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

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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

SOT-FSSS Webinar – Aug 2016
Stevia Product Launches Continue to Grow 2009-2014

Global New Product Launches with Stevia

- 2009 = 367
- 2010 = 584
- 2011 = 1,072
- 2012 = 1,934
- 2013 = 2,940
- 2014 = 4,405

Source: Innova Database
Growth in Stevia-related R&D

### Stevia-related publications
1984-2014*

*PubMed (NCBI-NIH)

#### Areas of research include:
- Stevia plant propagation
- Improved extraction techniques
- Enzymatic modification
- Identification of novel glycosides
- Microbial synthesis

[Bar graph showing the number of publications per year from 1984 to 2014]
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

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>R-1</th>
<th>R-2</th>
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</thead>
<tbody>
<tr>
<td>Dulcoside A</td>
<td>DLC</td>
<td>βglc-</td>
<td>αrha-βglc-</td>
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<tr>
<td>Rebaudioside A</td>
<td>Reb A</td>
<td>βglc-</td>
<td>(βglc)2-βglc-</td>
</tr>
<tr>
<td>Rebaudioside B</td>
<td>Reb B</td>
<td>H-</td>
<td>(βglc)2-βglc-</td>
</tr>
<tr>
<td>Rebaudioside C</td>
<td>Reb C</td>
<td>βglc-</td>
<td>(βglc, αrha)-βglc-</td>
</tr>
<tr>
<td>Rebaudioside D</td>
<td>Reb D</td>
<td>βglc-/βglc-</td>
<td>(βglc)2-βglc-</td>
</tr>
<tr>
<td>Rebaudioside F</td>
<td>Reb F</td>
<td>βglc-</td>
<td>(βglc, βxyl)-βglc-</td>
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<tr>
<td>Rubusoside</td>
<td>Rub</td>
<td>βglc-</td>
<td>βglc-</td>
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<tr>
<td>Stevioside</td>
<td>Stv</td>
<td>βglc-</td>
<td>βglc-/βglc-</td>
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<tr>
<td>Steviolbioside</td>
<td>Stb</td>
<td>H-</td>
<td>βglc-/βglc-</td>
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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

• Over 40 glycosides of commercial interest found in the leaf

• Common metabolic pathway of steviol glycosides described

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)
Common Metabolic Pathway

Steviol

Successive removal of glucose by bacterial glucosidases

COLON

Absorbed via hepatic portal system

LIVER

Conjugation by UGTs

Excretion

Glucuronide
**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


**Selected 3rd Party Expert Reviews – Affirming Safety**

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

• Food Safety Specialty Section – Society of Toxicology
Questions?