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Afssa – Request no. 2008-SA-0108
Related request no. 2006-SA-0231

Maisons-Alfort, 11 September 2008

OPINION

of the French Food Safety Agency (Afssa) on a provisional two-year authorisation for the use of steviol, an extract of *Stevia rebaudiana*, as a food sweetener under article 5 of Directive 89/107/EEC, further to Afssa's opinion of 12 October 2007.

THE DIRECTOR GENERAL

On 11 April 2008, the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF) requested the French Food Safety Agency (Afssa) to issue an opinion on a provisional two-year authorisation for the use of steviol, an extract of *Stevia rebaudiana*, as a food sweetener under article 5 of Directive 89/107/EEC, further to Afssa's opinion of 12 October 2007, sent by the C2 office.

Afssa had issued an initial opinion on this request on 12 October 2007, in which it was considered that current scientific knowledge and the application dossier submitted indicated that the health risk of using steviol glycosides, extracts of *Stevia rebaudiana*, as a food sweetener, could not be estimated with any accuracy. This view was justified in particular by the findings of pharmacological studies in humans and animals which evoked the existence of pharmacological effects associated with the oral consumption of these substances. As a result, Afssa considered that proof of the consumer safety of using steviol glycosides as a sweetener had not been furnished.

Afssa received new information on 17 April 2008, followed by additional information on 30 May 2008 consisting of several scientific articles under publication. This opinion only concerns the new information supplied on one of the components of *Stevia rebaudiana* extracts, rebaudioside A. Its conclusions must be compared to those drawn in Afssa's opinion of 12 October 2007.

After consulting the "Additives, Flavourings and Processing Aids" Scientific Panel, which met on 12 June and 10 July 2008, Afssa issues the following opinion:

<u>In terms of chemical composition, manufacturing processes and product purification</u>

The initial commercial product assessed presented a 95% purity level of steviol glycosides (g/100g of dry matter) composed of: stevioside: 63-73%; rebaudioside A: 13-17 %; rebaudioside C: 7-10%, dulcoside A: 3-6 %. The impurities were composed of minor steviosides and other molecules from stevia leaves (sterebin, flavanoids and polysaccharides). Rebaudioside A was the sweetest stevioside.

In the new information assessed for this opinion, the petitioner specifies that the new product comprises 96% dry matter, comprising 98% rebaudioside A.

The manufacturing process described in the previous dossier contained the following stages: Grinding of leaves, concentration, precipitation/clarification and neutralisation of the liquid phase by citric acid, followed by filtration, discolouration and adsorption on resins. In this file, neither the rebaudioside A extraction and purification process nor the analytical method used to determine rebaudioside A concentration are described. As a result, the dossier supplied did not provide any information guaranteeing that the commercial product proposed actually presents the claimed purity level of rebaudioside A.

In general, the composition of extracts in steviol glycosides can vary somewhat, depending on plant variety, climate conditions during growth and the purification and extraction method. It is

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therefore essential to ensure reproducibility in terms of the quantitative composition of the extracts likely to be used to purify rebaudioside A.

In terms of stability

The general information presented in this dossier reports that steviol glycosides present temperature and pH stability. Some of the documents supplied in this dossier indicate that, under acidic conditions (pH 2-4), steviol glycosides are stable for 8 hours at a temperature of 80°C. However, these documents also report that, at 100°C and pH 3-4, steviol glycosides can break down, in the range of 4 and 8% and 10 and 40% respectively. The main breakdown product reported is isosteviol.

In the previous dossier, glycoside stability during technological treatments at high temperatures (equal to or above 60°C in a pH < 3 and > 9 solution, or above 140°C in a solid state) had not been proven. Rebaudioside A stability has not been documented in this dossier (e.g. by the presentation of stability curves based on temperatures and pH conditions, established in model systems and/or certain food matrices proposed) regarding potential applications in products that require heat treatment through cooking or stabilisation at temperatures above 100°C (e.g. biscuits, pastries, sweets, soups and some tinned food).

In terms of new data on metabolism

Two recent publications, among those supplied in this dossier , describe the toxicokinetics of steviols in rats (Roberts. 2008) and humans (Wheeler. 2008).

In rats, single doses of rebaudioside A, stevioside and steviol marked radioactively were administered. The kinetic parameters determined (Tmax, Cmax, AUC) showed that the three molecules presented similar metabolic profiles as they were rapidly absorbed and eliminated 72 hours after ingestion. The predominant metabolite of the three molecules was steviol, and in lower proportions, steviol glucoronide. Elimination is mostly through faeces, in the form of steviol, and only 2% of the dose administered was eliminated through urine.

In the study on humans, single doses of rebaudioside A and stevioside were administered orally. The parameters determined (Tmas, Cmax, AUC, renal clearance and half-life) showed that the two molecules are metabolised predominantly into steviol glucoronide. Elimination of steviol glucoronide, mainly through urine, accounted for between 59 and 62% of the rebaudioside A and stevioside administered respectively. No steviol glucoronide was detected in faeces.

In terms of new pharmacological and toxicological data

Pharmacological effects:
In the previous opinion, the potential side effects of steviol glycoside consumption by diabetics and people suffering from high or low blood pressure were questioned. Indeed, some publications suggested that consumption of these products had a vasodilator effect on the aortic arch isolated in rats and that stevioside had antihypertensive properties in rats and dogs. Other publications showed that stevioside and steviol could stimulate the insulin secretion in vitro of islets of Langerhans isolated in mice and by the β cells, and that stevioside induced antihyperglycaemic, insulinotropic and glucagonostatic (suppression of glucagon secretion) effects in diabetic rats.

Two recent publications of randomised and controlled clinical trials, supplied in this dossier, describe the impact of rebaudioside A on blood pressure (Maki et al. 2008a) and the effects of rebaudioside consumption in men and women with type 2 diabetes (Maki et al. 2008b).

The study of the effect on blood pressure was carried out on 100 people (18 to 73 years old, predominantly women), split into two groups, one receiving rebaudioside A in the form of a 250mg capsule, 2 capsules twice a day, i.e. 1000mg/day for 4 weeks, and the other a placebo. The study participants initially presented normal and low-normal systolic and diastolic blood pressure. The study's findings showed that none of the parameters measured (systolic and diastolic pressure, average blood pressure, cardiac frequency) differed statistically between the placebo group and the group exposed to rebaudioside A.

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The study of effects in type 2 diabetics was conducted on 122 people (33 to 75 years old, similar numbers of men and women), split into 2 groups, one receiving rebaudioside A in the form of 250mg capsules, 2 capsules twice a day, i.e.1000mg/day for 16 weeks, and the other a placebo. No significant difference in the blood parameters measured, glycosylated haemoglobin, fasting glycaemia, insulin or C-peptide, could be detected between the placebo group and the group exposed to rebaudioside A.

It can therefore be concluded from these studies that exposure to rebaudioside A, at the 1000mg/day dose (or ~17 mg/kg bw/day, for a person weighing 60kg) has not demonstrated any adverse effects on blood pressure in people with normal or low-normal blood pressure, or in type 2 diabetics. No information has been supplied on people with high blood pressure.

Toxicological effects:
Of the studies recently published, two subchronic toxicity studies (4 and 13 weeks) were conducted on Han Wistar rats with rebaudioside A presenting a purity level of 97% (Curry and Roberts 2008).

The first, four-week, study (range finding to determine the doses to be used in the thirteen-week study) comprised at the end of the study doses in the region of 2,600, 5,500, 8,200 and 11,700mg of rebaudioside A/kg body weight (bw)/day in males and of 2,900, 6,200, 9,200 and 13,100mg of rebaudioside A/kg bw/day in females (n=10 animals/group/gender). At the highest dose, in females, reduced body weight gain was observed that could be attributed to the palatableness of the food and its lack of calories. An increase in creatininaemia was reported in all treated males and females with the 2 highest doses. Effects unrelated to the administered doses show some minor variations in plasma bile acid levels and in suprarenal weight in females. A drop in bile acid levels is also reported in males at the two highest doses. At testicular level, weight loss (-7% compared with the control group) was observed at the highest dose. However, no histological alteration was reported after examination of the testicles, epididymis and seminiferous tubules.

The second, thirteen-week, study comprised at the end of the study doses in the region of 690, 1,500 and 3,150mg of rebaudioside A/kg/bw/day in males and in the region of 980, 1,900 and 3,700mg of rebaudioside A/kg bw/day in females. It was conducted in pre-pubescent rats aged from 40 to 45 days (n=20/group/gender). As in the previous study, reduced body weight gain compared with the control group was observed in males at the highest (-20%) and intermediate doses (-9%). Some modifications in motor behaviour (gripping strength, in particular) were reported as decreased in males, but without any real dose-effect relationship and with an increase in females. A few non dose-dependent variations, some of which appear to be gender-specific, were reported for plasma creatinine and urea, bile acid, triacylglycerols and cholesterol. Very moderate weight loss of some organs was observed at the highest dose: the epididymis in males, and ovaries, heart and kidneys in females. Based on the weight variations of organs, a NOAEL (No Observed Adverse Effect Level) of 1,473mg/kg bw/day could be proposed .

Mutagenesis:
Amongst the recent studies one publication provides an exhaustive review of existing data on the genetic toxicity of steviol and steviol glycosides (Brusik 2008). The vast majority of tests carried out report negative findings, which makes it possible to conclude that there is no genotoxic risk for humans associated with the consumption of steviol glycosides. Abundant studies both *in vitro* and *in vivo* have been conducted on diverse substances related to steviol using genetic toxicity tests measuring gene or chromosome alterations or primary DNA damage. For all these studies, the purity level of each sample tested should be taken into account.

The few positive results reported with steviol glycosides or steviol can be interpreted as stemming from methodological or interpretation problems associated with the interference of cytotoxic effects in *in vitro* tests, or with the use of specific bacterial strains or with the purity of the *S. rebaudiana* extract batches tested.

Reproduction and development:
The previous opinion mentioned that, in a toxicity study on reproduction conducted with extracts of *S. rebaudiana*, the exposure of rats through drinking water provoked an infertility, lasting for two

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months. Several studies on these extracts reportedly confirm the existence of effects on the male reproductive system: reduction in spermatogenesis, in seminal vesicle weight and in the testicular cell population. Moreover, this opinion recalled that steviol administered at oral doses of 500 and 1000mg/kg bw/day to hamsters provoked a reduction in the number of living foetuses per litter and in the average foetal weight.

Of the studies published recently, a two-generation study on Han Wister rats was conducted with rebaudioside A (97% purity) with food concentrations of 7,500, 12,500 or 25,000 mg/kg of food, corresponding respectively to 586 and 669, 975 and 1,115 or 2,048 and 2,237 mg of rebaudioside A/kg bw/day for males and females of the F0 generation and corresponding to 734 and 798, 1,254 and 1,364 or 2,567 and 2,768 mg of rebaudioside A/kg bw/day for males and females of the F1 generation (Curry, Roberts, Brown 2008). For the F0 generation, during the gestation and lactation phases the females were exposed to maximum doses of 713 and 1,379, 1,169 and 2,388 or 2,381 and 5,019 mg of rebaudioside A/kg bw/day respectively. For the F1 generation, during the gestation and lactation phases the females were exposed to maximum doses of 625 and 1406, 1058 and 2394 or 1406 and 4,893 mg of rebaudioside A/kg bw/day respectively.

Slight weight loss, associated with a transitional drop in food intake, was observed in females of the F1 generation treated with the two highest doses and in males of the F1 generation as well as males and females of the F2 generation treated with the highest dose. Moderate weight gain of the liver (around +10%) was observed in rats of the F0 generation and in females of the F1 generation treated with the 2 highest doses. Weight loss of the thymus (-3 to -12%) and of the spleen (-6 to -27%) was also reported for all the male and female rats treated with the two highest doses for the F1 generation and with the highest dose for the F2 generation. Regarding the reproduction function parameters (oestrous cycle, fertility, gestation indicator, sperm production), no significant treatment effect was observed. The study's authors proposed a NOAEL between 2,048 and 2,273 mg/kg bw/day.

In terms of estimating exposure

In the initial request, the stevia extracts were put forward as an ingredient in all processed foods (drinks, dairy products, sauces, sweets, biscuits, etc.). However, since the usage doses per foodstuff were not stated, it was impossible to estimate the exposure resulting from use of steviols as a sweetener for the French population.

No calculation of rebaudioside A exposure is put forward in the new dossier submitted for examination.

Of the studies published recently, a predictive study estimated rebaudioside A exposure by using data published in the literature on sweetener consumption in different countries (Renwick 2008). This method made it possible to predict that average and high consumer exposure levels for the general population would be 1.3 and 3.4 mg/kg bw/day respectively; for children, 2.1 and 5.6 mg/kg bw/day and for diabetic children, 3.4 and 4.5 mg/kg bw/day.

These findings only remain exposure predictions, as the method does not take account of new potential uses of a sweetener, since it replaces current intense sweetener uses with those supposedly attributable to the new sweetener, by retaining an "equivalent" sweetening potential. Moreover, the diet surveys on sweetener intakes used in this publication date from the early 2000s and do not take account of any increases in the consumption of sweetened drinks.

With these limits, the exposure predictions quoted in this publication show that the highest exposure intakes to rebaudioside A, stemming from its use as a sweetener, would stay a maximum of 263 and 365 times below the lowest NOAELs identified in recent animal studies (1,473 and 2,048 mg/kg bw/day). These predictions also indicate that this exposure would stay three times below the doses presenting no physiological effect on blood pressure or blood parameters linked to diabetics, measured in human studies (~ 17 mg/kg bw/day).

Conclusion

The observations made in the first opinion of 12 October 2007 concerned the findings of studies conducted with non-purified extracts of *Stevia rebaudiana*.

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The new dossier presented only concerns the use of rebaudioside A with a purity level higher than 97%. It has not been possible to set a acceptable daily intake (ADI) for rebaudioside A insofar as the only new data available to Afssa are publications that do not contain the unprocessed (raw) data of these studies. That said, based on the assessment of the findings of the last studies presented, not showing any adverse effects (thirteen-week toxicity study, two-generation reproduction study, critical analysis of mutagenesis studies and pharmacological studies in humans), and on predictive exposure calculations, Afssa considers that the use of rebaudioside A, extracted from *Stevia rebaudiana*, with a purity level higher than 97%, does not present a risk for consumers. This conclusion only concerns rebaudioside A, which was the focus of the most recent studies published, with a purity level higher than 97%.

However, Afssa makes the following remarks under the technological and chemical terms: a) it is important that the stability of rebaudioside A, depending on the pH level and temperatures of use under the food applications considered, can be assured, b) the intended foodstuffs, use conditions and doses of use per foodstuff shall have to be defined, c) chemical specifications must be drawn up for rebaudioside A, and d) the production method and necessary analytical inspection ensuring a rebaudioside A purity level higher than 97% must be defined.

Pascale BRIAND

Key words: Sweetener, rebaudioside A, *Stevia rebaudina*, food additive

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