International Stevia Council Meeting with EFSA

Parma, Italy - 11 June 2018

INTERNATIONAL STEVIA COUNCIL

ISC Delegation

Present at the meeting:

- Marta Baffigo, Cargill
- Henk Aalten, DSM
- Maria Teresa Scardigli, ISC

Present via conf call:

- Nicole Cuellar-Kingston and Zheng Yang, Cargill
- Sachin Bhusari, Coca-Cola
- Jeanine Van de Wiel, DSM
- Sidd Purkayastha, Pure Circle
- Hadi Omrani, Sweegen
- Ashley Robert, Intertek

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Background

- 24 April 2018: letter from EFSA seeking further information on the determination and quantification of kaurenoic acid in the food additive steviol glycosides (E 960) and requesting an *ad hoc* meeting.
- 11 June 2018: an *ad hoc* is scheduled between industry representatives and EFSA to discuss possible presence of the impurity kaurenoic acid in the food additive (E 960).
- In response to EFSA's inquiry and in preparation for the upcoming meeting, 4 industry representatives namely, DSM, Cargill, PureCircle, and Sweegen have provided data regarding analytical methods and results of the analysis for kaurenoic acid in various commercially available samples of stevia leaf extract.

Data from industry representatives on Kaurenoic Acid levels in various commercially available samples of stevia leaf extract

Number of Samples Tested	Methodology	LOD (ppm)
40	LC-MS	From <0.25 to <1

LC-MS = liquid chromatography- mass spectrometry; LOD = level of detection

No kaurenoic acid was detected at levels of detection ranging from <0.25 to <1 ppm.

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Corroborating Evidence of the lack of Kaurenoic Acid (KA) Genotoxicity

- *In silico* Quantitative Structure Activity Relationship (QSAR) analysis using three independent tools (Leadscope, CaseUltra, ToxTree) predict a lack of bacterial mutagenicity and mammalian genotoxicity potential. The *In silico* QSAR results are corroborated by the results of *In vitro* mutagenicity screening of KA.
- A recent study by Cano et al (2017) reported a lack of KA genotoxicity when tested at non-cytotoxic concentrations.
- The fruit cherimoya grown and consumed in localised areas of Europe since the end of the 19th century has been shown to contain levels of up to 60mg of KA per 100g of fresh fruit.
- KA therefore has a long history of consumption supporting a lack of human health concerns in regards to genotoxic potential.

Summary

- No measurable levels of KA in stevia leaf commercial samples using sensitive LC-MS methodology
- A mini Ames study conducted with KA showed no mutagenic potential at non-cytotoxic concentrations
- Negative mini Ames results supported by *in silico* QSAR analysis and recently published mutagenicity study (Cano et al 2017)
- KA would not induce a significant increase in the micronucleus frequency in TK6 cells in a definitive study

In conclusion:

- the results from the recent analysis and review indicate no safety concerns based upon a lack of KA in the commercial products and the fact that KA is shown to be non-mutagenic at non-cytotoxic concentrations and thus there is NO need to change the current EU SG specification.

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