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Perspectives on Low Calorie Intense Sweeteners with a Focus on Aspartame and Stevia

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Author's contribution

This paper is a translation into English of two earlier papers in Dutch (Verhagen H. (2012). Voeding Nu 10: 10-11; Peters S. and Verhagen H. (2012) Voeding Nu 11: 21-23), with updates. HV and SP wrote the original texts in Dutch. HV did the translation. CL integrated and updated the work. All authors read, improved and approved the final manuscript.

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ABSTRACT

The safety of some food additives/E-numbers, including low calorie (intense) sweeteners (LCS), is constantly the subject of dispute and controversy. However, since LCS have been assigned an acceptable daily intake (ADI) and an E-number following extensive assessment of available safety and toxicological data, consumer safety is assured. These substances have been carefully evaluated, for example by the European Food Safety Authority (EFSA), leading to the conclusion that they are essentially safe when consumption is below ADI levels. Although, intake data indicate that general consumption of LCS is relatively low, many people appear to remain concerned about their safety, particularly aspartame (E951). More recently, stevia (steviol glycosides, E960) has been marketed as a "natural" alternative to aspartame. However, it is unclear whether stevia can live up to its promises. With regards to public health, the real risk within our diet is not the safety of food additives, but rather more likely to be the potential impacts of consuming too much energy and/or an unhealthy dietary pattern.

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1. INTRODUCTION

Hazard and risk are essentially different concepts. A hazard is a potential source of an adverse health effect; however it is only considered a risk when the potential for excessive exposure exists. In principle, any substance that we are exposed to, for example via the diet, can be a health risk when exposure levels become too high. This applies to everyday substances like water, oxygen and salt and therefore logically applies to food additives such as low calorie sweeteners (LCS). LCS are substances that can be added to our food and beverages in order to provide a sweet taste without additional calories and they are now widely used in a variety of products. Toxicology is concerned with establishing the level at which intake/exposure is safe and above which the risk of adverse health effects may be increased. Acceptable Daily Intake (ADI) is the term used to describe the safe level of intake for food additives, and this has been defined as, "the daily intake of a chemical, which during an entire lifetime appears to be without appreciable risk on the basis of all known facts at that time" [1]. The ADI is expressed as mg per kg body weight (bw) (Table 1).

The ADI is typically determined following assessment of available data from *in vitro* and *in vivo* studies (usually from animal studies). The highest level of intake that does not cause apparent adverse effects for the most sensitive endpoint in the most sensitive species of test animals is established and identified as the "no observed adverse effect level" (NOAEL). The ADI is then calculated after applying a large safety/uncertainty factor in order to account for inter-species differences (e.g. between rat and humans) and intra-human differences (e.g. between the most sensitive and least sensitive individuals) [1]. In the case of most food additives, including LCS, this means applying an overall safety factor of 100 to the NOAEL; a factor of 10 for inter-species differences and a further factor of 10 for intra-human differences [2] as illustrated in Figure 1. This wide margin of safety is designed to ensure that at daily consumption below the ADI over an entire lifetime, no health risks are to be expected. Any potential hazard, therefore, is not an actual risk as a result of the application of these safety factors.

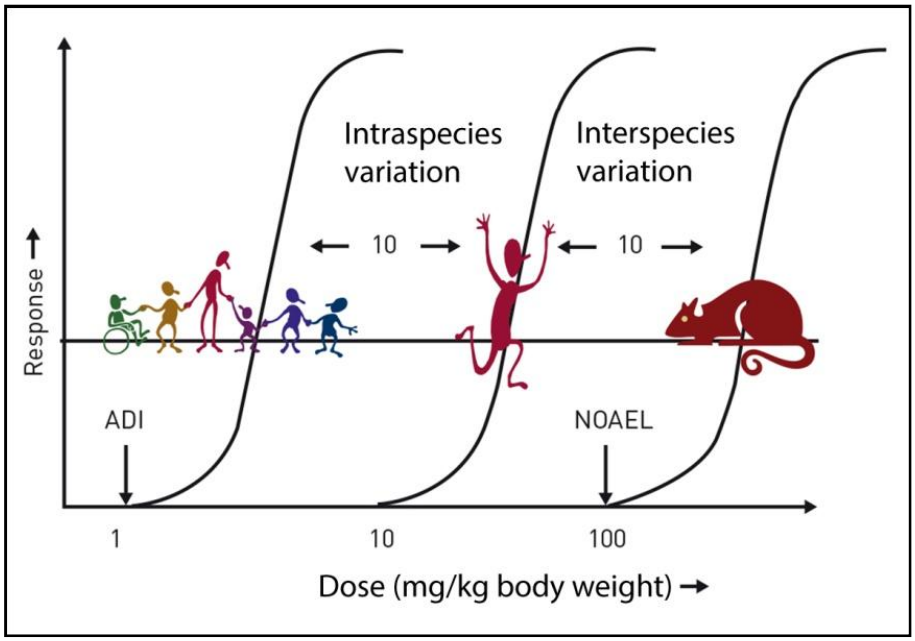

Figure 1. A safety factor of 100 is typically used to calculate and ADI from the NOAEL (10 for interspecies differences and 10 for intraspecies differences)

However, before a food additive can be approved for use in foods, a second step in the safety evaluation of the additive application is required. It is necessary to demonstrate that, under the proposed conditions of use, levels of consumption will not exceed the ADI. Hence, when a food additive has been approved and assigned an E-number, this should provide additional assurances that the ADI will not be exceeded.

2. RISK ASSESSMENT IN THE EU/EFSA

The safety of all LCS that have been permitted on the (EU) market have been extensively evaluated by the European Food Safety Authority (EFSA) or its predecessor the EU Scientific Committee on Food (SCF). "EFSA is the keystone of European Union (EU) risk assessment regarding food and feed safety. ….. EFSA was set up in January 2002, following a series of food crises in the late 1990s, as an independent source of scientific advice and communication on risks associated with the food chain. EFSA was created as part of a comprehensive programme to improve EU food safety, ensure a high level of consumer protection and restore and maintain confidence in the EU food supply" [3]. The establishment of EFSA has been laid down in Regulation EC/178/2002 [4] following the recommendations in the 2000 EU White paper on food safety [5], which also recommended that risk assessment is done independently from risk management. It is important to note that "EFSA's most critical commitment is to provide objective and independent science-based advice and clear communication grounded in the most up-to-date scientific information and knowledge. EFSA's independent scientific advice underpins the European food safety system.……. EFSA's activities are guided by a set of core values. These are: excellence in science, independence, openness and transparency, and responsiveness" [3].

Over the years, a number of LCS have been approved for use as food additives in the EU; these are listed in Table 1.

Permitted food additives are periodically re-evaluated in the EU and by 2020 this work must be completed [6] under EU Regulation EU/257/2010 [7]. The use of some food additives are frequently a cause for concern within some sections of society, especially after the publication of new scientific findings which may receive unbalanced (media) attention. One of the most well-known examples of this has been aspartame, whose latest re-evaluation was brought forward in 2011 at the request of the European Commission as a result of ongoing intense interest around its safety. In order to demonstrate transparency and promote debate within the EU on the matter, a public consultation on the draft opinion was carried out [8]. EFSA concluded that " ….. aspartame was not of safety concern at the current aspartame exposure estimates or at the ADI of 40 mg/kg bw/day. Therefore, there was no reason to revise the ADI of aspartame. Current exposures to aspartame - and its degradation product DKP (diketopiperazine) - were below their respective ADIs". In 2013 EFSA published its final Opinion on the safety of aspartame, again confirming its safety at the current levels of use [9].

Table 1: overview of LCS approved for use in the EU

Sweetener	E-number	Sweetness[c]	ADI (mg/kg body weight)	Year of approval
Acesulfame K	E 950	200	9	1984
Aspartame	E 951	180-200	40	1984
Cyclamic acid and its salts[a]	E 952	30	7	1984
Saccharin and its salts[b]	E 954	300-500	5	1977
Sucralose	E 955	600	15	2000
Thaumatin	E 957	2000-3000	'not specified'	1984
Neohesperidine DC	E 959	1900	5	1988
Steviol glycosides	E 960	300	4 (expressed as steviol equivalents)	2011
Neotame	E 961	7000	2	2009
Salt of aspartame acesulfame K	E 962	350	Aspartame: 40; acesulfame K: 9	2000
Advantame	E 969	37000	5	2014

[a]Sodium and Calcium salts, [b] Sodium, Potassium and Calcium salts, [c] Relative to sucrose

3. APPLICATION OF LCS IN SOFT BEVERAGES

Food additives that have been evaluated and considered to be safe for consumption by humans under the proposed conditions of use are allocated an “E-number” (E = European). Although this is meant to signify a guarantee for safe use, E-numbers are sometimes wrongly viewed as representing non-natural ingredients or even a risk to human health even though it is possible for a food additive to be of natural origin or be nature-identical.

With regards to manufacturers' LCS use, maximum usable dosage (MUD) levels for each sweetener have been specified for relevant food and beverage products which must not be exceeded. The actual levels used in products, however, will not always equate to the MUD as this will also be influenced by additional factors such as the intrinsic sweetening properties of the sweetener and the desired taste of the final product. Additionally, it is possible for some LCS to be used in combination as they have been shown to work synergistically to enhance the sweetness intensity or mask undesirable aftertastes. Quite often, this will result in a lower level of LCS being used in products than would be required if they were being used in isolation. Table 2 illustrates the volume of low calorie sweetened beverages that would need to be consumed in a day before an individual would surpass the ADI, assuming that the MUD has been used.

It remains important to monitor actual consumption levels of LCS in order to ensure intakes remain below the ADI and, within Europe, Member states have been instructed to do this on a regular basis as per EU Directive 94/35/EC [10]. Available data from a number of European countries such as the UK, France, Belgium and the Netherlands, indicate that indeed the consumption of LCS is (far) below the ADI, with only cyclamate intake among young children potentially exceeding the ADI [11].

4. FOCUS ON ASPARTAME AND STEVIA

Aspartame is a methyl ester of two amino acids (the building blocks of proteins); aspartic acid and phenylalanine. Gram for gram, it has the same energy content as sucrose, however because it is up to 200 times sweeter, very small amounts are required to achieve the same level of sweetness hence the labels "low calorie" or "intense". As the most commonly used LCS, it can be found in around 6,000 products worldwide, however food consumption surveys have consistently shown that intake levels do not exceed the ADI [9].

Animal studies have reported no negative effects from aspartame when administered in doses of up to 4,000 mg/kg-bw (a very high dose as because tests for toxicity normally do not exceed 1,000 mg/kg-bw). On the basis of the NOAEL at 4000 mg/kg-bw the ADI was established at 40 mg/kg-bw following the application of a safety factor of 100 [9,12].

Following ingestion, aspartame is broken down into its component parts; aspartic acid, phenylalanine and methanol which can also be obtained, quite often in greater quantities, from commonly consumed foods and beverages such as meat, poultry and fruit juices. Upon absorption, all three components are metabolized in the same way as they would be if obtained from the normal diet [13]. At current estimates of aspartame intake, the amount of absorbed phenylalanine and aspartic acid does not reach physiologically significant levels within the general population. The final component of aspartame metabolism is methanol which can be very toxic at high levels of intake. Excess methanol is converted to formic acid via formaldehyde in the liver, and it is this which ultimately produces the toxic effects of methanol.

Table 2. Lifetime daily consumption of light beverages for a 25 kg child and a 70 kg adult before surpassing the ADI

Low calorie sweetener	Child (bw = 25 kg)[a]	Adult (bw = 70 kg)[a]
Aspartame	1,67 L	4,66 L
Acesulfame K	0,64 L	1,80 L
Cyclamate	0,70 L	1,96 L
Saccharine	1,56 L	4,37 L
Sucralose	1,25 L	3,50 L
Steviol Glycosides	1,25 L	3,50 L

[a]*(calculated via www.zoetstoffen.nl).*

At high enough levels of intake, it can cause metabolic acidosis, blindness and poisoning of the central nervous system [14]. Fortunately, the body is capable of dealing with a certain amount of methanol; a single bolus of 2g of methanol can be tolerated without any negative effects [15]. It has been estimated that from one litre of soft drink sweetened with aspartame, 55 mg of methanol would be formed which is less than what is naturally found in one litre of fruit juice [9] and well within the 2g shown to be safe, therefore the risk of methanol toxicity from aspartame is negligible. Furthermore, the liver naturally processes 22 mg of formaldehyde per minute (around 50 grams per day) as part of normal biochemical processes [15] so the contribution of aspartame to the level of formaldehyde within the body is also negligible. A large amount of methanol would have to be consumed at once for it to become toxic, therefore if it is assumed that 55 mg methanol would be formed per litre of an aspartame sweetened soft drink, more than 36 litres of that beverage would have to be consumed at one sitting in order to exceed the safe amount of two grams. It is clear that at this volume of intake, one would develop problems other than methanol poisoning and this provides a good illustration of the importance of making a proper distinction between the terms “hazard” and “risk”.

4.1 Aspartame and Cancer

In 2006 and 2007, two studies were published by the Italian Ramazzini Institute which purported to prove that aspartame causes cancer in rats [16,17]. Substances which could potentially be genotoxic carcinogens in humans are not permitted and, although these were the only studies which provide support for such cancer claims, EFSA commissioned a panel of independent experts to examine the results of these studies. Based on the data from the first study, EFSA concluded that the tumours found were not as a result of the aspartame treatment, but of chronic inflammation of the lungs in the rats. Furthermore, it was found that the statistics appeared to be flawed and the results were not consistent. In order to assist in the assessment of the second study, EFSA made a number of requests for additional data but only some of this was subsequently made available to the panel. Another study, published by the same institute [18], claimed to demonstrate a dose response relationship between exposure to aspartame and the incidence of hepatocellular and alveolar/bronchiolar carcinomas in male Swiss mice. Once again, EFSA evaluated the results of this work and concluded that there were a number of flaws in the study design and how the study findings were reported, therefore it was decided that there was no reason to reconsider their original opinion on the use of aspartame at the permitted levels [19].

It is also worth noting that, upon examination of other publications by the Ramazzini Institute, the rats used by this group tend to develop cancer from many of the substances to which they are exposed [20-22], including Coca cola which does not contain aspartame [23]. To date, hundreds of safety studies have been carried out on aspartame and in none but the Ramazzini studies were any associations with genotoxicity or cancer reported [9]. In short, there are no indications that aspartame is carcinogenic, nor is this to be expected because the substances into which aspartame is metabolized in the body are known and occur naturally in food and in the body.

4.2 Diketopiperazine (DKP)

Diketopiperazine (DKP) is the major degradation product of aspartame and may form when a soft drink containing aspartame is stored for a long period of time at room temperature. DKP has an ADI of 7.5 mg/kg-bw. In the studies that were carried out to establish the ADI for aspartame, DKP was concurrently administered to the test animals in a ratio of 3:1 (aspartame: DKP) with no effects observed [9]. The safety of DKP has also been evaluated and confirmed in no less than thirteen toxicological studies [13]. It is also worth noting that DKPs are the most frequently occurring peptide derivatives in nature and can be found in many commonly consumed protein containing foods. EFSA included DKP in its recent Opinion on the safety of aspartame and concluded that with exposure estimates of up to 5.5mg/kg-bw at the 95th percentile for the general population, there was no reason to reconsider the current ADI [9].

4.3 Obesity and Premature Births

Based on epidemiological studies, it is regularly incorrectly reported (usually in the media) that there is a positive relationship between LCS beverage (hence, aspartame) intake and obesity. However, many of these studies do not consider the possibility of reverse causality, since people who are overweight or obese often tend to consume LCS beverages as part of a strategy to

manage their weight more effectively. It is therefore necessary to view apparent associations between LCS beverage intake and the development of obesity with caution. A number of studies have also been published which have investigated a possible association between intake of LCS beverages and the risk of preterm delivery [24,25]. Overall the evidence does not conclusively support the case of negative effects ASB intake on pregnancy outcomes [26] and additional studies would be necessary to establish a causal link. It is also worth noting that most epidemiological studies investigating the health effects of LCS simply consider intakes of all LCS collectively and do not consider the intake levels of individual LCS in isolation. This is a major weakness in this type of research as it may result in erroneous associations or mask any true associations that may exist.

4.4 Phenylketonuria

Phenylalanine, an essential amino acid, is formed from aspartame metabolism. However, patients with phenylketonuria (PKU), an inborn error of metabolism which is diagnosed by means of the neonatal heal prick test, are unable to break down the amino acid properly resulting in high concentrations in the blood. High levels of phenylalanine in children can result in a number of potentially very negative health effects such as impaired brain development. For this reason, the ADI is not applicable to PKU patients and as such, products containing aspartame must carry a warning that they are a source of phenylalanine. This warning, however, does not relate to any potential danger of aspartame to those in the general population.

4.5 Stevia as Aspartame's Alternative?

Although "natural" is not synonymous with healthy or nontoxic, there is an appetite within the market for a natural alternative to aspartame. One such alternative may come in the form of the leaves of a shrub native to South America; the Stevia rebaudiana plant of the chrysanthemum family. The leaves of this plant contain a large number of sweet diterpene glycosides known as steviol glycosides [27]. These can be isolated by a process of water extraction followed by purification and crystallization and have been used as a sweetener in various countries for many years.

In 2008, an ADI of 4mg/kg-bw (expressed as steviol equivalents) was established at the 69th Joint FAO/WHO Expert Committee on Food Additives (JECFA) [28], however it was not until 2011 that the use of steviol glycosides was approved in Europe following a positive Opinion by EFSA [29]. The primary reason for this delay was due to the timing of the submission of the safety dossier by the petitioners to EFSA. As per JECFA specifications [30], the final sweetening mixture must consist of not less than 95% of nine named steviol glycosides with the most abundant being stevioside and/or rebaudioside A. Thus, following isolation, the sweetener mixture is approximately 300 times sweeter than sucrose and is therefore classified as a low calorie intense sweetener. Steviol glycosides have been allocated an E-number (E960), indicating that they fulfil the same safety requirements as aspartame and any other approved food additive.

4.6 Metabolism, Safety and Application of Steviol Glycosides

Following ingestion, both stevioside and rebaudioside A experience similar metabolic fates; both are very poorly absorbed, if at all, and instead are converted to their common aglycone, steviol, via bacterial hydrolysis in the large intestine. A large proportion of steviol is then absorbed while the remainder is excreted in the faeces. Very limited amounts of free steviol are found in the plasma as it undergoes extensive first pass metabolism in the liver and is excreted in the urine predominantly as steviol glucuronide [31].

As with all approved food additives, extensive safety and toxicological testing has been conducted via *in vitro* and *in vivo* studies in order to establish the safety of steviol glycosides. Although the safety of steviol glycosides have been established, some *in vitro* studies have shown that the aglycone, steviol, and some of its oxidative derivatives exhibit genotoxic activity. These results, however, have not been replicated *in vivo* with doses of up to 8000 mg/kg-bw [32] therefore the associated risk is negligible. The ADI for steviol glycosides was established following analysis of data from a 2 year carcinogenic study in the rat in which a NOAEL for stevioside of 967 mg/kg-bw, equating to 388 mg/kg-bw steviol. Following the application of a safety factor of 100 the ADI was therefore set at 4 mg/kg-bw, to be expressed as steviol equivalents [30].

Can the new low calorie sweetener E960 completely replace the other LCS as a natural alternative? Although this would prove to be an attractive option for some, it does not seem likely. This is mainly due to the fact that E960 has a liquorice-like aftertaste which would negatively affect the taste of products too much for it to be used in isolation. Nevertheless, two of the major soft drinks manufacturers, Coca-Cola Company and PepsiCo, have each claimed to have developed a new beverage product in which stevia has replaced 30% of the sugar without compromising on taste. Officially, these products can be marketed as light (or diet) products as they contain 30% less sugar than the regular varieties, albeit that manufacturers may not do this as consumers tend to equate the label "light" in soft drinks with "calorie-free." The "stevia drinks" may have to be marketed differently, possibly by exploiting the perceived advantages of sweetening the products with a sweetener that has been derived from a natural source rather than using artificial alternatives.

4. CONCLUSION

All approved food additives, including LCS are safe to use in the permitted quantities. The decision of which sweetener(s) to use in products will be dependent upon a number of factors including the permitted MUD and the properties of the sweetener. The impact of substituting LCS for sugar in commonly consumed food and beverage products on health, particularly weight status, has been the subject of debate in recent years. Recent research [33] has shown that children who drank a soft drink sweetened with sweeteners instead of a soft drink with sugar in a placebo controlled double blind study had a lower body weight and/or consumed fewer calories. This suggests that LCS could indeed make a positive contribution to public health, as previously calculated [34].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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