



JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

**Sixty-third meeting
Geneva, 8-17 June 2004**

3.1.6 Steviol glycosides

Explanation

Steviol glycosides are natural constituents of the plant *Stevia rebaudiana* Bertoni, a member of the Compositae family. The leaves of *S. rebaudiana* Bertoni contain at least ten different glycosides, the major constituents being stevioside and rebaudioside A. The material evaluated at the present meeting contains not less than 95% glycosylated derivatives of steviol, primarily stevioside, rebaudiosides A and C and dulcoside A, with minor amounts of rubusoside, steviolbioside, and rebaudiosides B, D, E and F. At its fifty-first meeting (Annex 1, reference 149), the Committee evaluated toxicological data on stevioside and the aglycone steviol. The Committee noted several shortcomings in the available information and requested that specifications should be developed to ensure that the material tested is representative of the material of commerce. Further information was required on the nature of the substance tested, on the metabolism of stevioside in humans and on the activity of steviol in suitable studies of genotoxicity in vivo.

There is no single common or trivial name in common usage for the evaluated mixture of glycosylated derivatives of steviol. At its thirty-third meeting (Annex 1, reference 83), the Committee developed guidelines for designating titles for specification monographs.

According to these guidelines, the title of a monograph should, in such circumstances, be selected from the available scientific, common and trivial names. The name chosen must be nonproprietary and should be a scientifically accurate description of the substance. In addition, the name should communicate to the consumer an accurate description of the substance, within the scope of existing names for food additives. At its present meeting, the Committee established that the evaluated material of commerce for which specifications were developed should be known as "steviol glycosides". The Committee reviewed additional biochemical and toxicological data on the major glycosylated derivatives of steviol and on the aglycone, steviol.

Chemical and technical considerations Steviol glycosides are obtained by extracting leaves of *Stevia rebaudiana* Bertoni with hot water, followed by solvent purification of the water-soluble extract. Ion-exchange resins may also be used during the purification process. *Stevia* extracts generally contain a high percentage of stevioside and rebaudioside A, and smaller amounts of other steviol glycosides. The composition of the extracts depends on the composition of the leaves, influenced by soil and climate conditions, and on the manufacturing process. The data on analytical chemistry available to the Committee indicated that commercial products contain at least 95% steviol glycosides. However, the remainder of the material was not identified. The impurities occurring in steviol glycosides consist primarily of compounds extracted from the *Stevia* leaves. Results of analysis of *Stevia* preparations support the setting of maximum limits of 1mg/kg for both arsenic and lead.

Different methods, mainly involving liquid chromatography, are currently available for the identification and determination of the principal steviol glycosides. Stevioside and rebaudioside A are reasonably stable at the elevated temperatures used in food processing, and do not undergo browning or caramelization when heated. No information on the hydrolytic stability of steviol glycosides in acidic foods was available to the Committee.

Toxicological data

After oral administration, steviol glycosides are poorly absorbed in experimental animals and in humans. Intestinal microflora metabolize steviol glycosides to the aglycone, steviol, by successive hydrolytic removal of glucose units. Data reviewed by the Committee at its current and fifty-first meetings (Annex 1, reference 149) indicated that this process is similar in rats and humans. The hydrolysis of rebaudioside A to steviol was slower than that of stevioside. In humans treated orally with stevioside, small amounts of steviol were detected in the plasma, with considerable interindividual variability. The major route by which steviol is metabolized in humans in vivo appears to be via conjugation with glucuronide and/or sulfate. Studies with liver microsomal preparations indicated that steviol is also metabolized to a number of hydroxy and dihydroxy derivatives via cytochrome P450 (CYP)-dependent pathways.

Stevioside and/or steviol affected a variety of biochemical parameters in models in vitro, indicating possible mechanisms of antihypertensive and antiglycaemic effects that involve modulation of ion channels. High concentrations (e.g. 1 mmol/l) of stevioside were required to produce a maximal increase in insulin secretion,

while steviol was effective at a concentration that was about three orders of magnitude lower. Stevioside also affected a variety of biochemical parameters in different animal species in vivo, mostly with parenteral administration; these studies were considered by the Committee to be of limited relevance to dietary exposure.

No new long-term studies of toxicity or carcinogenicity were available at the present meeting. At its fifth-first meeting, the Committee noted that oral administration of stevioside (purity, 95.6%) at a dietary concentration of 2.5%, equal to 970 and 1100mg/kgbw per day in male and female rats, respectively, for 2 years was not associated with toxicity. Reduced body-weight gain and survival rate were observed with stevioside at a dietary concentration of 5%. In a new study, stevioside was found to inhibit the promotion of skin tumours by 12-O-tetradecanoylphorbol-13-acetate (TPA) in a model of skin carcinogenesis in mice.

The Committee reviewed new data on genotoxicity that considered together with data reviewed by the Committee at its fifth-first meeting, allowed a number of conclusions to be drawn. Stevioside and rebaudioside A have not shown evidence of genotoxicity in vitro or in vivo. Steviol and some of its oxidative derivatives show clear evidence of genotoxicity in vitro, particularly in the presence of a metabolic activation system. However, studies of DNA damage and micronucleus formation in rats, mice and hamsters in vivo indicate that the genotoxicity of steviol is not expressed at doses of up to 8000mg/kgbw.

One new study of developmental toxicity was available at the present meeting. Adverse effects on the reproductive apparatus, which could be associated with impaired fertility, were observed in male rats given a crude extract of *S. rebaudiana*, at a dose corresponding to 1.34 g of dried leaves. However, at its fifth-first meeting, the Committee reviewed a number of studies of reproductive and developmental toxicity with stevioside (purity, 90% or 96.5%). Doses of up to 2500mg/kgbw per day in hamsters and 3000mg/kgbw per day in rats had no effect in studies of reproductive toxicity. No teratogenic or embryotoxic effects were observed in rats given stevioside at a dose of up to 1000mg/kgbw per day by gavage. The Committee considered that the adverse reproductive effects associated with administration of aqueous extracts of *S. rebaudiana*, noted at the present and fifth-first meeting, were unlikely to be caused by steviol glycosides.

Stevioside is being investigated as a potential treatment for hypertension and diabetes. Administration of stevioside at a dose of 750 or 1500 mg per day for 3–24 months resulted in decreased blood pressure in hypertensive patients, and no adverse effects. These studies, in a limited number of subjects, provided some reassurance that stevioside at a dose of up to 25mg/kgbw per day (equivalent to 10mg/kgbw per day, expressed as steviol) for up to 2 years shows no evidence of significant adverse effects in these individuals.

There is no information on the effects of repeated administration of stevioside on blood pressure in normotensive individuals. A small study in 12 patients with type-2 diabetes showed that a single dose of 1g of stevioside reduced postprandial glucose concentrations and had no effect on blood pressure.

Intake

The Committee evaluated information on exposure to steviol glycosides, 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 (4). All the intake results are presented in terms of equivalents of steviol, based on a conversion of 40% from the steviol glycoside, stevioside (relative molecular mass: steviol, 318, steviosid, 805).

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

The Committee evaluated estimates of exposure per capita derived from disappearance (poundage) data supplied by Japan and China. The Committee also evaluated estimates of exposure to steviol glycosides based on the replacement of all dietary sugars in the diets for Japan and the USA. Table 1 summarizes the exposures to steviol glycosides (as steviol) evaluated or derived by the Committee.

The Committee concluded that the replacement estimates were highly conservative and that intake of steviol glycosides (as steviol) would be likely to be 20–30% of these values.

Table 1

Summary of estimates of exposure to steviol glycosides (as steviol)

Estimate	Exposure (mg/kg bw per day)
GEMS/Food (International) ^a	1.3–3.5 (for a 60kg person)
Japan, per capita	0.04
Japan, replacement estimate ^b	3
USA, 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 USA would be replaced by steviol glycosides on a sweetness equivalent basis, at a ratio of 200: 1

Evaluation

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 970mg/kgbw per day in a long-term study 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 12.5–25mg/kgbw per day (equivalent to 5–10mg/kgbw per 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 0–2mg/kgbw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970mg/kgbw per day (or 383mg/kgbw per 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.

A toxicological monograph was prepared, incorporating summaries of the key toxicological data on the evaluation of stevioside conducted by the Committee at its fifty-first meeting. New tentative specifications were prepared, accompanied by a Chemical and Technical Assessment. In order to be able to remove the tentative designation from the specifications, the following further information for commercially available products was required by 2007:

- analytical data on distribution and concentrations of all component steviol glycosides, including those that were not identified in the tentative specifications;
- method of analysis for the determination of all component steviol glycosides, including those that were not identified in the tentative specifications;
- the nature and concentration of the fractions that do not contain steviol glycosides;
- the quantities of residual solvents from isolation and purification steps of the manufacturing process;
- the hydrolytic stability of the steviol glycosides in acidic foods and beverages.

SUMMARY AND CONCLUSIONS

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held in Geneva, Switzerland, from 8 to 17 June 2004. The purpose of the meeting was to evaluate certain food additives and ingredients, flavouring agents, and a natural constituent of food.

Dr John Larsen, Division of Toxicology and Risk Assessment, Danish Institute of Food and Veterinary Research, Søborg, Denmark, served as Chairman and Mrs Inge Meyland, Danish Institute of Food and Veterinary Research, Søborg, Denmark, served as Vice-Chairman.

Dr Manfred Luetzow, Food Quality and Standards Service, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, and Dr Angelika Tritscher, International Programme on Chemical Safety, World Health Organization, served as joint secretaries.

The present meeting was the sixty-third in a series of similar meetings. The tasks before the Committee were (a) to elaborate further principles for evaluating the safety of food additives; (b) to evaluate certain food additives, ingredients, and flavouring agents; (c) to review and prepare specifications for selected food additives and flavouring agents; (d) to evaluate a natural constituent of food.

The report of the meeting will appear in the WHO Technical Report Series. Its presentation will be similar to that of previous reports, namely, general considerations, comments on specific substances, and recommendations for future work. An annex will include detailed tables (similar to the tables in this report) summarizing the main conclusions of the Committee in terms of acceptable daily intakes (ADIs) and other toxicological recommendations. Information on specifications for the identity and purity of certain food additives examined by the Committee will also be included.

The participants in the meeting are listed in Annex 1. Further information required or desired is listed in Annex 2. General considerations, that contain information that the Committee would like to disseminate quickly are included in Annex 3.

Toxicological monographs or monograph addenda on most of the substances that were considered will be published in WHO Food Additives Series No. 54.

New and revised specifications for the identity and purity of the compounds will be published in FAO Food and Nutrition Paper Series 52, Addendum 12.

More information on the work of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is available at:

Toxicological recommendations and information on specifications

1. Food additives and ingredients evaluated toxicologically

Food additive	Specifications a)	Acceptable daily intake (ADI) and other toxicological recommendations
Steviol glycosides	N, T	0–2 mg/kg bw (temporary)

a) N: new specifications prepared
T: tentative specifications

Steviol glycosides

The Committee required additional information 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. In order to be able to remove the tentative designation from the specifications, further information for commercially available products is required on:

- Analytical data on distribution and concentrations of all component steviol glycosides, including those that are not identified in these tentative specifications.
- Method of analysis for the determination of all component steviol glycosides, including those that are not identified in these tentative specifications;
- The nature and concentration of the fractions that do not contain steviol glycosides.
- The quantities of residual solvents from isolation and purification steps of the manufacturing process.
- The hydrolytic stability of the steviol glycosides in acidic foods and beverages.



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