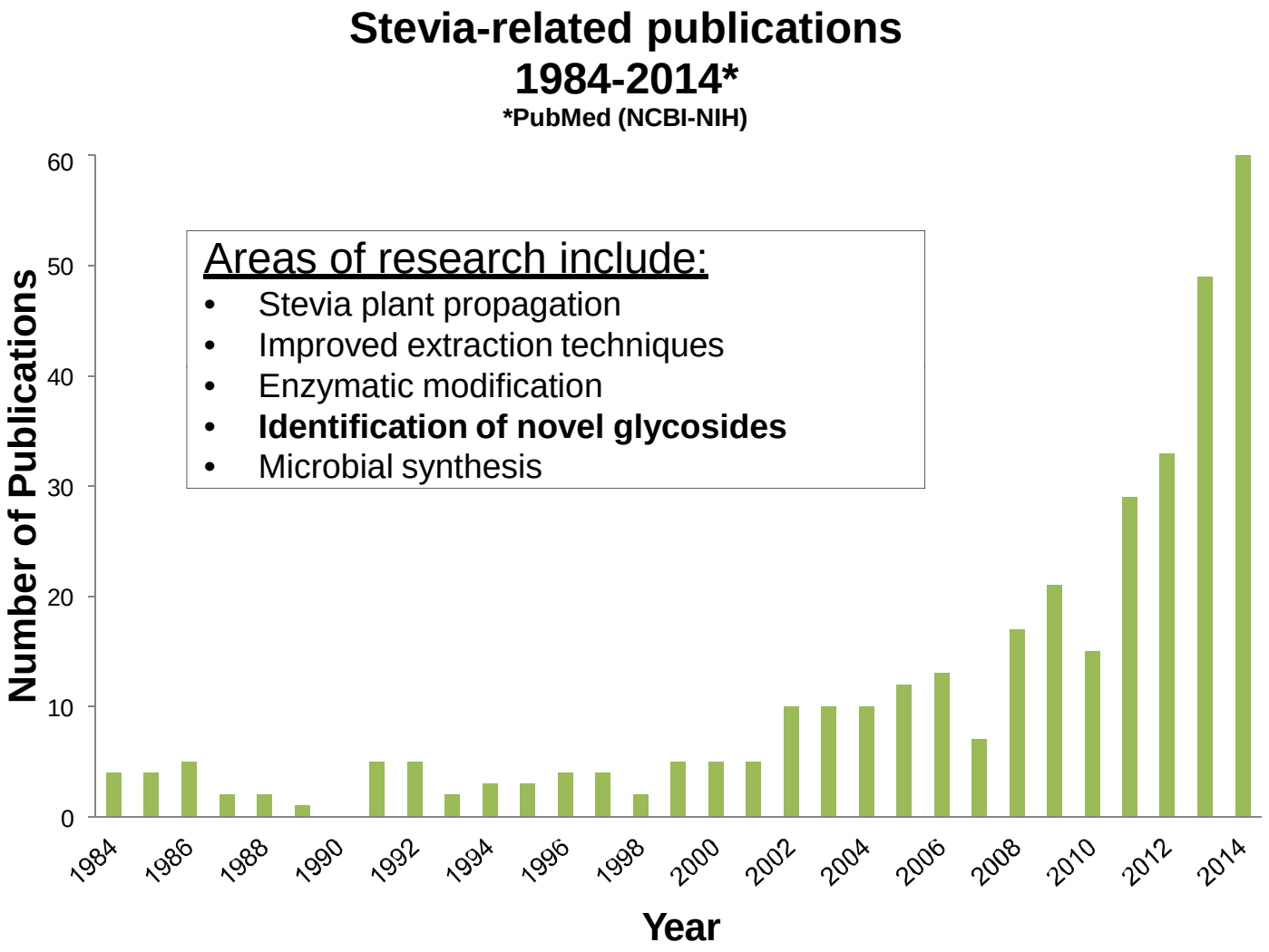
- Shrub in the *Asteraceae* family native to northeastern Paraguay
- First discovered by indigenous people who used plant's leaves to sweeten drinks
- By 1800s, stevia consumption established throughout South America, including Brazil and Argentina
- Food researchers worldwide have been working with stevia for decades
  - In 1931, French food chemists isolated compounds (steviol glycosides) that give stevia its sweet taste
  - Japan has been using stevia commercially for over three decades
  - Stevia-sweetened food and beverages are now available to consumers on every continent
- US market opened in 2008

Stevia Product Launches Continue to Grow 2009-2014



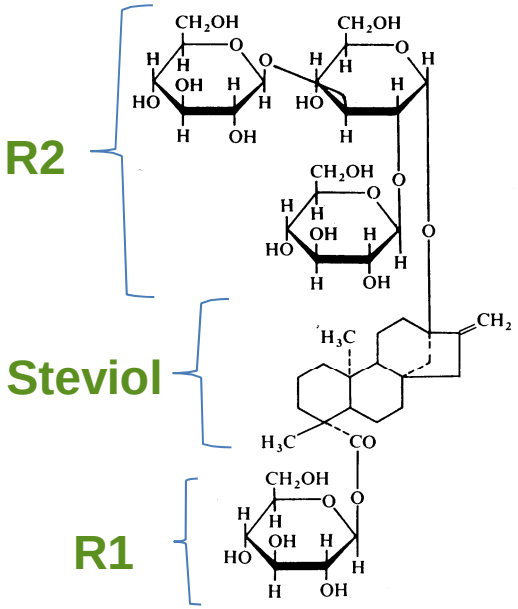
Source Innova Database

Growth in Stevia-related R&D



Sweet Components of the Leaf

- Family of sweet molecules called steviol glycosides
- Over 40 glycosides of commercial interest identified in the leaf
- Joint Expert Committee on Food Additives (JECFA-2010) – 9 primary



Name	Abbreviation	R-1	R-2
Dulcoside A	Dlc	βglc-	αrha-βglc-
Rebaudioside A	Reb A	βglc-	(βglc)2-βglc-
Rebaudioside B	Reb B	H-	(βglc)2-βglc-
Rebaudioside C	Reb C	βglc-	(βglc, βrha)-βglc-
Rebaudioside D	Reb D	βglc-βglc-	(βglc)2-βglc-
Rebaudioside F	Reb F	βglc-	(βglc, βxyl)-βglc-
Rubusoside	Rub	βglc-	βglc-
Stevioside	Stv	βglc-	βglc-βglc-
Steviolbioside	Stb	H-	βglc-βglc-

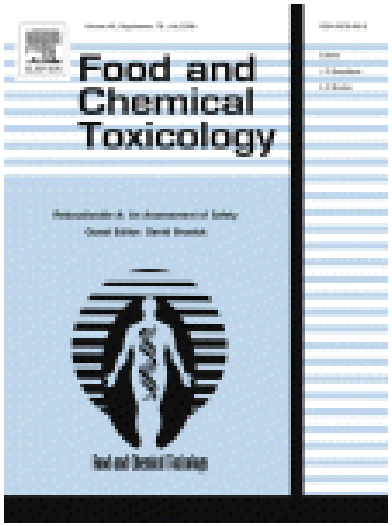
Functional Attributes

- Plant-derived, high potency, zero calorie, good tasting sweetener
- 200-350 times sweeter than sucrose
- Good solubility in water
- Non-caloric, non-glycemic
- Heat and pH stable in food and beverage systems
- Safe for use in individuals with diabetes
- No adverse effects on oral hygiene
- Low levels of use due to high potency
- Ease of combinations with other sweeteners

Safety of Steviol Glycosides

- Majority of published safety data on Rebaudioside A (Reb A) and Stevioside – most abundant in the stevia leaf

- Food and Chemical Toxicology – Volume 46 (2008) – Rebaudioside A: An Assessment of Safety

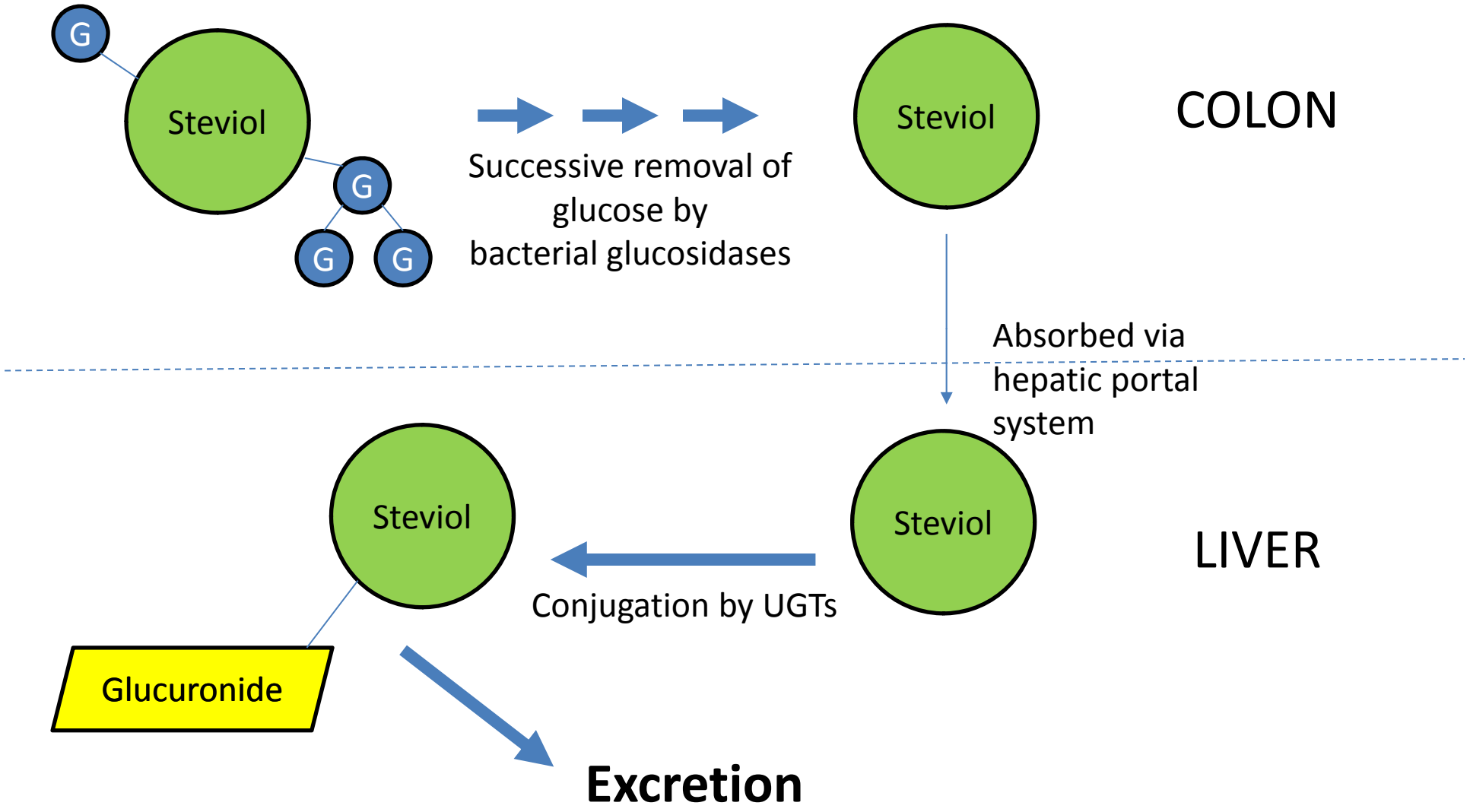


List of studies taken from FDA Redbook 2000 Guidance
Study Type
Genetic Toxicity Tests
Short-term rodent study
Sub-Chronic rodent
Sub-Chronic non-rodent
One-Year non-rodent
Two-Year rodent chronic toxicity/carcinogenicity
Reproduction study
Developmental Toxicity
Metabolism and Pharmacokinetic studies
Human Clinical Trials (safety only)

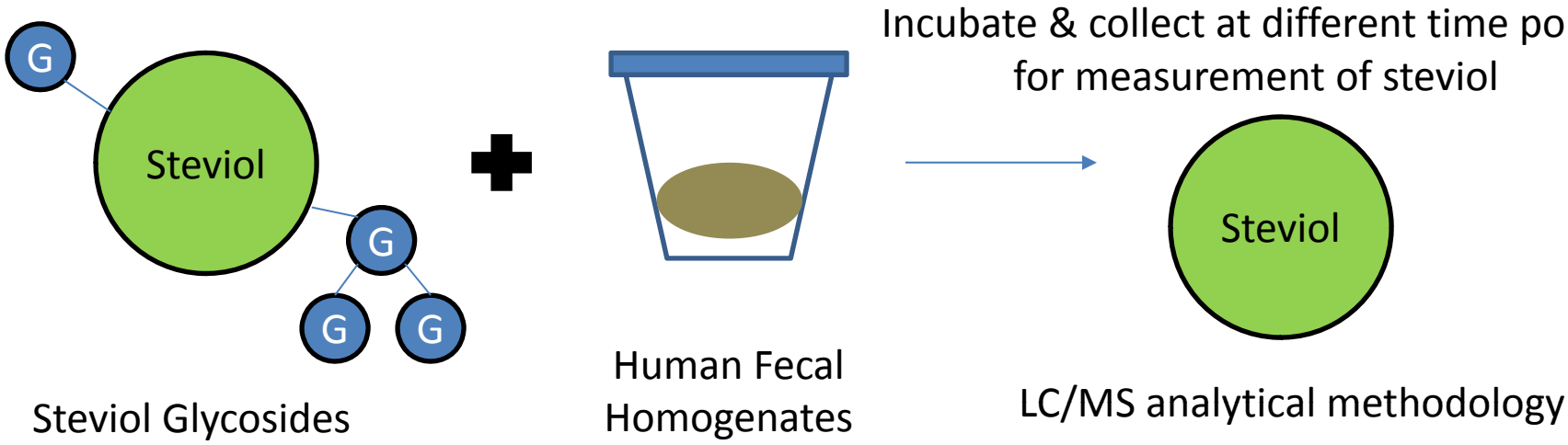
- Over 40 glycosides of commercial interest found in the leaf

- **Common metabolic pathway of steviol glycosides described**

Common Metabolic Pathway



In Vitro Metabolism Assay



- Remarkable similarity between different steviol glycosides with respect to the rate of hydrolysis to steviol, particularly during the first 24 hours
- Number and location of the sugar units attached to steviol does not significantly impact the rate of hydrolysis
- The major steviol glycosides as well as the many “minor” steviol glycosides recently identified share a common metabolic fate

Purkayashta et al., 2016. Regul Toxicol Pharmacol.

Published Studies* Evaluating Steviol Glycoside Metabolism

Purkayastha S, Markosyan A, Prakash I, Bhusari S, Pugh G Jr, Lynch B, Roberts A. Steviol glycosides in purified stevia leaf extract sharing the same metabolic fate. *Regul Toxicol Pharmacol*. **2016** Jun;77:125-33.

Purkayastha S, Bhusari S, Pugh G Jr, Teng X, Kwok D, Tarka SM. In vitro metabolism of rebaudioside E under anaerobic conditions: Comparison with rebaudioside A. *Regul Toxicol Pharmacol.* **2015** Aug;72(3):646-57.

Purkayastha S, Pugh G Jr, Lynch B, Roberts A, Kwok D, Tarka SM Jr. In vitro metabolism of rebaudioside B, D, and M under anaerobic conditions: comparison with rebaudioside A. *Regul Toxicol Pharmacol*. **2014** Mar;68(2):259-68.

Nikiforov AI, Rihner MO, Eapen AK, Thomas JA. Metabolism and toxicity studies supporting the safety of rebaudioside D. *Int J Toxicol.* **2013** Jul;32(4):261-73.

Roberts A, Renwick AG. Comparative toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol in rats. *Food Chem Toxicol*. **2008** Jul;46 Suppl 7:S31-9.

Renwick AG, Tarka SM. Microbial hydrolysis of steviol glycosides. *Food Chem Toxicol.* **2008** Jul;46 Suppl 7:S70-4.

* -http://www.ncbi.nlm.nih.gov/pubmed (PubMed Search on SG Metabolism)

Selected 3rd Party Expert Reviews – Affirming Safety

Food and Chemical Toxicology	Published safety studies conducted with rebaudioside A and stevioside
Joint FAO/WHO Expert Committee on Food Additives (JECFA)	Comprehensively examined the safety data and set a permanent ADI for steviol glycosides
Food and Drug Administration (FDA)	Issued no-objection letter for rebiana affirmed as Generally Recognized as Safe (GRAS); FDA has responded to over 40 separate GRAS notifications to date
European Food Safety Authority (EFSA)	Reviewed safety of steviol glycosides Expert panel's Scientific Opinion officially published on 14 April 2010 and approval granted in November 2011
Health Canada	Reviewed safety of steviol glycosides and considered it safe for consumption in foods by the general population Added to List of Permitted Food Additives in November 2012
Mercado Común del Sur (MERCOSUR)	Steviol Glycosides added to Positive List of Food Additives – GMC 11/06

Conclusions

- The current database of safety studies for individual steviol glycosides can be extended to all glycosylated derivatives of steviol (containing sugar moieties such as glucose, xylose, rhamnose, fructose and deoxyribose). The number and location of the sugars does not influence rate of hydrolysis & metabolism.

- Metabolism is independent of method of production (leaf extract, chemical synthesis, bioconversion and fermentation-derived)

- Available data agrees with establishment of a group acceptable daily intake (ADI) to assess the safety of steviol glycosides as opposed to individual ADIs.

- Broad scientific consensus on use of *in vitro* metabolism assay to bridge safety data (peer review publications, international regulatory agency approvals)

- The use of the *in vitro* metabolism assay to substantiate the safety of steviol glycosides aligns with the 3Rs of animal testing (Replacement, Reduction and Refinement).

Acknowledgements

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Questions?