Stevia and steviol glycosides

Properties, techniques, uses, exposure, toxicology, pharmacological effects

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Summary

- Steviol glycosides are given the number (E-) 960 by the Codex Alimentarius.

- In part I of the book, a detailed description and identification are given of the different steviol glycosides, including spectroscopic data, physical state and the sweetness compared with 0.4 % sucrose (Chapter 1). The specification of 95 % pure steviol glycosides is explained in Chapter 2 and a general scheme of the manufacturing process is given in Chapter 3. Chapter 4 is dedicated to validated techniques for the analysis of steviol glycosides. This enables food inspectors to control the quality of steviol glycosides on the market. Also the *Stevia* Industry will benefit from these techniques, as the analysis of steviol glycosides must be accurate. This way, only a good quality product will be available on the market. The development of an internal standard (IS) is also described, that will enable corrections to be made for losses during purification processes required when analysing complex foods.

- Part II describes the use of the validated techniques in the extraction of leaves (Chapter 5), in the quality control of steviol glycosides (Chapter 6) and in the analysis of different foods, beverages and complex foods (Chapter 7). Chapter 8 is dedicated to the reaction and fate of steviol glycosides in food, whereas Chapter 9 discusses the technological needs and proposed uses of the sweetener. In Chapter 10, the exposure is calculated using 2 scenarios, *viz*., a worst case (all sugar replaced) and a more realistic 30 % sugar substitution.

- Part III gives a detailed survey of the toxicological data. Many toxicological studies with steviol glycosides have been done by different independent laboratories in different countries. Nearly all the studies confirm the safety of steviol glycosides as a food additive.

- Absorption and metabolism studies (chapter 11) revealed that the uptake of steviol glycosides is extremely low. Enzymes of the digestive tract are not able to degrade steviol glycosides into steviol, nor is the stomach juice. Only the group of bacteroides of the colon are able to degrade steviol glycosides to steviol. Some of this steviol is excreted with the faeces, the rest is absorbed by the colon and glucuronated in the liver. The kidneys filter the steviol glucuronide into the urine and it is excreted. No accumulation of derivatives occurs in the body. Besides steviol glucuronide, no other derivatives could be detected. In the blood, only steviol glucuronide was detected, but no free steviol. However, the Milan research group demonstrated the presence of stevioside in blood, but the amounts were too small to be quantified.

- Acute and chronic toxicity studies showed that steviol glycosides have a very low toxicity. No toxicity was observed in chronic tests (chapter 12).

- Genotoxicity studies (chapter 13) revealed that steviol glycosides did not induce gene mutations in bacteria. However, after metabolic activation, it was shown that so far unknown steviol metabolites caused mutations in a very sensitive strain of *Salmonella typhimurium* TM677, but not in other strains or in other bacteria. Steviol glycosides did not induce gene mutations in mammalian cells *in vitro.* However, after metabolic activation of steviol (99 % purity), some gene mutation and chromosomal aberration was found in Chinese hamster lung fibroblasts at high concentrations above 300 µg/ml that, however, will never be reached in the plasma, as the administration of high doses, up to 750 mg daily, did not reveal traces of steviol in the blood (detection limit of the steviol derivative analysed: 100 pg). Moreover, the metabolism in rodents and humans is totally different. However, no sign of enhancement of mutation frequency by steviol was observed in the mouse lymphoma L5178Y $tk^{+/-}$-3.7.2C gene mutation assay (MOLY), whereas the

positive control showed its expected stimulation. Steviol glycosides, as well as steviol, were inactive in all the different chromosome aberration tests used.

Because of the positive score in 2 genotoxicity tests at relatively high concentrations, free steviol was also tested *in vivo* in different animal systems. No signs of harmful effects were ever found. This corresponds well with the metabolism studies with volunteers and the lack of traces of steviol in the blood. Therefore, the general conclusion is that the use of steviol glycosides as a sweetener is safe. In animal models it was shown that high doses of steviol glycosides inhibited tumour formation. However, due to the extremely small absorption by the intestines and the small amounts needed for sweetening purposes, the advantages of oral steviol glycosides as chemo-preventive agents seem to be less relevant.

- In chronic toxicity and carcinogenicity studies (chapter 14) with steviol glycosides and free steviol, and using different animal models, no harmful effects were observed.

- There were no observable effects of steviol glycosides or steviol on reproduction, or on developmental toxicity (chapter 15). The study by Planas and Kuć (1968) has, in the past, led to some confusion and controversy on fertility. However, these results were refuted by Shiotsu (1996) who did more reliable experiments with many more animals. Many other authors have described the lack of effects of steviol glycosides on male and female reproduction. The significance of the administration of only one extremely large dose of crude *Stevia* extracts to young rats, should be questioned. In this case, extracts of fresh *Stevia* leaves amounting to more than 50 % of the body weight were daily administered to the young rats. In various studies on prenatal developmental toxicity, steviol glycosides and steviol

were without effect, even when very large doses were administered, nor was there any effect on postnatal developmental toxicity. Steviol glycosides are not intended for use in infant formulae, follow-on formulae or weaning foods.

- Many studies of chapter 16 revealed that there was no effect on bio-availability of nutrients from the diet. There is a clear beneficial effect of the use of steviol glycosides on caries formation, most probably due to the substitution of sucrose in the food by a non-cariogenic substance. Although there may be pharmacological effects of large doses of steviol glycosides on lowering blood glucose, the lowering of hypertension and prevention of atherosclerosis, these effects will not occur when steviol glycosides are used in small doses for sweetening purposes. To provoke these beneficial effects, large amounts have to be administered (eg. 250-500 mg thrice a day, e.g. in capsules). Steviol glycosides are unlikely to give rise to nutritional, microbiological, toxicological and/or allergenicity problems.

Part IV is a general discussion.

- A dietary exposure assessment estimated that for the majority of consumers the ADI (0-4 steviol equivalents) was not exceeded when steviol glycosides were added to the range of foods requested in the application. The estimated exposure for high consumers (children aged 2-6 years) marginally exceeded the ADI. However, this estimate is based on very conservative assumptions and when a dietary exposure estimate was undertaken with concentrations of steviol glycosides that reflected a more realistic level of use, it was estimated that dietary exposure for high consumers (children aged 2-6 years) was only 50 % of the ADI. Moreover, the ADI value used is rather low, as much higher values might be considered as suggested by Table 42.

- The toxicological studies reveal that steviol glycosides are safe and should be authorised as food additives in Europe. There are no public health and safety concerns for steviol glycosides when used as a food additive at the maximum levels proposed in Table 28. There will be a continuous follow-up of the quality on the European market. Moreover, the group of steviol glycosides is not a new food additive and is already approved in many countries in other continents. It can be estimated that **daily** over 150 million of people consume steviol glycosides as such or as components of dried *Stevia* leaves.

- The introduction of steviol glycosides on the European market would have many benefits for the consumer through a reduction of energy intake, the possibility to consume all natural products and even totally organic meals are possible. Moreover, steviol glycosides are very sweet, and only small amounts need to be used. The steviol glycosides are very stable and can be cooked and baked up to 200°C without breakdown. They are also safe for PKU patients.

- There will also be a beneficial impact in the whole EU; translated as social benefits, health benefits through a reduction of medical care costs, and of course there are large economic and industrial benefits. Besides the possibility of growing *Stevia* in regions where tobacco is now grown, there will be job creations in European agronomy, research institutes and the food industry. There is also a beneficial environmental impact, as sustainable techniques can be used for growing and processing *Stevia*. Furthermore, the European companies will gain competitiveness with foreign companies in using and developing products derived from *Stevia*, certainly now that steviol glycosides are already approved in many countries.

- In May 23rd 2007, the Food Standards of Australia New Zealand decided to publish the draft of its assessment report including an authorisation on its website. In this document they write explicitly that they did not wait until after JECFA had evaluated additional studies on potential pharmacological effects of steviol glycosides, as FSANZ considered the safety of steviol glycosides used as a sweetener completely proven. Dr. O'Calaghan (2006) also concluded that there were no risks in using steviol glycosides. He made a study of published literature on pharmacological effects. As a specialist in hyper- and hypotension, he concluded that there is no risk in using *Stevia*, not even in the case of hypotension. FSANZ has concluded that steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day (or about 4 mg SVeq./kg BW). The adequacy of the existing database and a new study in humans provides a basis for revising the uncertainty factors that were used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened, so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore, a full ADI of 0-4 mg steviol equivalents/kg bw/day, derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study, has been established.

- In 2003, *Stevia rebaudiana* was approved for use as an active and/or excipient ingredient in listed medicines in Australia (FSANZ, 2007). Stevioside is permitted in listed medicines only in conjunction with the use of *Stevia rebaudiana* (it is not approved as an ingredient in its own right). There have been no known ad verse

effects for stevioside reported to the Therapeutic Goods Administration to date. This very important information comes from FSANZ itself.

- From an extended review of the scientific literature, an ADI value between 0 and 10 mg steviol equivalents/kg BW can be suggested. JECFA (2008) recommended a final ADI of 0-4 steviol equivalents (safety factor 100x). FSANZ fixed a value of 0-4 mg steviol equivalents/kg BW. Finally, in December 2008, the FDA (USA) accepted the GRAS status of rebaudioside A and, in 2009, for the mixture of steviol glycosides. In September 2009, the French authorities authorised rebA (>97 % purity) as a food additive, excluding its use as a table top sweetener. However, in January 2010 rebA was also authorised as a table top sweetener.