Special Article

Biological fate of low-calorie sweeteners

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With continued efforts to find solutions to rising rates of obesity and diabetes, there is increased interest in the potential health benefits of the use of low- and no-calorie sweeteners (LNCSs). Concerns about safety often deter the use of LNCSs as a tool in helping control caloric intake, even though the safety of LNCS use has been affirmed by regulatory agencies worldwide. In many cases, an understanding of the biological fate of the different LNSCs can help health professionals to address safety concerns. The objectives of this review are to compare the similarities and differences in the chemistry, regulatory status, and biological fate (including absorption, distribution, metabolism, and excretion) of the commonly used LNCSs: acesulfame potassium, aspartame, saccharin, stevia leaf extract (steviol glycoside), and sucralose. Understanding the biological fate of the different LNCSs is helpful in evaluating whether reports of biological effects in animal studies or in humans are indicative of possible safety concerns. Illustrations of the usefulness of this information to address questions about LNCSs include discussion of systemic exposure to LNCSs, the use of sweetener combinations, and the potential for effects of LNCSs on the gut microflora.

INTRODUCTION

Sweetness is a characteristic of foods and beverages that humans perceive through taste receptors as early as infancy. A wide variety of structurally diverse compounds are perceived as sweet.[1] Low- and no-calorie sweeteners (LNCSs) have been substituted for carbohydrate sweeteners, as a means of reducing caloric intake and helping diabetics control blood sugar levels, for over a century. From the very earliest uses of saccharin and, later, cyclamate, there have been concerns about the safety of LNCSs. Research and reviews demonstrating their safety are numerous and include assessments by government and international food safety authorities; however, the safety of LNCSs continues to be a topic of public debate.

Although all LNCSs induce perceptions of sweetness, they do not share common absorption profiles, metabolic fates, or excretion pathways. These properties of LNCSs are critical components of their safety assessment, but there are significant differences among the LNCSs, and this aspect of their safety profile is often not recognized. The common LNCSs acesulfame potassium, aspartame, saccharin, and sucralose are included in this review. The expanding use and market for stevia leaf extract (steviol glycosides) warranted inclusion of this high-intensity sweetener as well. These compounds are very diverse in their structure, metabolism, and history of use. Saccharin use predates the establishment of food and drug safety regulations by many decades, but it has a long and controversial history.[2] Aspartame has become a widely used LNCS, especially in beverages.

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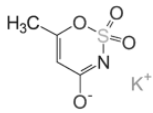
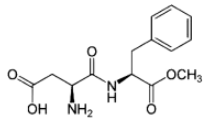
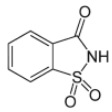
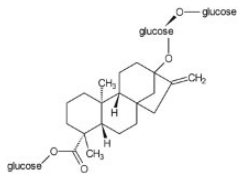
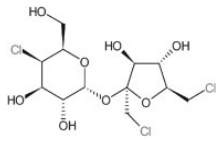
Although its metabolism is simple and well documented, the safety of aspartame remains a topic of attention despite global approval and a recent and comprehensive reevaluation by the European Food Safety Authority (EFSA), which concluded that aspartame was "not of safety concern" at current consumption levels.[3] Acesulfame potassium is widely used, but primarily in combination with other LNCSs. As a result, acesulfame potassium is little known to consumers and even to many food technologists and nutritionists. Stevia-based sweeteners were used by indigenous people of South America for years, but highly purified stevia leaf extracts have only recently been permitted as a legal food ingredient in most developed countries.

As shown in Table 1, LNCSs are a diverse group of compounds structurally. In addition, the fate of these compounds within the human body following consumption of foods and beverages sweetened with LNCSs differs. This is critically important to understand, as there are many examples in the scientific literature in which biological or dietary effects observed in studies with one LNCS are incorrectly extrapolated to all LNCSs without supporting scientific evidence.

The LNCSs included in this review are designated *high-intensity sweeteners* because their sweetening potencies are many times higher than that of sucrose (Table 1). This means that, in comparison with the sucrose (or another caloric sweetener) that the LNCS is replacing, very little of the LNCS is actually present in food or beverage. For example, aspartame has 200 times the sweetening potency of sucrose, meaning that when solutions of sucrose and aspartame are compared, the same sweetness associated with a sucrose solution will be associated with an aspartame concentration 200 times lower than the concentration of sucrose.[4] Therefore, very little of an intense sweetener is actually present in the "diet" food or beverage. In most cases, the maximum sweetness levels that can be achieved with intense LNCSs is less than what can be achieved with sucrose due to other "off tastes" from the LNCS, such as bitterness or metallic tastes.[5] This will be further discussed below.

Lower levels of LNCS use for equivalent sweetening power means that the amount of the LNCS or its metabolites that will be absorbed, metabolized, or excreted is also very low compared with that of caloric sweeteners. The very low levels of use typically result in vast differences between the highest estimated human exposure from usual LNCS intake and the exposure levels shown to cause an adverse effect in animal studies. In fact, most adverse effects in LNCS animal safety studies

Table 1 **Comparison of low- and no-calorie sweeteners**

Sweetener (E no.)	Compound classification	Structure	Sweetness intensity[a]	Approximate amount that replaces 25 g of sugar
Acesulfame K (E 950)	Oxathiazinone dioxide		≈200 times	125 mg
Aspartame (E 951)	Methylated dipeptide		≈200 times	125 mg
Saccharin (E 954)	Benzoic acid sulfimide		≈300 times	80 mg
Steviol glycosides (E 960)	Glycosylated diterpenes	 (stevioside shown)	200–300 times	80–125 mg
Sucralose (E 955)	Chlorinated disaccharide		≈600 times	40 mg

[a]Sweetness as compared with sucrose on a gram-for-gram basis.

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are due to a caloric or nutrient imbalance caused by the addition of a large amount of LNCS, an essentially non-nutritive ingredient, to the diet, which displaces and dilutes nutritive ingredients.

Toxicological testing employs a range of concentrations to characterize dose–responses of potential adverse effects. High doses are required to ensure any potential adverse effects are identified, while lesser concentrations are included to identify a dose that does not cause adverse effects or the no observed adverse effect level (NOAEL). As will be discussed below, NOAELs are used to establish acceptable daily intakes (ADIs) with appropriate safety factors.

Prior to the regulatory approval of an LNCS, extensive studies are undertaken to elucidate the biological fate of the LCNS in the body (called *toxicokinetics*), including absorption, distribution, metabolism, and excretion (ADME) following ingestion. An understanding of the ADME of the individual LNCS is key to extrapolating the results of preclinical safety studies conducted in animals to human risk assessment and the establishment of an ADI. For example, comparison of the ADME characteristics in animal species, such as mice and rats, with the ADME characteristics observed in humans will be used to determine which species is the most appropriate for safety studies, as this species will be most predictive of possible toxicity in humans. Often, this is the basis for selecting the results from a specific safety study that will be selected to establish the ADI, although studies in multiple species will have been conducted.

The ADI is an important and often misinterpreted value. The ADI is defined as the amount of a food additive, expressed on a body weight basis, that can be consumed daily over a lifetime without appreciable health risk.[6] The ADI is not a threshold between safe and unsafe; rather, it is a calculated value, derived by dividing the NOAEL observed in toxicology studies by a safety factor. The NOAEL is the daily amount consumed in long-term, repeated-dose studies that was shown to have no adverse effects in the animals; in other words, it is a daily intake level that is too low to cause any biological effects. The safety factor is established by regulatory agencies and convention to ensure protection of the most susceptible and sensitive individuals in an entire population, including children and pregnant women.[7] Often, the safety factor used is 100, resulting in the ADI being set at a level 100 times lower than the NOAEL, ensuring a wide margin of safety. For example, if the amount shown in animal studies to have no effect when consumed daily for the majority

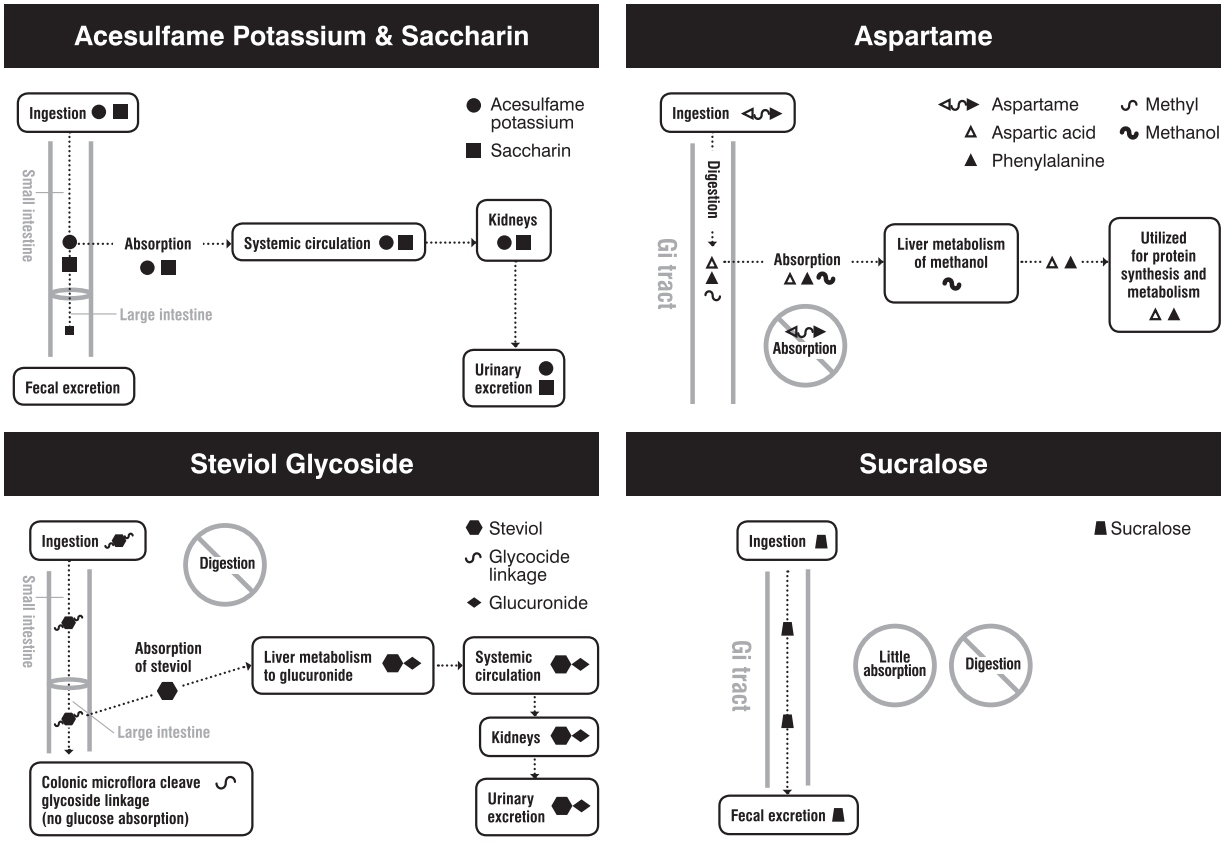

Figure 1 **Comparison of the major routes of absorption, digestion, metabolism, and excretion of acesulfame potassium, saccharin, aspartame, steviol glycosides, and sucralose.** See text for detailed descriptions of these processes and any other minor routes that may exist.

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of the animal's lifetime was 4000 mg per kilogram of body weight, the NOAEL would be 4000 mg/kg/d and, with a 100-fold safety factor, the ADI would be 40 mg/kg/d. Thus there is a 100-fold reduction from the amount shown to have no effect to the established ADI. This is a much greater safety factor than exists for most nutrients and naturally occurring food components. Therefore, the ADI is a level of daily intake considered safe for everyone, including those with the highest potential exposure to an ingredient.[7] An understanding of the metabolism and disposition of ingested LNCSs adds additional assurance that occasional consumption above the ADI is highly unlikely to cause any adverse effects. It should be noted that the process of safety evaluation of LNCSs for use as food additives is the same for all LNCS, regardless of whether the LNCS is from a natural source.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION OF LNCSS

The sections that follow provide a brief review of the chemistry and regulatory status of each LNCS, followed by a detailed review of studies investigating the toxicokinetics of that LNCS. An overview of the major pathways of the ADME of these sweeteners is provided in Figure 1. For more information on toxicokinetics, Dybing et al.[8] provide an excellent overview of toxicokinetic modeling and the biological processes that affect the fate of compounds contained in food ingested into the body, as well as an explanation of how such information is used in safety assessments.

Acesulfame potassium

Chemistry and regulatory status. Acesulfame potassium (acesulfame K [ACK], or E950) is a non-nutritive sweetener belonging to the oxathiazinonedioxide class of chemicals whose sweetness properties were discovered in 1967, when a researcher accidentally tasted a newly synthesized compound. Acesulfame potassium, whose relative sweetness is approximately 200 times that of sucrose, was selected as the best potential oxathiazinonedioxide-class sweetener because of its superior sweet taste, high water solubility, and stability.[4,9,10] The stability of ACK in foods, especially its resistance to thermal degradation, makes it an attractive non-nutritive sweetener for foods and beverages.[11,12]

The Food and Agriculture Organization of the United Nations–World Health Organization (FAO-WHO) Joint Expert Committee on Food Additives (JECFA) established an ADI for the safe use of ACK based on the results of extensive human and animal toxicology and safety studies.[13] Subsequent approvals for the use of ACK as an LNCS occurred in 1985 and 1988 by the European Union's Scientific Committee for Food and the US Food and Drug Administration (FDA), respectively (FDA, 1988; Scientific Committee for Food, 1985).[4,14] All approvals concurred that neither rats nor dogs exhibited adverse effects when fed diets containing up to 3% ACK for up to 2 years, which, based on body weights, corresponded to NOAELs of 1500 mg/kg/d and 900 mg/kg body weight/day for rats and dogs, respectively. Differences in human ADIs stemmed from which species better represented humans and, thus, which NOAEL should form the basis of the ADI calculation. The initial approval by JECFA established an ADI of 0 to 9 mg/kg/d on the basis of the 2-year dog study.[13] The JECFA later reevaluated available data and revised the ADI to 0 to 15 mg/kg/d on the basis of the 2-year study in rats, concluding the rat study better represented humans because exposures in the rat study began in utero and because chronic exposure for 2 years represented a greater portion of the rats' lifespan compared with the same exposure duration in dogs.[15] The Scientific Committee for Food assigned an ADI of 0–9 mg/kg/d on the basis of the study in dogs, while the FDA set an ADI of 0–15 mg/kg/d on the basis of the study in rats.[4,14] Later evaluations reaffirmed the safe use of ACK as a non-nutritive sweetener with the same previously established ADIs.[14,16]

Absorption, metabolism, and excretion. Acesulfame potassium is a hydrophilic, organic acid derivative that, once ingested, is rapidly and almost completely absorbed into the systemic circulation.[12,17] Absorbed ACK is distributed via the blood to tissues throughout the body. In rats, the highest tissue concentrations were observed in absorption and excretion organs (ie, gastrointestinal tract, urinary bladder, and kidneys). Concentrations in remaining organs were similar to blood concentrations, and concentrations of all organs decreased in parallel with blood concentrations.[18]

Acesulfame potassium can transfer across the placenta and appear in fetal tissues at low concentrations. When pregnant mice were administered a single, large intragastric dose of 20 mg (about 400 mg/kg of body weight, or 37–44 times the ADI), the peak ACK level in amniotic fluid was observed in amniotic fluid collected 5 hours after intragastric ACK administration.[19] Following a single oral dose of radiolabeled ACK at 10 mg/kg to pregnant rats late in pregnancy, fetal ACK levels were low when maternal blood concentrations were at their highest (ie, fetal concentrations were 7% and 33% of the highest maternal blood concentrations observed 0.5 hour and 1.5 hours after dosing, respectively).[15] The placenta tissue concentrations were higher than those in the fetus, thus the placenta acts as a protective barrier, which was also confirmed by the lack of

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change in the amniotic fluid ACK concentrations. These studies confirm that maternal exposure to ACK does not pose a risk to the fetus.

Human, animal, and in vitro studies have shown that ACK is not metabolized in humans or animals prior to excretion. In radiolabeled studies, only intact ACK (and no metabolic products) was detected in serum, urine, feces, and/or bile following oral administration. In addition, no metabolism was detected following 24-hour incubations with urine and fecal samples of human and animal origin.[12–18,20]

Absorbed ACK is excreted primarily via the kidneys into urine within 24 hours after consumption. Human studies using radiolabeled ACK demonstrated that most (98%) of a 30-mg dose is excreted within 24 hours of ingestion; nearly all (>99%) excretion occurred via the urine, with less than 1% excreted in feces. Excretion in animals is similar to that in humans, whereby absorbed ACK is excreted largely in the urine within 24 hours after consumption. In rats and dogs given radiolabeled ACK orally, ≥82% of ingested doses were excreted in urine (with the remainder excreted fecally). In rats, results after either a single dose or 60 consecutive days of consumption were similar, demonstrating that repeated consumption does not affect ACK kinetics.[13,15,16,20]

Acesulfame potassium is also excreted in the milk of lactating animals. In lactating rats administered a single oral dose of 10.6 mg of radiolabeled ACK per kilogram of body weight, approximately 1.6% of the maternal dose was excreted in milk within 24 hours of dosing and 0.16% was excreted in milk during the second day after dosing; the mean milk concentration over 48 hours was about 6.3 times the maternal blood concentration.[15] Zhang et al.[19] evaluated ACK concentrations in milk following intragastric administration of 20 mg of ACK (about 400 mg/kg, or 37–44 times the ADI) to mice and reported that the highest ACK concentration in milk (about 360 μg/mL) occurred 9 hours after dosing. The recent report of detection of low levels of ACK in human breast milk corresponds with and supports the results of these earlier animal studies in which low concentrations of ACK were repeatedly detected in milk 48 hours after administration.[21]

Acesulfame potassium injected intravenously in rats has a blood elimination half-life of about 14 minutes, which demonstrates that ACK is rapidly cleared from the general circulation. In rats given ACK orally at 10 mg/kg, maximum blood levels (0.7 μg/mL) occurred about 0.5 hour after dosing and then declined slowly, likely because of continued absorption from the gastrointestinal tract (resulting in a blood elimination half-life = 4.8 hours). Rapid absorption and excretion of ACK also occurs in humans: blood levels (0.2–0.3 μg/mL) peak 1 to 1.5 hours after ingestion of a single oral dose of 30 mg (about 0.4 mg per kilogram of body weight) followed by a blood elimination half-life of 2.5 hours. In dogs, maximum blood levels (about 7 μg/mL) occur 1 to 1.5 hours after ingestion of a single oral dose of 10 mg per kilogram of body weight, followed by a blood elimination half-life of 1.3 hours.[15,18,20] Acesulfame potassium absorption in pigs was also relatively rapid, with maximum blood levels (0.35–0.72 μg/mL) reached 1 to 2 hours after oral administration of a single dose of 3.6 to 4.5 mg/kg.[15,16]

Repeated exposures did not alter the fast absorption profiles of ACK in rats and dogs. Unlabeled ACK was administered either as part of the diet in rats (840 or 1325 mg/kg/d) or as daily bolus doses in dogs (900 or 1500 mg/kg/d) for 2 weeks prior to 24-hour toxicokinetic assessments. The 24-hour areas under the curve in both species were proportional to dose and were 848 to 934 μg · h/mL and 1521 to 1671 μg · h/mL in rats and 2149 to 3819 μg · h/mL and 3065 to 5722 μg · h/mL in dogs for the lower-dose and the higher-dose group, respectively. Dogs, whose bolus exposure regimen allowed assessment of peak plasma concentrations, exhibited peak levels of 180 to 311 μg/mL and 273 to 491 μg/mL for the lower dose and the higher dose, respectively. Following a continuous exposure paradigm, rats had steady-state plasma concentrations of 16 to 71 μg/mL and 30 to 119 μg/mL.[16]

Acesulfame potassium absorption and excretion profiles have not been evaluated in children specifically; however, excretion of drugs largely excreted unchanged in the urine occurs in infants and children at rates similar to, or greater than, those in adults.[22] Therefore, excretion rates of ACK in children are expected to be similar to rates observed in adults. In rat toxicity studies, exposures that began in utero and continued throughout the lifetime of the offspring occurred without adverse effects.[14,15] The highest exposure concentration in the study that included evaluation of all rat life stages, 3% ACK in diet (or 1500 mg per kilogram of body weight per day), is the NOAEL used for the basis of ADIs set by the JEFCA and the FDA. Species and age differences are accounted for in safety assessments through the use of a 100-fold uncertainty factor to convert the NOAEL observed in an animal study to the ADI (ie, the ADI is calculated by dividing the NOAEL by 100).

Potassium in acesulfame potassium. Acesulfame potassium is structurally a potassium salt, and individuals on (or recommending) potassium-restricted diets may question whether ACK intake should be monitored for its contribution to total potassium intake. No potassium-specific data were reported in ACK safety and toxicology studies, which focused on characterizing

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all potential adverse effects associated with ACK without evaluating effects on circulating potassium levels. Some ACK studies indicate dissolution of ACK into free acesulfame and free potassium but do not examine the extent of dissolution (eg, a reduction in digoxin toxicity was attributed the potassium content of the dose administered in guinea pigs, and a 90-day study in rats was performed using equivalent dietary potassium levels to elucidate possible effects from ingestion of potassium alone).[15,20] In absorption, metabolism, and excretion studies, acesulfame was clearly not metabolized prior to excretion, but results were unclear whether the excreted moiety was intact ACK or the acesulfame anion.

More evidence for dissolution is provided by the aspartame-acesulfame salt; it dissociates into an anion (negatively charged acesulfame) and a cation (positively charged aspartame) in saliva in the mouth or when added to aqueous foods. Because the 2 ions released by the aspartame-acesulfame salt are the same 2 ions derived from the approved sweeteners (ACK and aspartame), the Scientific Committee for Food concluded that use of the salt raised no additional safety considerations.[16] The acesulfame-aspartame salt does not contain potassium.

Since no studies were found that report the expected dissociation constant for potassium from ACK, the most conservative approach for estimating dietary contributions would be to consider the worst-case contribution of dietary potassium. Using the ADI of 0 to 15 mg/kg/d, the upper range for a 60-kg individual would be 900 mg of ACK per day.[23] Because ACK is 20% potassium by weight, consumption of 900 mg of ACK per day, would, at most, add 180 mg of potassium to an individual's daily intake. To illustrate contributions from individual portions of beverages, the highest amount of ACK contained in an 8-oz diet soda was found to be 41 mg, which correlates to 61 mg of ACK in 12-oz serving.[24] Therefore, consumption of a 12-oz can of diet soda would, at most, add 12 mg of potassium to an individual's daily intake.

Aspartame

Chemistry and regulatory status. Aspartame (also referred to as E951) is a low-calorie sweetener used extensively worldwide as a tabletop sweetener and in a wide variety of foods and beverages, including chewing gum, yogurt, desserts, and nutritional bars. Its calorie content per gram is similar to that of sucrose (≈4 calories per gram), but aspartame's sweetening intensity is approximately 200 times that of sucrose. As a result, only a small amount of aspartame is needed to achieve sweetness, leading to virtually no calories from aspartame in sweetened products.

Aspartame is a white, odorless crystalline molecule whose structure is quite simple, being a methyl ester of a dipeptide containing 2 amino acids that occur widely in fruits, vegetables, nuts, and dairy products, namely, *L*-aspartic acid and *L*-phenylalanine.[25] The JECFA established an ADI for aspartame of 40 mg/kg/d in 1981, on the basis of results of extensive chemical analyses, toxicology testing, and human clinical studies. Aspartame was approved for use in foods and beverages and as a tabletop sweetener by the FDA and several other regulatory agencies at around the same time.[3,26,27] Currently, aspartame is approved for use as a low-calorie sweetener food additive in over 90 countries worldwide.[26] The ADI for aspartame established by the FDA is 50 mg/kg/d.[27]

Current estimates show that, although aspartame intakes have increased since the 1980s, they remain well below the ADI. The most current data in the United States showed an average intake of 4.9 mg/kg/d and a 95th percentile intake of 13.3 mg/kg/d.[28] In the recent (2013) review of aspartame by the EFSA, mean exposure based on 26 studies in 17 European countries was estimated to be 1.2 to 5.3 mg/kg/d, while the highest consumers reached 1.9 to 15.6 mg/kg/d.[3] Even at the highest levels of consumption, intakes are less than 50% of the ADI.

Absorption, metabolism, and excretion. Aspartame is solely consumed orally through intake of beverages, foods, and chewing gum, with a small amount used in oral pharmaceutical preparations (tablets and liquids). Starting in the 1970s, several in vitro, in vivo, and clinical studies, reviewed extensively elsewhere,[28] elucidated the pharmacokinetics of aspartame and established that, after ingestion, aspartame is quickly digested into its 3 main components: phenylalanine, aspartic acid, and methanol.[29,30]

The breakdown of aspartame occurs in the gastrointestinal tract by esterases and peptidases, and none of the intact, unhydrolyzed aspartame reaches the bloodstream.[25,31] Aspartame is digested in both the gastrointestinal lumen and the inside intestinal mucosal cells to methanol, aspartic acid, and phenylalanine, which are all absorbed into the bloodstream.[25,29,30] Only the digestion products, ie, methanol (≈10% weight of aspartame) and the 2 amino acids phenylalanine (≈50%) and aspartic acid (≈40%), reach the circulation, and they are in the same form as when absorbed into the body from natural food sources such as fruits, vegetables, and protein foods such as meat, fish, eggs, dairy, or legumes.[25]

The amounts of these digestion products are much lower than those obtained from many other natural dietary sources.[3,25] For example, the amount of methanol in tomato juice is 6 times greater than that derived from

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aspartame in diet cola.[25] The amino acids aspartate (ie, anion of aspartic acid) and phenylalanine are very common in the diet, found in foods such as lean protein, beans, and dairy, with 100 g of chicken providing an almost 40 times greater intake of aspartate and a 12.5 greater intake of phenylalanine than a diet soda.[25] In the body, the 3 digestion products follow their normal metabolic pathways, being broken down further, taken up by tissues in the body, or excreted. Thus, due to the rapid digestion of aspartame in the gastrointestinal lumen and small intestinal mucosal cells before reaching the bloodstream, the intact aspartame molecule is never present in internal tissues in the body or breast milk.[3,25,28] The absence of aspartame in the breast milk of lactating women consuming aspartame was recently confirmed.[21]

Many studies have investigated the metabolism of aspartame in a variety of animals, including rodents, dogs, rabbits, pigs, and monkeys, as reviewed by Magnuson et al.[28] The majority of these studies use radiolabeled [^{14}C] incorporated into 1 of the 3 components of aspartame – the aspartic acid, phenylalanine, or methyl moiety – to track the metabolism, distribution, and excretion of aspartame and its resulting digestion products. In the 1970s, Oppermann and Ranney[32–35] began metabolism studies in rodents, rabbits, and monkeys, using radiolabeled aspartame doses of 10 to 1600 mg/kg/d and found that 100% was cleaved to methanol, aspartic acid, and phenylalanine before entering the portal circulation, with no aspartame detected in the plasma. After digestion, the components are processed in the body the exact same way as if they came from other dietary sources, ie, they are distributed to the rest of the body, undergo further metabolism, and are utilized or excreted as outlined below. There was little difference in the metabolism of aspartame across species, regardless of where the [^{14}C] was incorporated. To further elucidate the enzymes responsible for aspartame metabolism, Hooper et al.[31] incubated solutions of 1 mM aspartame or aspartame analogues with human and pig intestinal and kidney microvillar membranes in the absence and presence of peptidase inhibitors and enzymes. Aminopeptidase A and, to a lesser extent, aminopeptidase W were found to be the major peptidases involved in aspartame hydrolysis.

Research on the metabolism of aspartame in humans in the late 1970s and throughout the 1980s has provided evidence that, as shown in animals, aspartame is fully digested into methanol, aspartic acid, and phenylalanine in human adults, adolescents, children, and infants and does not reach the general circulation as the intact molecule.[3] This is important from a safety assessment perspective because studies in which aspartame has either been injected into the body or added directly to cells in culture, thereby bypassing the process of digestion, do not provide biologically plausible exposure scenarios and subsequently do not provide useful information relevant to the safety of dietary consumption of aspartame. The complete digestion of aspartame into the same digestion products that are commonly found in much higher levels in foods has been a critical component of the safety evaluation of aspartame and was again emphasized in a recent EFSA review of the safety of aspartame, which concluded that aspartame poses no health concerns to the general population, to children, or to pregnant and breastfeeding women.[3]

Metabolism of aspartame digestion products. Methanol from aspartame enters the portal circulation and is quickly metabolized to formaldehyde by catalase-peroxidase (in rodents) or alcohol dehydrogenase (in primates and humans), as reviewed in Butchko et al.[25] Formaldehyde is then oxidized to formic acid by formaldehyde dehydrogenase in a matter of minutes, with the half-life of formaldehyde being 1 to 2 minutes. Formic acid is excreted from the body in the urine, or is further metabolized to carbon dioxide and excreted through the breath. Because of the negative health effects of rapid consumption of high levels of methanol sufficient to cause methanol toxicity, many animal and human studies have investigated the metabolism of methanol from aspartame (details below). Methanol toxicity occurs when pathways of formic acid metabolism are overwhelmed, resulting in a build-up of concentrations in blood. Studies in humans, however, have shown that there are no changes in baseline blood formate (the anion of formic acid) levels following consumption of aspartame-containing products, even with very large single doses of aspartame or repeated chronic exposure over time (reviewed in Magnuson et al.[28] and summarized below). Additionally, the FDA concludes that the safe level of methanol intake in humans is 7.1 to 8.4 mg/kg/d, roughly 25 times higher than the level of methanol derived from aspartame in the 90th percentile of intake.[36] Furthermore, methanol is obtained from many other dietary sources; for example, the average methanol concentration in various fruit juices was 140 mg/L, resulting in consumption of 28 mg of methanol from a 200-mL serving of juice.[37] This issue was addressed extensively in the recent EFSA reevaluation of aspartame safety.[3] Methanol is both present in free form and produced from other natural food components, such as pectin, during the digestion of many foods and beverages, including fruits, fruit juices, coffee, vegetables, and alcoholic beverages.

In normal human adults, blood methanol concentrations were undetectable after administration of aspartame at 34 mg/kg/d, equivalent to 10 times the 90th

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percentile of dietary aspartame intake.[38] Increases in blood methanol were detected only after doses of aspartame exceeded 100 mg/kg/d, equivalent to approximately 12 L of diet soda in a 60-kg person.[38] At extreme doses of aspartame (200 mg/kg/d), blood methanol levels rise, but not blood formate levels.[29] Urinary formate levels increased with this high dose, peaking 8 hours after aspartame ingestion, indicating that the human body is able to quickly process and eliminate the formate generated from even extreme doses of aspartame.[28]

Potentially sensitive populations have also been investigated. One-year old infants were as effective as adults in digesting aspartame, as their blood methanol concentrations were similar to or lower than those found in adults after receiving aspartame at 34 to 100 mg/kg.[39] Individuals with liver disease, a population sensitive to methanol toxicity, had blood methanol, blood formate, and urinary formate concentrations similar to those of healthy control groups after receiving aspartame at 15 mg/kg.[40] Additionally, individuals heterozygous for phenylketonuria (PKU) gene mutations who were given 600 mg of aspartame hourly for 8 hours did not differ from control subjects in levels of blood methanol, blood formate, or urinary formate.[41] Long-term studies in adults have also confirmed the inability of dietary aspartame to generate sufficient methanol to cause harm, with administration of 75 mg/kg every day for 6 months causing no detectable change in blood methanol, blood formate, or urinary formate.[42]

There are many allegations of toxicity of aspartame attributed to the production of methanol resulting from the digestion of aspartame. However, this is biologically implausible when the exposure to methanol produced from aspartame is compared with the exposure to methanol from other sources. For example, the average methanol concentration in various fruit juices was 140 mg/L,[37] resulting in consumption of 28 mg of methanol from a 200-mL serving of juice. During its recent review of aspartame safety, the EFSA conducted an extensive analysis of food intake surveys and concluded that, even using conservative aspartame intake estimates, aspartame contributed less than 10% of total daily methanol exposures.[3]

The 2 other digestion products of aspartame are amino acids. In the body, aspartate (ie, the carboxylate anion of aspartic acid) is converted in enterocytes to oxaloacetate through transamination before reaching the portal circulation and entering the free amino acid pool (see review in Magnuson et al.[28]). Oxaloacetate and aspartate are interconverted in the body and can participate in the urea cycle and gluconeogenesis.[3] Aspartate can also be used to generate other essential amino acids (methionine, threonine, isoleucine, lysine) and acts as a neurotransmitter by stimulating the *N*-methyl-D-aspartate receptors.[43,44] Excess aspartate is excreted in the urine. High blood aspartate has been associated with neuronal necrosis in animal toxicity studies, and therefore extensive work has been done to assess the level of aspartate produced from aspartame and reaching the general circulation. Several animal and human studies (details below) have shown that it is not possible to raise blood aspartate levels in humans through consumption of aspartame products because of rapid metabolism and incorporation of aspartate into proteins.[45] Additionally, the 90th percentile intake of aspartame provides approximately only 2% of the dietary intake of aspartate by adults and children.[25]

In normal adults, aspartame doses of 34 and 50 mg/kg caused no significant change in plasma levels of aspartate or other related amino acids, including asparagine, glutamate, and glutamine.[45–47]

Long-term studies in adults given aspartame at 75 mg/kg every day for 24 weeks found no effect on fasting plasma aspartate concentrations.[42] In a study with 1-year-old infants, aspartame at doses of 34 and 50 mg/kg did not change plasma concentrations of aspartate or other amino acids tested.[48] Lactating women have also been studied, with aspartame given at 50 mg/kg having no biologically significant effect on levels of aspartate or phenylalanine in breast milk.[49]

The essential amino acid phenylalanine is absorbed by mucosal cells in the gastrointestinal tract and enters the portal circulation to the liver, where it can be partially converted to the amino acid tyrosine by phenylalanine hydrolases.[50] Phenylalanine reaching the systemic circulation can be distributed throughout the body, including the brain, where it is needed for normal growth and development.[25] As mentioned previously, it can also be converted to tyrosine and, eventually, into the catecholamine neurotransmitters dopamine, norepinephrine, and epinephrine.[50] Phenylalanine intake in excess of need by the body is excreted in the urine. If phenylalanine accumulates and reaches extremely high concentrations in the body, such as in individuals with the phenylketonuria genetic disorder and impaired metabolism of phenylalanine, neurological problems can result. Thus, many studies (detailed below and reviewed in Magnuson et al.[28]) have investigated the level of phenylalanine produced from aspartame as well as potential neurological effects in both normal populations and individuals with phenylketonuria. At the 90th percentile of aspartame intake, plasma phenylalanine levels were within the normal postprandial range in the general population and well below the levels associated with negative health outcomes.[45]

The effect of phenylalanine derived from aspartame in humans was thoroughly investigated, particularly in

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individuals heterozygous for phenylketonuria. In normal adults, bolus doses of aspartame given at 34 mg/kg (representing the 99th percentile of dietary intake) and 50 mg/kg increased plasma phenylalanine concentrations from baseline (5–6 μmol/dL) to 11 μmol/dL and 16 μmol/dL, respectively, which are similar to the plasma phenylalanine concentrations normally observed in a postprandial state (≈12 μmol/dL).[30] After extreme doses of aspartame (100 mg/kg and 200 mg/kg), phenylalanine levels peaked at 20.0 μmol/dL and 48.7 μmol/dL, respectively.[30] Although these levels are high for normal adults, they are within the normal range for individuals with benign phenylketonuria (24–48 μmol/dL).[25] Further studies have looked at the potential for aspartame to cause increases in plasma phenylalanine in individuals with moderate and severe phenylketonuria. When individuals with moderate phenylketonuria were given aspartame at 34 mg/kg and 100 mg/kg, plasma phenylalanine rose to 16 ± 2 μmol/dL and 42 ± 2 μmol/dL, respectively, while levels in normal adults were 11 ± 3 μmol/dL and 20 ± 7 μmol/dL.[47,51] Lower doses (10 mg/kg) that more reasonably reflect actual aspartame consumption showed that plasma phenylalanine levels in normal individuals reached 6 μmol/dL (up from baseline of 4.5 μmol/dL), while levels in individuals heterozygous for phenylketonuria reached 8 μmol/dL (up from a baseline of 6.9 μmol/dL).[52] People with severe phenylketonuria had baseline levels of 137 μmol/dL and displayed no change in blood levels after aspartame consumption.

Other possibly sensitive populations, including normal infants and lactating women, have also been studied. In lactating women, aspartame given at the high dose of 50 mg/kg increased milk phenylalanine concentrations approximately fourfold and remained within the normal postprandial range.[28,49] In 1-year-old infants, plasma phenylalanine levels reached 9.4, 11.6, and 22.3 μmol/dL, respectively, after administration of aspartame at 34, 50, and 100 mg/kg, compared with levels in adults of 11.1, 16.2, and 20.2 μmol/dL, respectively.[25,39]

To better mimic real-world conditions, aspartame doses of 10 mg per kilogram of body weight were administered every 2 hours for 6 hours (3 doses) in normal adults and did not result in significant accumulation in the plasma.[53] Long-term studies have also shown that, in normal adults, children, adolescents, and individuals heterozygous for phenylketonuria, aspartame (30–77 mg/kg or 1800 mg) given every day for 13 to 21 weeks caused no change in fasting plasma phenylalanine levels.[54]

Saccharin

Chemistry and regulatory status. Saccharin (also known as E954) is one of the most widely known LNCSs, with use beginning in 1900. It was discovered serendipitously in 1878 by Remsen and Fahlberg, who were studying the chemistry of cyclic sulfonamides.[55] Initially, saccharin was considered a drug because it was used to manage diabetes, but its use in food increased during both World Wars because of sugar rationing and limitations.[55] Saccharin has a long and complicated regulatory history, especially in the United States.[56] Once almost banned from the market, saccharin is now a widely approved food additive. For example, in 2014, approved uses of saccharin were extended from only tabletop sweeteners to use in unstandardized foods and beverages in Canada, where saccharin use had been quite limited compared with use in other countries.[57]

Saccharin is approximately 300 to 500 times sweeter than sugar.[58] Two methods have been used to synthesize saccharin commercially.[59] The Remsen–Fahlberg process involves oxidation of o-toluenesulfonamide to saccharin, whereas the Maumee process involves the diazotization of anthranilic acid to form saccharin and is the current commercial process.[60] Saccharin is an acid that can be converted into a salt with sodium hydroxide or calcium hydroxide. Saccharin salts vary in their water solubility and can be used in products with a broad range of pH values, but they are less stable at temperatures above 125°C, which limits their usefulness in baked foods.[55]

Reviews of published surveys of intake have shown that the average daily intakes of saccharin by even high consumers is below the ADI and is equivalent to about 600 mg of sugar per kilogram of body weight per day, or about 2 mg of saccharin per kilogram of body weight.[61,62] Intakes by high consumers of saccharin (90th percentile or higher) are orders of magnitude lower than the amounts used in many animal studies and are well below the saccharin ADI of 0 to 5 mg/kg/d established by the JECFA[63] and the Scientific Committee for Food.[64]

Absorption, metabolism, and excretion. The toxicokinetics of saccharin as derived from animal and human studies was extensively reviewed previously.[65] Saccharin is a water-soluble acid with a pK_a of 1.8, and absorption is increased in animal species with lower stomach pH, such as rabbits and humans, compared with those with a higher stomach pH, including rats.[65–67] In humans, approximately 85% to 95% of ingested saccharin is absorbed and eliminated in the urine, with the remainder excreted in the feces.

Absorbed saccharin binds reversibly to plasma proteins and is distributed via the blood to the body organs. With the exception of those in the kidney, the concentrations of saccharin in the tissues of rats fed diets containing up to 10% saccharin are lower than those in plasma.[68] The concentrations of saccharin in body

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tissues are in equilibrium with those in the blood plasma and decrease in parallel with the levels in the general circulation. Saccharin can transfer across the placenta and appears in fetal tissues of rats, monkeys, and humans. Following a single oral dose given in late pregnancy, the tissue levels of saccharin in fetal rats are similar to or slightly higher than those in maternal tissues. The concentrations in the fetal kidney, urinary bladder, and amniotic fluid exceeded those in fetal plasma, indicating in utero renal excretion. The concentrations in fetal tissues decreased more slowly than the concentrations in maternal tissues. In pregnant rats fed a diet containing 5% saccharin, the steady-state concentrations in fetal tissues, apart from the urinary bladder, were lower than corresponding maternal levels.[69] These data show that the fetus represents a slow equilibration tissue compartment that does not accumulate saccharin during repeated administration.

Studies performed in the 1950s showed that, in animals and humans, saccharin is excreted primarily in urine without undergoing detectable metabolism. Studies with radiolabeled saccharin in the early 1970s indicated limited metabolism (about 1%) to a hydrolysis product and to carbon dioxide, but later extensive research using radiolabeled saccharin under a wide range of conditions showed that it is not metabolized by animal species or by humans.[65]

Saccharin directly injected intravenously is rapidly eliminated from the general circulation, with a plasma elimination half-life of about 40 minutes in rats and 70 minutes in humans, demonstrating that saccharin is rapidly cleared from the general circulation.[68,70] After administration of saccharin to rats by oral gavage, plasma levels peak in 30 to 60 minutes, after which they decline slowly, primarily due to slow and continued absorption from the gastrointestinal tract, such that a plasma half-life cannot be defined. The plasma concentration–time profile after oral dosage to humans is complex and shows rapid initial elimination during the first 10 hours, followed by slower elimination; the slow phase of elimination was not seen after intravenous administration and is therefore determined by prolonged absorption from the gastrointestinal tract (flip-flop kinetics).[65]

Dose-dependent differences in plasma clearance (the best measure of the body's ability to eliminate the compound) were observed when comparing low intravenous doses (1, 20, and 50 mg/kg) with high doses (up to 1000 mg/kg) of saccharin in rats. Plasma clearance was halved with intravenous bolus doses above 200 mg per kilogram of body weight and with intravenous infusions giving constant plasma concentrations of saccharin greater than 200 to 300 μg/mL.[68] Following administration through addition in the diet, there was a nonlinear relationship between high saccharin concentrations in the diet and plasma and tissue concentrations predicted on the basis of results observed at lower dietary concentrations. Elevated concentrations present in the plasma and tissues of rats fed diets containing more than 3% saccharin indicated saturation of excretion mechanisms (discussed below) when such high dietary concentrations are used in toxicity studies.

Following absorption, saccharin is excreted unchanged, primarily in urine, which is the principal method of plasma clearance. Active tubular transport, which is the primary mechanism of renal elimination of saccharin, is a saturable process that is inhibited by the drug probenecid. Probenecid pretreatment reduced the plasma clearance by about 60% in rats and 35% in humans.[68,70] Saturation of renal excretion occurs when rats are fed very high dietary levels (>3% in the diet) and results in the excessive accumulation of saccharin.[68]

The elimination of saccharin has not been specifically evaluated in children, but Dorne et al.[22] reported that infants and children eliminate drugs that are largely (60%–100%) excreted in urine unchanged at rates similar to, or greater than, those observed in adults. The ADI for saccharin is based on the NOAEL from a 2-generation feeding study, which includes all life stages. Furthermore, any age-related and species differences observed in safety assessment are accounted for by the use of a 100-fold uncertainty factor to convert the NOAEL into the ADI.

Stevia leaf extract

Chemistry and regulatory status. Stevia leaf extract is a non-nutritive sweetener derived from the plant *Stevia rebaudiana* Bertoni, which contains one or more sweet-tasting compounds called steviol glycosides. Steviol glycosides can also be produced de novo via fermentation.[71] Four major and at least 6 less prevalent steviol glycosides have been isolated from the leaves of *S. rebaudiana*.[72,73] The most abundant steviol glycosides are stevioside and rebaudioside A; others include various rebaudiosides (ie, D, B, M). However, a large number of minor steviol glycosides, some with rare α1,4 glucosyl linkages and rhamnose or xylose side chains, have recently been identified in stevia leaf extracts.[72,74,75] Enzyme-modified steviol glycosides have been created that add α1,4 glucosyl linkages to naturally occurring steviol glycosides (Government Reference Number [GRN] 337, GRN 375, GRN 452).[76–78] All steviol glycosides contain a common chemical core, the diterpene steviol, which is also the final product of their metabolism by bacteria in the colon.[79,80]

Stevia leaves were used by indigenous people in South America as both a food sweetener and medicine, followed centuries later by stevia's introduction for the

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same uses in Japan in the 1970s.[73] After initially being banned in the United States in 1991 because of gaps in required safety information, stevia was eventually permitted for use only as a dietary supplement in 1995. The FDA and food safety authorities in Europe, Australia, Canada, and other countries banned stevia use as a sweetener as a result of safety concerns that could not be resolved by the studies available at that time. The JECFA declined to establish a full ADI until specification problems and safety gaps were resolved. From 1999 to 2007, stevia was evaluated by the JECFA on several occasions, and the gaps in the available safety and metabolism data were widely reported.[4,81,82] Once appropriate safety, metabolism, and clinical studies were conducted and made public in 2008, purified steviol glycosides were given a full ADI by the JECFA and were approved or permitted by the FDA and many other national food safety authorities.[73] Approval in the European Union and Canada followed in late 2011 and 2012, respectively.

The metabolism of different steviol glycosides to steviol by intestinal bacteria was known at a relatively early stage in the development of stevia as a food ingredient.[83] Because all steviol glycosides are metabolized to a common metabolic end product, the JECFA established an ADI for all steviol glycosides on the basis of the amount of steviol each glycoside produced after hydrolysis, called the steviol equivalent. The temporary ADI was set at 0 to 2 mg of steviol equivalents per kilogram of body weight per day, which was later increased to 0 to 4 mg of steviol equivalents per kilogram of body weight per day.[84,85] In order to compare the exposure, metabolism, and safety of the various steviol glycosides (such as stevioside and rebaudiosides A D, B, and M), each one can be converted to steviol equivalents on the basis of its molecular weight vs that of steviol.[79] For example, 12 mg of rebaudioside A converts to 4 mg of steviol equivalents.

Absorption, metabolism, and excretion. Enzymes and acid present in the upper gastrointestinal tract do not hydrolyze steviol glycosides. Hutapea et al.[86] reported no metabolism of stevioside incubated with salivary and pancreatic amylase, pepsin, gastric secretion, and intestinal brush border enzymes from rats, mice, and hamsters. Nikiforov et al.[87] demonstrated similar results with rebaudiosides A and D. Although hydrolysis of stevioside to glucose and the aglycone steviol was first reported in the early 1930s,[88] elucidation of the bacterial metabolism of ingested stevioside and rebaudioside A to steviol was not reported until much later.[83]

In vitro studies of absorption, metabolism, and excretion. Wingard et al.[83] and, later, Hutapea et al.,[86] Gardana et al.,[89] and Koyama et al.[90] demonstrated that stevioside and rebaudioside A were degraded in vitro by cecal, colonic, or fecal bacteria to steviol. Steviolbioside is an intermediate in the degradation of stevioside and rebaudiosides A and M that is quickly converted to steviol.[89,91] Rebaudiosides B, D, E, and M have all been shown to have the same metabolic endpoint (steviol) as stevioside and rebaudioside A when exposed to human fecal bacteria in vitro.[87,91,92] Steviol is completely resistant to bacterial degradation.[89] Koyama et al.[90] demonstrated that enzyme-modified steviol glucuronides containing α1,4 glucosyl linkages are also metabolized to steviol by fecal bacteria. Gardana et al.[89] appear to be the first to report that *Bacteroides* species were the only bacteria capable of hydrolyzing steviol glycosides to steviol.

Steviol epoxide was found during in vitro bacterial metabolism of stevioside using fecal samples from mice and humans, but not rats and hamsters.[86] The epoxide was also shown to be rapidly converted to steviol in fecal samples from both species. Gardana et al.[89] failed to find steviol epoxide in their in vitro study and attributed the difference in findings to their use of a more specific analytical method. Steviol epoxide has not been found in in vivo studies using radiolabeled steviol glycosides or steviol.[93] Isosteviol has also been reported in in vitro bacterial metabolism studies but is likely to be an artifact.[94]

In vivo studies of absorption, metabolism, and excretion. In vivo studies of steviol glycoside metabolism have been conducted in rats, mice, pigs, chickens, and humans. Radioactivity was observed in the feces and bile of Wistar rats administered labeled stevioside, indicating the presence of enterohepatic circulation of metabolites.[95,96] Oral administration of steviol to rats resulted in rapid absorption of steviol into the portal plasma. After administration of steviol glycosides, however, steviol detection in portal plasma was much slower and its presence was sustained over a period of hours.[96] This is consistent with observations by numerous authors that steviol glycosides are metabolized slowly by colonic bacteria, leading to a long slow increase in portal and plasma levels of steviol or its metabolite, steviol glucuronide, depending on the species.

In a study reported by Roberts and Renwick,[93] 5 mg/kg of [^{14}C]-labeled rebaudioside A and the molar equivalent amounts of [^{14}C]-labeled stevioside or [^{14}C]-labeled steviol were administered orally to Sprague-Dawley rats. Each of the compounds was labeled in the steviol moiety for consistency. Radioactivity from the 2 steviol glycosides increased slowly in plasma over a period of hours, peaking at 2 to 8 hours post dosing. The predominant metabolite found in plasma was steviol,

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indicating that the rat is a good model for safety assessment of steviol glycosides, as the rat metabolism also produces the common metabolite of steviol, following administration of all steviol glycosides. Pharmacokinetics and excretion of radioactivity after [^{14}C]-steviol dosing followed a pattern similar to that of steviol glycosides, but at a more rapid rate. Excreted radioactivity was found almost exclusively in the bile of bile duct–cannulated rats and in the feces in noncannulated rats. Less than 2% of the radioactivity administered for any of the test compounds was found in urine. The predominant metabolites found in bile and feces were steviol glucuronide and steviol, respectively. The authors confirmed that neither of the steviol glycosides tested was metabolized or absorbed in the upper gastrointestinal tract, but both were absorbed as steviol following bacterial degradation in the colon. Steviol was then glucuronidated by the liver and transported via bile back to the intestinal tract, where it was again metabolized to steviol by bacteria and then excreted. Over 95% of the radioactivity (originating from the administered test compounds) was found in feces, and no significant residual radioactivity was found in any body organ 96 hours after dosing.

Nikiforov et al.[87] reported similar metabolism and excretion results for rebaudioside A and D in Sprague-Dawley–derived rats dosed at dietary levels up to 2000 mg/kg/d. At this dose, most of the rebaudioside A and D passed through the gut unchanged because of the slow pace of bacterial hydrolysis. Plasma metabolites consisted primarily of conjugated steviol, with smaller amounts of free steviol and virtually no rebaudioside A or D. Other intermediary compounds of rebaudioside A metabolism, such as rebaudioside B, steviolbioside, and stevioside, were not detected in plasma. The reason for the larger amounts of conjugated and free steviol in plasma compared with the amounts reported by Roberts and Renwick[93] is probably the very large oral dose used in this study, which overwhelmed biliary excretion rates and the normal barriers to organic anion excretion in rats.

Results similar to those observed in rats have been reported in metabolism–excretion studies in chickens and pigs, although the gut bacteria in chickens may be less capable of converting stevioside and other steviol glycosides to steviol than the gut bacteria in rats and humans.[94,97]

The metabolism of steviol glycosides is similar in animals and humans. Wheeler et al.[80] demonstrated that stevioside and rebaudioside A are hydrolyzed to steviol in the colon, and steviol is absorbed. Absorbed steviol is then transported to the liver and glucuronidated. Peak plasma levels of steviol glucuronide were found in humans 8 hours after administration of stevioside and 12 hours after administration of rebaudioside A. The plasma half-life of steviol glucuronide was approximately 14 hours. Plasma steviol was detected just above the quantitative limit of 100 ng/mL at only 1 time point and in only 1 of 8 subjects tested for both sweeteners, and none was detected in the remaining subjects.[80] There is no evidence that glucose removed from the glycosides in the colon is absorbed, and it is presumably quickly utilized by colonic bacteria.[79]

Steviol glucuronide in bile is excreted by rats almost exclusively in feces as steviol following bacterial metabolism.[95] Over 95% of the radioactivity from labeled stevioside and rebaudioside A administered orally to rats was accounted for in feces.[93] In humans, the major metabolite is steviol glucuronide, which is excreted mostly in urine.[80,98] This species difference in excretion is due to a difference in molecular weight thresholds for biliary excretion of organic anions (reviewed in Carakostas et al.[79]). In humans, systemic exposure to steviol from typical consumer exposures to steviol glycosides appears to be minimal.

Children have not been specifically evaluated for their ability to metabolize steviol glucuronides. However, Dorne et al.[99] reported that children 2 years of age and older are able to glucuronidate and excrete 15 drugs at rates similar to, or greater than, those observed in adults. The same hepatic glucuronidation process evaluated for drug metabolism in this study is responsible for steviol glucuronidation. Toxicity studies in which young rats were exposed to steviol glucuronides via diet or to metabolites via milk have not indicated any adverse effects of steviol glycoside ingestion by immature animals.[79] The JECFA ADI was established using the NOAEL of 970 mg/kg/d from a conservatively interpreted 2-year carcinogenicity study with stevioside. These data suggest that the current ADI provides a very wide margin of systemic safety for free steviol following typical human exposures to steviol glycosides.[73]

Sucralose

Chemistry and regulatory status. Sucralose is an intensely sweet compound that has a sweetening potency approximately 600 times that of sucrose, which means that the addition of very small amounts of sucralose can be used to replace sugar to sweeten foods and beverages.[100]

Structurally, sucralose is similar to sucrose and is produced from sucrose by replacing the hydroxyl groups in the 4, 1′, and 6′ positions with chlorine. The chemical name for sucralose is 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-

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galactopyranoside. Sucralose has also been described as 4,1′,6′-trichlorogalactosucrose and trichlorosucrose. According to the European Union food additive numbering system, sucralose is E 955.

Sucralose was first approved in 1989 by the JECFA, which established a temporary ADI (t-ADI) of 0 to 3.5 mg/kg/d. After further studies were conducted, an ADI of 0 to 15 mg/kg/d was allocated by JECFA in 1991. Sucralose is now widely approved by international regulatory agencies as a food additive for sweetening purposes.[101]

Sucralose is a highly water-soluble compound that is stable at high temperatures and has negligible effect on pH or viscosity, making it an ideal sweetener for use in beverages and other foods, including those undergoing heat treatment such as baked goods.[102] Unlike some organochlorine compounds, to which sucralose has erroneously been compared, sucralose is highly water soluble and only poorly soluble in lipids.[102]

Sucralose is also not digested into monosaccharides or metabolized for energy by the body; therefore, sucralose contributes no calories and does not affect blood glucose levels. The change of three hydroxyl groups in sucrose to chlorine in sucralose results in a change in the conformation of the molecule, such that the glycosidic enzymes that hydrolyze sucrose and other carbohydrates are unable to cleave sucralose. These properties make sucralose suitable for use in producing sweetened foods and beverages that are appropriate for diabetics and that contain fewer or no calories.

Absorption, metabolism, and excretion. The absorption, metabolism, distribution, and excretion of sucralose have been evaluated in several species, including mouse,[103] rat,[104] dog,[105] rabbit,[106] and human.[107] The fate of orally administered sucralose has been shown to be similar in all species evaluated, with very low levels of absorption and little to no metabolism reported.

Acute studies of absorption, metabolism, and excretion. In the mouse, oral doses of (^{14}C)-sucralose ranging from 100 to 3000 mg/kg were completely excreted within 72 hours. There was no difference between male and females.[103] Between 94% and 99% of the radioactivity in the feces was present as unchanged sucralose, indicating there is little to no breakdown of sucralose or metabolism by gut bacteria. Urine contained primarily unchanged sucralose and 2 minor metabolites that were determined to be the same minor urinary metabolites in dogs and humans.[103]

Following an intravenous dose (mimicking 100% absorption) of radioactive sucralose to the mouse, the presence of sucralose (22% of total dose) in feces indicated excretion into the gastrointestinal tract by some mechanism, possibly through bile.[103] Using comparison of urinary excretion following intravenously administered sucralose with urinary excretion following orally administered sucralose, absorption of an oral dose of sucralose was determined to be about 20% of the administered dose in the mouse.

Following oral administration of (^{14}C)-sucralose in the rat, over 90% of the radioactivity was excreted in the feces, with less than 10% excreted in the urine, demonstrating that absorption is very low, regardless of the oral dose administered (50 to 1000 mg/kg).[104] Following intravenous administration of radioactive sucralose, over 90% of the radioactivity recovered from the urine was unchanged sucralose, demonstrating little to no metabolism. Fifteen minutes after an intravenous dose of (^{36}Cl)-sucralose, whole-body autoradiography demonstrated that most of sucralose was distributed to the liver, blood, kidney, and small intestine. After 6 hours, the concentration was lower in all organs except the large intestine. At no time was there evidence of sucralose uptake into the central nervous system. Thin-layer chromatography of urine samples identified 2 minor metabolites (<1% of the total dose), but the levels in urine were too low for structural analysis to be performed.[104]

In rabbits, the time of excretion was more prolonged than in other species, although the metabolic fate of sucralose was similar, with the majority being excreted unchanged in the feces.[106] One possible explanation for the species difference in rate of excretion is the extensive coprophagy in the rabbit, which facilitates oral recycling of the sucralose excreted in the feces.[106]

Male and female dogs were administered oral (10 mg/kg) and intravenous (2 mg/kg) doses of (^{14}C)-sucralose, and radioactivity was determined in plasma, urine, and feces to assess pharmacokinetics and metabolism.[105] Further characterization of metabolites was conducted using enzyme hydrolysis studies and mass spectrometry. Following intravenous administration, radioactivity was excreted rapidly, primarily in the urine. In contrast, oral administration resulted in radioactivity being excreted primarily in the feces (68.4% of total dose), with a lesser amount (26.5%) excreted in the urine after 5 days. Comparison of urinary excretion following an oral dose with that following an intravenous dose showed that approximately 35% of the oral dose was absorbed in dogs. In both routes of administration, the major component of urinary excretion was unchanged sucralose, with the remaining radioactivity present in one metabolite, which was determined to be a glucuronide conjugate of sucralose.

In a study with 8 healthy men (aged 30–48 years), (^{14}C)-sucralose was administered at a dose of 1 mg/kg in drinking water following an overnight fast.[107] In a

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second study, a higher dose (10 mg/kg) was administered to 2 men.[107] Radioactivity in blood, urine, and feces was monitored for 5 days. The majority of the radioactivity was recovered from the feces, representing between 70% and 90% of the total dose administered. Urinary recovery averaged 14.5% of the total dose following administration of 1 mg/kg, indicating low oral absorption. Slightly lower urinary recovery in 2 subjects following consumption of 10 mg/kg suggests the absorption of sucralose may be lower at higher doses.[107]

To assess the metabolism of sucralose in humans, thin-layer chromatography and gas chromatography, in combination with mass spectrometry analysis, were used to detect and determine the structure of the radiolabeled compounds in urine and feces. Unchanged sucralose was the major component of radioactivity found in urine and represented essentially all (>99%) of the radioactivity found in feces. Two metabolites were detected in urine, representing approximately 2% of the total dose, and were identified as glucuronide conjugates of sucralose. The pharmacokinetics further indicated that the limited metabolism of sucralose occurs within the body tissues, as opposed to in the gut lumen.[107]

Chronic studies of absorption, metabolism, and excretion. Chronic exposure to high levels of sucralose (3% of diet) for 18 months did not alter the percentage of sucralose excreted in the urine or feces in rats.[104] This study demonstrated that chronic exposure did not result in an adaptation of metabolic enzymes or of gut microflora to result in an ability to metabolize or utilize sucralose over time.

In summary, sucralose is poorly absorbed, undergoes little metabolism, and is excreted primarily unchanged in the feces in all species, including humans. Based on the totality of all toxicokinetic studies, there is no evidence that sucralose is selectively or actively transported across the placenta or across the blood–brain barrier. There is no evidence of either dechlorination or hydrolysis of sucralose in any species. The low level of absorption, in combination with systemic clearance of sucralose, indicates there is very low likelihood that chronic consumption would result in accumulation of sucralose in the body.[108]

DISCUSSION

Despite extensive safety testing and excellent understanding of the absorption, distribution, metabolism, and excretion of the LNCSs reviewed above, the safety and efficacy of LNCSs has become controversial, resulting in many health professionals being hesitant to recommend the use of foods and beverages containing these sweeteners, even when patients or clients are facing the certainty of serious adverse chronic health effects due to obesity and uncontrolled diabetes. This review aims to provide health professionals with a better understanding of both the likely exposure and the biological fate of these compounds so they may be better equipped to judge the relevance of the experimental design and potential health consequences of purported biological effects of various LNCSs.

The similarities and differences in the absorption, distribution, metabolism, and excretion of the 5 LNCSs reviewed above are summarized in Figure 1. This figure illustrates the major pathways of the absorption, distribution, metabolism, and excretion of these LNCSs, while minor other pathways are described in the sections above. There are considerable differences in the absorption patterns of the different LNCSs. Saccharin and ACK are the only 2 sweeteners that are absorbed extensively as intact molecules; aspartame is completely digested by digestive enzymes, and only the digestion products of aspartame are absorbed; the majority of sucralose is not absorbed; and only the steviol metabolite of steviol glycosides is absorbed.

The LNCSs can be divided into 2 main groups on the basis of their metabolism. Saccharin, ACK, and sucralose belong to the first group, which consists of LNCSs that undergo virtually no metabolism following either minimal absorption (sucralose) or extensive absorption (ACK and saccharin). Aspartame and steviol glycosides comprise the second group of compounds, which are first digested/metabolized in the intestinal tract before absorption, after which only their digestion breakdown products are absorbed systemically and metabolized. Methanol and amino acids resulting from digestion of aspartame are metabolized in the same manner as these same digestion products from fruits, vegetables, and protein-containing foods. Steviol glycosides are not metabolized by human enzymes, but only by the bacteria that normally inhabit the lower intestinal tract. The only metabolite, steviol, is absorbed and rapidly conjugated in the liver to facilitate excretion in the same way that many endogenous and exogenous compounds such hormones, bilirubin, medicines, and environmental compounds are conjugated prior to elimination.

In all cases, elimination is rapid, with no bioaccumulation of either LNCSs or their metabolites in the body. The sweetness intensity of LNCSs means very little is actually used in foods and beverages. The low exposure, along with the absorption, distribution, metabolism, and excretion profiles of LNCSs, results in systemic exposure that is short and minimal, as discussed in detail below. Common concerns about tissue accumulation of LNCSs and consequent adverse effects

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or chronic disease are clearly unwarranted and can be addressed using the toxicokinetic data, which demonstrate that all LNCSs are eliminated completely without change, are changed to facilitate rapid elimination, or are not ever absorbed intact at all.

There are a number of current questions regarding LNCSs that highlight the importance of an understanding of exposure and metabolism of these compounds. It is important that nutritionists and other health professions are familiar with LNCS metabolism so that they can be authoritative sources of scientifically sound information for their clients and the public.

Consumer exposure to LNCSs

Prior to approval of use of LNCSs, potential exposures by the highest-use consumers are calculated on the basis of dietary survey data and proposed levels of use of the LNCS in various foods and beverages. Maximum use levels for different food categories are set to ensure that even high-use consumers are unlikely to exceed the ADI.

There are several reasons why many people overestimate actual exposures to LNCSs. One reason is that most people are unaware that consumer-use formulations, including tabletop sweetener packets or tablets, as well as LNCS sugar substitutes for baking, are highly diluted with filler compounds for ease of measurement by the consumer. As discussed earlier, only about 1/200th or less of a LNCS is needed to achieve the same sweetness level of sugar. As it would not be convenient for individuals to try to measure out 1/200th of a teaspoon or 1/200th of a cup of sweetener, the consumer formulations typically comprise only 1% to 3% LNCS and 97% to 99% filler (such as dextrose), allowing consumers to measure amounts similar to those typically used for sugar. Many consumers incorrectly assume that all of the content in the packet is the LNCS and that they are adding an amount somewhat similar to sugar itself.

There are also suggestions that this misunderstanding exists in the scientific arena as well, on the basis of recent references to "massive" consumption of LNCSs.[109] To investigate exposure to LNCSs further, the amount of LNCS that actually enters the body (ie, systemic exposure) was estimated on the basis of reported intakes and absorption of sweeteners. The intake of LNCSs has been the subject of a large number of population surveys around the world. Renwick[62] reviewed the intake survey data and converted consumption of each sweetener to "sucrose equivalents," which allows comparison of the consumption of LNCSs with varying sweetness (and, therefore, varying levels of use) by comparing each one to the amount of sucrose needed to replace the LNCS sweetness or its sucrose equivalent. Sucrose equivalents are calculated by multiplying the reported dietary exposure for an LNCS by the relative sweetness intensity of that LCNS compared with sucrose. Using this approach, the average daily intake for all LNCSs was 255 mg of sucrose equivalents per kilogram of body weight per day for a nondiabetic adult and 280 mg of sucrose equivalents per kilogram of body weight per day for diabetic adults.[62] Although other sweetener intake surveys have been published more recently, the advantage of the Renwick[62] approach is that it allows intake comparisons across LNCSs with different sweetness intensities and levels of use. Using the information provided above on the percentage of each LNCS that actually enters the body through absorption, the internal or systemic exposure to LNCSs is shown in Table 2.

The systemic exposures ranged from 0 to 76 mg of LNCS per day for a nondiabetic individual (Table 2).

Table 2 **Average daily systemic exposure to low- and no-calorie sweetener or sweetener metabolite in a nondiabetic adult, assuming the entire daily intake of sweetener is from one sweetener only (Renwick[62] estimate)[a]**

Sweetener	Sweetener intensity as compared with sucrose	Absorption in humans	Amount of LNCS or LNCS metabolite per day entering body of a 60-kg nondiabetic individual
ACK	200 times	100%	76 mg of ACK (no metabolism)
Aspartame	200 times	0% as aspartame (because of complete digestion) 100% as digestion products	0 mg of aspartame, 69 mg of amino acids, 8 mg of methanol
Saccharin	300 times	85%	43 mg of saccharin (no metabolism)
Steviol glycosides (rebaudioside A)[b]	200 times	0% as steviol glycoside, assuming 100% absorption of steviol	25 mg of steviol (metabolized to glucuronide)
Sucralose	600 times	15%	4 mg of sucralose (no metabolism)

Abbreviations: ACK, acesulfame potassium; LNCS, low- and no-calorie sweetener.
[a]Example of systemic exposure calculation based on sucralose data: 255 mg of sucrose equivalents per kilogram of body weight per day (Renwick estimate) divided by 600 (sweetness intensity compared with sucrose) multiplied by 15% (absorption) multiplied by 60 kg of body weight = 4 mg.
[b]An additional factor is needed for steviol glycosides to adjust for the proportion of the steviol glycoside that represents steviol. Rebaudioside A: 255 mg/kg/d multiplied by 0.33 (correction for steviol, which constitutes about one-third of the molecular weight of rebaudioside A) divided by 200 (sweetness intensity) multiplied by 60 kg of body weight = 25 mg of steviol.

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This would be only slightly higher for diabetics (ie, up to 84 mg of LNCS per day), on the basis of an intake of 280 mg of sucrose equivalents per kilogram of body weight per day. To put these values into perspective, compare these amounts with the typical consumption of salt, a common flavoring compound: consumption of 2300 mg of sodium per day results in consumption of about 6000 mg of salt.

Use of LNCS combinations

Blends of intense sweeteners are becoming more popular in food and beverage formulations because mixtures provide synergistic enhancement of sweetness intensity and improved sweetness quality beyond those afforded by individual sweeteners. In addition, the use of sweetener blends results in lower amounts of each individual LNCS being used, further lowering exposure to each compound. Selection of the type and ratio for mixed sweeteners is based on the desired flavor (eg, cola vs orange) and/or application (eg, beverages vs baked goods). The common use of each LNCS in LNCS blends is discussed below. Knowledge of the toxicity and toxicokinetics of each sweetener allows for assessment of the likelihood of any potential adverse interaction due to the use of the blends. Consistently, no evidence for mixtures to represent a safety concern has been found. On the contrary, the use of diverse sweeteners will result in overall reduced exposure to each LNCS because of consumption of lower amounts of individual LNCSs and the different toxicokinetic pathways in the body (see Figure 1).

Acesulfame potassium is often used in combination with other LNCSs. Sweeteners mixed with ACK in low-calorie beverages include aspartame, sucralose, sodium saccharin, and/or sodium cyclamate. Sweeteners mixed with ACK in foods processed at high temperatures (eg, baked goods, canned fruits, confectionary items, and fruit-flavored, pasteurized dairy products) include sucralose, aspartame, aspartame/saccharin, and/or sugar alcohols such as sorbitol, maltitol, lactitol, and isomalt.[12,110]

Acesulfame potassium, saccharin, and sucralose are biologically inert, do not undergo significant metabolism in animals or humans, and once absorbed are simply excreted unchanged in urine and/or feces. Therefore, coingestion of ACK, saccharin, or sucralose with combinations of other sweeteners does not affect the elimination of these LNCSs and, likewise, should not affect the absorption and/or metabolism of other sweeteners. Although these 3 sweeteners are excreted in urine, this is a common pathway for the elimination of all water-soluble compounds from the body, and renal excretion would not be saturated at the systemic exposure (Table 2) found following ingestion of these sweeteners in foods.

Aspartame is most commonly blended with ACK, especially in beverages. There is no likely site of interaction, for the reasons described above for ACK and because aspartame is completely digested before absorption. The safety of this mixture was also confirmed by the approval of use of the acesulfame-aspartame salt, which is used as a replacement for simple ACK and aspartame mixtures because of its increased stability.[16,111]

Food and beverage manufacturers typically use purified stevia leaf extract or purified steviol glycoside sweeteners when they want to market a product with a direct or implied "all-natural" claim, and thus combinations with synthetic low-calorie sweeteners are rare. Combinations with caloric sweeteners like sucrose or high-fructose corn syrup and polyols (eg, erythritol) are the most common combinations found on the market. As with other more common combinations of synthetic sweeteners, combinations of purified stevia leaf extracts with other sweeteners produce a cleaner sweet taste with reduced off-tastes.[73]

Common caloric sweeteners that are combinations of glucose and fructose (sucrose and high-fructose corn syrup) are directly absorbed in the upper intestinal tract and are used primarily for energy. If molecules of glucose or fructose traversed the small intestine unabsorbed, they would likely be quickly utilized for energy by colonic bacteria. Interference with steviol glycoside hydrolysis by *Bacteroides* species would be unlikely. Over 90% of ingested erythritol is absorbed in the small intestine and excreted unchanged via urine.[112] Simple sugars and erythritol are not glucuronidated, and thus there is little likelihood of metabolic interference by combinations of steviol glycosides with these sweeteners.

LNCSs and gut microflora

The role of the gut microflora in human health is currently an area of extensive research for many different health endpoints and dietary components. This research has recently included LCNSs.[109] In the present review, the absorption, distribution, metabolism, and excretion of each of LNCS was considered, as was previously published data, to assess the potential for each LNCS to affect the gut microflora in the lower gastrointestinal tract.

Acesulfame potassium. As described above, the predominant ACK profile is fast absorption followed by urinary excretion, which greatly limits the amount of ACK likely to reach the cecal or colonic bacteria. In vitro, high concentrations of ACK inhibited anaerobic

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glucose fermentation in cecal bacteria isolated from rats; the median effective dose (ED50) was 260 ± 56 mM.[113] However, concentrations of this magnitude are unlikely to ever occur in humans because of rapid absorption. In addition, the ED50 was more than 450 times greater than concentrations expected in foodstuffs; for example, the representative amount of ACK in 12-oz (355-mL) sugar-free sodas is 40 mg, resulting in a concentration of 0.56 mM.[114]

Aspartame. Aspartame is completely digested into amino acids and methanol, which are absorbed in the small intestine. Neither aspartame nor its digestion products ever reach the colon; thus, aspartame itself cannot directly affect gut microbiota.[3] Given this knowledge, it is critical to carefully examine the study design and other parameters that may be responsible for the changes in gut microflora observed in studies of animals fed aspartame. Palmnäs et al.[115] reported differences in the gut microflora in rats fed either a low-fat (12%) or a high-fat (60%) diet and given either plain or aspartame-sweetened water. Notably, rats given aspartame-containing water consumed 17% to 25% fewer calories from consumption of their diets, resulting in significantly less fat, protein, fiber, and other nutrients, which are well known to alter gut microflora. In the study by Suez et al.,[109] food consumption was reported for only 4 of 20 animals and for only 72 hours of the 11-week study. Nonetheless, up to a 50% reduction in food intake in mice given drinking water containing LNCSs, including aspartame, sucralose, and saccharin, is evident in graphs provided in the supplemental data. Thus, it is impossible to assess the contributions to changes in gut microflora by LNCSs separately from those resulting from changes in food intake and diet composition in these studies. Furthermore, as such dramatic reductions in food intake do not occur in humans consuming LNCSs, the significance of such studies to human health is limited.

Steviol glycosides. Gardana et al.[89] reported no consistent effect of stevioside or rebaudioside A on anaerobic fecal cultures taken from healthy human subjects. Specifically, no changes to members of Bacteroidaceae or to *Clostridia* species were reported. The microbial hydrolysis of steviol glycosides, as well as the potential effect of steviol glycosides on gut microbiota, was reviewed by Renwick and Tarka,[116] who found no reason to believe steviol glycosides adversely impact colonic bacteria.

Sucralose. As toxicokinetic studies demonstrated that the majority of sucralose is not absorbed and enters unchanged into the lower gastrointestinal tract, the potential for effects on gut microflora was assessed by the Scientific Committee for Food in 2000,[108] prior to the approval of sucralose. Unpublished studies on the stability of sucralose and its hydrolysis products (which can be generated with high temperature and acidic pH) as well as studies on metabolism and potential for adaptation were submitted and evaluated by the Scientific Committee for Food. On the basis of the high stability of sucralose and its resistance to hydrolysis, the Committee concluded that metabolic adaptation by microflora was highly unlikely.

Studies on the effect of sucralose on oral cavity pathogens and environmental microflora have shown sucralose to be non-nutritive to bacteria and resistant to degradation.[117,118] At low concentrations, sucralose has shown no effect on growth or survival of bacteria, but growth inhibition can occur at high concentrations (≈55 mM), with the effect dependent on the species of bacteria.[118] Bowen et al.[117] reported growth inhibition of some strains of oral bacteria at a concentration of 126 mM, but no effect at lower concentrations.

Two recent studies have purported that oral consumption of sucralose alters the gut microflora in male rats[119] and in male mice.[109] However, in both of these studies, the test material was not sucralose but was a consumer formulation of sucralose, which consists of approximately only 1% sucralose and 99% carrier, such as maltodextrin or another carbohydrate. Furthermore, Suez et al.[109] did not actually measure any parameter of gut microflora composition at any time in animals given commercial formulations of sucralose in drinking water. Thus, conclusions about sucralose must be considered only speculative.

Saccharin. Over 30 years ago, Sims and Renwick[120] showed that high concentrations of saccharin in the diet of rats affected gastrointestinal microbial activity. Feces from rats fed 7.5% saccharin in the diet for 3 months contained larger numbers of both anaerobes and aerobes than did those from rats fed the normal control diet. There was wide interanimal variability in the number of various bacteria present and no significant differences in any of the specific types of organisms measured. There was, therefore, no indication of saccharin having an organism-specific action on the composition of gut microflora.[120] Another study in male rats fed diets containing 7.5% sodium saccharin for 10 days reported an increase in the numbers of aerobic microbes, but no change in anaerobic microbes, in the cecum compared with the numbers in controls, but the controls were given 7.5% cellulose in the diet, which complicates the interpretation of this study.[121] The WHO summarized the many studies that followed observations of enlargement of the cecum resulting from feeding high dietary concentrations

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(5% wt/wt) of saccharin to rats, accompanied by an increase in the total numbers of microorganisms.[63,121,122] Thus, it should come as no surprise that newer "-omics" technology would identify changes in the gastrointestinal microbial population.

In summary, extrapolation of the effect of one LNCS on the gut microflora to all LNCSs is not appropriate, on the basis of well-documented differences in their chemistry, their movement through the body, and the amount of LNCS or LNCS metabolites that reach the gut microflora. Furthermore, careful control of other factors known to affect gut microflora, such as changes in food consumption, diet composition, and presence of carriers in LNCS formulations, is necessary in studies aiming to assess the potential effect of the very low amounts of LNCS that may actually reach the gut microflora in humans.

CONCLUSION

Although all LNCSs impart sweetness to foods and beverages, this is about the only trait they have in common, since they represent a diverse group of compounds with important differences in their metabolic fate. Understanding of the toxicokinetics (absorption, distribution, metabolism, and excretion) of LNCSs in both animal models and humans is a prerequisite for their approval, and thus an extensive body of data on these processes for all LNCSs is available in the scientific literature.

In many cases, the toxicokinetic information available for widely marketed LNCSs is significantly underutilized to assess the validity of reports of potential adverse effects of LNCSs on various biological processes and to answer important consumer concerns about LNCS safety. As LNCSs have the potential to be useful tools in the management of diabetes and excessive caloric intake, it is critical to use the existing knowledge of the absorption, metabolism, and excretion of these compounds to address the controversies surrounding their use. In many cases, safety concerns about existing LNCSs can be addressed with a basic understanding of the differences in various LNCSs, the metabolism of LNCSs, and the low systemic exposure to these compounds after their ingestion in foods.

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