Safety Assessment, Application and Recommended Dosage of Stevia

I. Basic Information

(I) Product Definition

Stevia (Steviol Glycosides, CAS: 57817-89-7) is a natural high-potency sweetener extracted and purified from the leaves of *Stevia rebaudiana Bertoni* of the Asteraceae family. It mainly contains 13 kinds of glycosides including Stevioside (Stv) and Rebaudioside A (Reb A). Its sweetness is 200~300 times that of sucrose, with an energy coefficient of ≈0 kcal/g (zero calorie). Its metabolism is insulin-independent with a Glycemic Index (GI) = 0 and no cariogenicity. It features heat resistance, acid and alkali resistance, and a slight astringent aftertaste in taste (significantly improved by high-purity Reb A).

(II) Production Processes

1. **Water Extraction-Alcohol Precipitation & Ion Exchange Method (Mainstream Process)**:
   Using dried stevia leaves as raw materials, the product is prepared through hot water extraction → flocculation filtration → ethanol precipitation → ion exchange resin purification → concentration → spray drying. The total glycoside purity is ≥95% and Reb A content can reach over 97%, making it the mainstream food-grade product.
2. **Enzymatic Conversion Method (Improved Process)**:
   Taking crude steviol glycosides as the substrate, α-glucosidase catalyzes the transfer of glucosyl groups to the steviol skeleton to form glucosylated steviol glycosides, which has higher sweetness and significantly reduced astringent aftertaste, suitable for high-end beverages and baking.
3. **Biosynthesis Method (R&D Stage)**:
   Using engineered yeast/Escherichia coli for fermentation and synthesis of steviol glycosides, it has high purity, few impurities and excellent environmental performance, but with high cost and low production capacity, it has not been applied in large-scale industrial production.

II. Safety Assessment

(I) Toxicological Assessment

1. **Acute Toxicity**:

   Oral LD_{50} in rats/mice > 34.77 g/kg·bw, classified as practically non-toxic; short-term high-dose feeding (10 g/kg·bw) caused no death or organ damage.

2. **Genotoxicity**:

   Ames test, in vitro mammalian cell chromosome aberration test and mouse bone marrow micronucleus test all showed negative results, with no risk of mutagenicity, teratogenicity or chromosome damage.

3. **Subchronic Toxicity (90 Days)**:

   The oral NOAEL of rats = 550 mg/kg·bw/day, with no abnormal weight, abnormal blood/biochemical indicators or target organ toxicity to liver and kidney.

4. **Chronic Toxicity and Carcinogenicity**:

   A 2-year lifelong feeding test on rats and mice (dose up to 100 times of ADI) showed no carcinogenicity or cumulative toxicity, and no pathological changes in liver, kidney and reproductive system.

5. **Reproductive and Developmental Toxicity**:

   Three-generation reproductive toxicity test showed no parental reproductive damage and no malformation/developmental retardation in offspring. Administration to pregnant mice until delivery resulted in normal body weight and bone development of fetal mice with no embryotoxicity.

6. **Human Tolerance**:

   Adults have good tolerance with daily intake **≤4 mg/kg·bw (steviol equivalent)**; excessive intake (> 8 mg/kg·bw/day) may cause transient gastrointestinal reactions such as abdominal distension, borborygmus and mild diarrhea in some populations, which can be rapidly relieved within 24~48 hours after discontinuation **; no allergic cases (except those allergic to Asteraceae plants)**.

(II) Safety Recognition by Global Authoritative Institutions

1. China (National Health Commission of the People's Republic of China / GB 2760-2024)

- Approved as a food additive (sweetener), allowed to be used in all categories of food such as beverages, baked goods, confectionery, dairy products, candied fruits and condiments in accordance with specified limits (calculated as steviol equivalent).

- Suitable for diabetics, obese people, children, pregnant women and lactating women, and safe for normal consumption of added food.
- Food safety indicators: heavy metals (lead ≤1 mg/kg), microorganisms (total bacterial count ≤1000 CFU/g) and total glycoside purity ≥95%, complying with national standard limits.

2. Codex Alimentarius Commission (CAC / JECFA)

- JECFA confirmed its safety in 2010 and set ADI=0~4 mg/kg·bw/day (steviol equivalent), serving as a global universal safety benchmark.
- Included in food additive standards (INS 960), permitted for use in all food categories with no restrictions on special populations.

3. Europe (EFSA)

- Safety confirmed by assessment in 2010 with ADI=4 mg/kg·bw/day (consistent with JECFA), recognized as non-genotoxic, non-carcinogenic and safe for reproduction and development.
- Approved for low-calorie beverages, sugar-free confectionery, baked foods and dairy products, with mandatory labeling of "contains steviol glycosides".

4. United States (FDA)

- High-purity steviol glycosides (Reb A≥95%) were recognized as GRAS in 2008 (21 CFR 172.802), permitted for use in beverages, baked goods, confectionery, oral care products and tabletop sweeteners.
- No need to be labeled as "artificial sweetener", and can be labeled as "natural sweetener (stevia extract)".

5. Other Regions

- More than 100 countries including Australia & New Zealand (FSANZ), Japan, South Korea, Canada and Brazil have approved its wide application as a food sweetener.

(III) Safety Conclusion

Stevia has complete toxicological data, a long global application history (since the 1970s in Japan) and excellent human tolerance, with no safety risks under normal consumption doses. Only excessive intake may cause mild gastrointestinal discomfort. It has no cumulative toxicity, zero calorie, no blood sugar impact and anti-caries effect, with no strict dietary taboos for special crowds, and is recognized as a high-safety natural sugar substitute.

Note: People allergic to Asteraceae plants should use with caution; pet food shall comply with local regulations.

III. Functional Characteristics and Application of Products

(I) Product Application Characteristics

1. **Ultra-high Sweetness & Zero Calorie**:
   200~300 times sweeter than sucrose with zero calorie (GI=0). It does not raise blood sugar or stimulate insulin secretion, meeting the demands of low-sugar/sugar-free, blood sugar control and weight management.
2. **Optimized Taste & Strong Stability**:
   High-purity Reb A (≥97%) has a taste close to sucrose with extremely mild astringent aftertaste; it is heat-resistant (stable at 120℃), acid and alkali-resistant (stable at pH 3~10), non-fermentable and unavailable to microorganisms, helping extend shelf life.
3. **Good Water Solubility & Excellent Compounding Performance**:
   Easily soluble in water/ethanol. Compounded with sucrose, erythritol, maltitol and fructose, it can optimize taste, reduce costs, enhance sweetness and achieve synergistic effects.
4. **Additional Functions & Wide Application**:
   Anti-caries (not fermented by oral bacteria to produce acid), bacteriostatic (bacteriostatic rate of about 43%), and flavor improvement (reducing the cloying taste of sucrose), suitable for multi-category food processing.

(II) Product Application Fields

1. Beverages (Core Application)

• Carbonated drinks, fruit and vegetable juices, tea drinks, functional drinks, plant protein drinks: 0.1~0.3 g/kg (steviol equivalent), replacing 30%~100% of sucrose, featuring zero sugar, low calorie and fresh taste.

• Bottled water, sugar-free coffee/milk tea: 0.05~0.2 g/kg, zero calorie with no impact on original flavor.

2. Bakery and Cereal Products

• Sugar-free biscuits, cakes, bread and pastries: 0.2~0.5 g/kg, high temperature resistance, non-browning and extended shelf life.

• Oatmeal, meal replacement powder and puffed food: 0.15~0.4 g/kg, low sugar and high fiber, meeting health-oriented demands.

3. Confectionery and Candied Fruits

• Sugar-free hard candy, gummy candy, chewing gum and chocolate: 0.3~0.8 g/kg, anti-caries, non-sticky teeth and long-lasting sweetness.

• Preserved fruits and candied fruits: 0.2~0.6 g/kg, moderate sweetness, retained fruit flavor and reduced sucrose dosage.

4. Dairy Products and Cold Drinks

• Sugar-free yogurt, flavored fermented milk and modified milk: 0.1~0.2 g/kg, optimized taste without affecting fermentation process.

• Ice cream, ice cream bars and smoothies: 0.15~0.3 g/kg, low calorie, non-cloying and good melt resistance.

5. Condiments and Others

• Soy sauce, vinegar, sauces, chicken essence/monosodium glutamate: 0.05~0.2 g/kg, enhancing umami, inhibiting bitterness and balancing overall flavor.

• Health products, dietary supplements and oral care products (toothpaste/mouthwash): 0.1~0.5 g/kg, natural, safe and compatible with sugar-free formulas.

(III) Recommended Dosage (Calculated as steviol equivalent, in accordance with GB 2760-2024 + industrial practice)

Food Category	Recommended Dosage (g/kg)	Sucrose Replacement Ratio
Beverages (including carbonated drinks / fruit & vegetable juices / tea drinks)	0.10~0.30	50%~100%
Baked Pastries (biscuits / cakes / bread)	0.20~0.50	40%~80%
Confectionery / Chewing Gum / Chocolate	0.30~0.80	60%~100%
Dairy Products (yogurt / modified milk / milk powder)	0.10~0.20	30%~60%
Candied Fruits / Preserved Fruits	0.20~0.60	40%~70%
Condiments (soy sauce / vinegar / sauces)	0.05~0.20	20%~50%
Cold Drinks (ice cream / ice cream bars)	0.15~0.30	50%~80%

Remarks:

• The dosage of high-purity Reb A (≥97%) can be reduced by 10%~20% based on the upper limit in the table to avoid astringent aftertaste;

• Compound application (e.g. stvioside + erythritol) can cut stevioside dosage by 30%~50% with better taste performance;

• Strictly comply with ADI=4 mg/kg·bw/day, and the maximum daily intake for adults (60kg) shall be **≤240 mg (steviol equivalent)**.