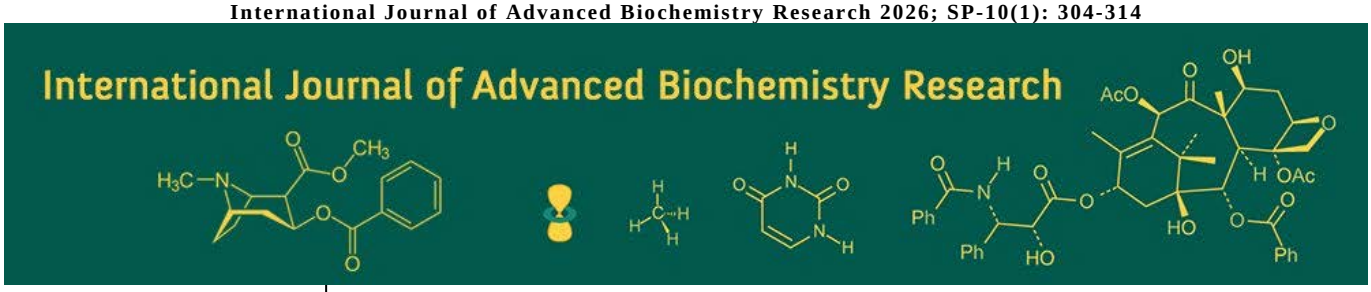

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(1): 304-314
www.biochemjournal.com
Received: 15-10-2025
Accepted: 17-11-2025

Vikram
ICAR-National Dairy Research Institute, Karnal, Haryana, India

Ridhi Pandey
ICAR-National Dairy Research Institute, Karnal, Haryana, India

Neha
ICAR-National Dairy Research Institute, Karnal, Haryana, India

Aman Nain
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Chirag Prajapati
ICAR-National Dairy Research Institute, Karnal, Haryana, India

Corresponding Author:
Chirag Prajapati
ICAR-National Dairy Research Institute, Karnal, Haryana, India

High-Intensity Sweeteners: A Critical Review of Safety, Metabolic Effects, and Regulatory Risk Assessment

Vikram, Ridhi Pandey, Neha, Aman Nain and Chirag Prajapati

DOI: https://www.doi.org/10.33545/26174693.2026.v10.i1Sd.6945

Abstract
High-intensity sweeteners (HIS) are widely used as sugar substitutes because they provide sweetness with little or no energy. They are commonly added to diet foods and beverages to support calorie reduction and glycaemic control. Over the past decades, regulatory authorities have evaluated these sweeteners and generally concluded that they are safe when consumed within established Acceptable Daily Intake (ADI) limits. Despite this, public concern and scientific debate about their long-term safety continue to grow. This review provides a critical and balanced overview of the safety, metabolic effects, and regulatory risk assessment of commonly used high-intensity sweeteners. It summarises their classification and key absorption, distribution, metabolism, and excretion (ADME) characteristics to clarify exposure pathways. Evidence from toxicological studies, animal experiments, human trials, and epidemiological research is reviewed in this paper. Particular attention is given to carcinogenicity, genotoxicity, reproductive and developmental effects, neurotoxicity, immunotoxicity, and gut microbiota-related metabolic outcomes. Most findings indicate that high-intensity sweeteners do not pose serious health risks when consumed within approved intake limits. However, emerging evidence suggests that some sweeteners may influence biological processes through indirect pathways. These effects are mainly linked to gut microbiota modulation and metabolic signalling. The review also highlights limitations of traditional ADI-based risk assessment frameworks. These include a focus on single compounds, reliance on overt toxicity endpoints, and limited consideration of cumulative exposure and population variability. Vulnerable groups such as children, pregnant women, and individuals with metabolic disorders are often underrepresented in long-term studies. Overall, current evidence supports the regulated use of high-intensity sweeteners within established limits. At the same time, continued research is needed. Long-term human studies, improved exposure assessment, and integration of emerging biological endpoints will be essential to strengthen future regulatory decisions and public health guidance.

Keywords: High-intensity sweeteners, non-nutritive sweeteners, safety assessment, Acceptable Daily Intake, metabolism, gut microbiota, regulatory risk assessment

1. Introduction
High-intensity sweeteners (HIS), also called non-nutritive sweeteners (NNS), provide intense sweetness with little or no energy. Their sweetness can range from about 30 times to more than 13,000 times sweeter than sucrose. This allows very small use of these sweeteners in foods and beverages (Whitehouse *et al*., 2008) [85]. As a result, HIS are widely used in “diet” and “sugar-free” products. They are often promoted for calorie reduction and glycaemic management. Their use is very common among people who are aiming for weight control and individuals with obesity or diabetes (Chattopadhyay *et al*., 2014; Mooradian *et al*., 2017) [7, 51]. HIS includes synthetic sweeteners such as aspartame, sucralose, saccharin, acesulfame potassium (Ace-K), neotame, advantame, and cyclamate. On the other hand, they also include plant-derived sweeteners, especially steviol glycosides from *Stevia rebaudiana* (Gwak *et al*., 2012; Prakash *et al*., 2014) [29, 63]. These sweeteners differ in structure, heat stability, and sensory profile which affect there use in food products. Natural-origin sweeteners such as steviol glycosides, and mogrosides from monk fruit are often viewed as “clean-label” options. However, botanical origin does not guarantee metabolic neutrality or long-termsafety. These compounds still require the same level of scientific scrutiny as synthetic sweeteners.

Regulatory agencies such as the U.S. Food and Drug Administration (FDA), the European Food Safety Authority (EFSA), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluate HIS before approval for food use. These agencies use toxicological datasets to set Acceptable Daily Intakes (ADIs). ADIs are typically derived from no observed adverse effect levels (NOAELs) with uncertainty factors applied (Renwick & Nordmann, 2007; EFSA, 2013) [66]. Regulatory assessments largely affirm that consumption within established ADI limits is safe; however, ongoing scientific debate persists regarding potential long-term and cumulative effects. This is partly due to the frequent and long-term nature of real-world exposure, where intake may occur repeatedly throughout the day from multiple products. Moreover, the growing incorporation of HIS into ultra-processed foods and beverages intensifies concerns regarding chronic low-dose consumption and the potential effects of combined exposures. (Debras *et al*., 2022; Sousa & Gloria, 2023) [12, 77].

This review presents a critical synthesis of the safety, metabolic effects, and regulatory risk assessment of high-intensity sweeteners (HIS). It integrates evidence from toxicological, mechanistic, epidemiological, and regulatory studies to provide a comprehensive evaluation. The review first outlines the classification and ADME characteristics of HIS to clarify exposure pathways, and then assesses key health outcomes. It also includes metabolic effects, gut microbiota alterations, reproductive and developmental toxicity, neurological effects, immunotoxicity, and organ-specific toxicity. Finally, the review discusses how existing risk-assessment frameworks, particularly ADI-based approaches, could be refined to better capture real-world exposure patterns and emerging biological endpoints (EFSA, 2013).

2. Classification of High-Intensity Sweeteners

High-intensity sweeteners (HIS) can be classified based on their origin as natural or artificial compounds. This classification is widely used in regulatory, nutritional, and scientific literature. All HIS share a common functional property as they provide intense sweetness at very low concentrations and contribute negligible energy to foods. Global consumption of HIS has increased steadily over recent decades. This trend is driven by rising rates of obesity, diabetes, and cardiovascular disease. Consumer demand for reduced-sugar and low-energy products has also played a major role (Chattopadhyay *et al*., 2014; Mooradian *et al*., 2017) [7, 51].

2.1 Natural High-Intensity Sweeteners

Natural high-intensity sweeteners are primarily derived from plant sources. The most widely used natural HIS are steviol glycosides obtained from *Stevia rebaudiana*. Other examples include mogrosides from monk fruit (*Siraitia grosvenorii*), glycyrrhizin from licorice, and protein-based sweeteners such as thaumatin and monellin. Among these, steviol glycosides are the most extensively studied and commercially important. Steviol glycosides consist mainly of stevioside and rebaudioside A. Newer purified fractions, such as rebaudioside M, have been developed to improve sensory quality. These newer compounds reduce bitterness and aftertaste compared with earlier stevia extracts (Gwak *et al*., 2012; Prakash *et al*., 2014) [29, 63]. The growing use of stevia-based sweeteners is also linked to consumer preference for plant-derived and “natural” ingredients. Natural origin often leads to a perception of improved safety. However, this perception is not scientifically sufficient. Plant-derived sweeteners contain bioactive structures that can interact with metabolic and microbial pathways. Therefore, natural HIS require the same toxicological and regulatory evaluation as synthetic sweeteners. Safety assessment must be based on dose, exposure, and biological effects rather than source alone.

2.2 Artificial High-Intensity Sweeteners

Artificial high-intensity sweeteners are synthetically produced compounds. They are designed to deliver sweetness without providing calories. This group includes aspartame, sucralose, saccharin, acesulfame potassium (Ace-K), neotame, advantame, and cyclamate. These sweeteners differ greatly in chemical structure and sweetness potency (Mora *et al*., 2023; Silva *et al*., 2021) [52, 75]. Artificial HIS are widely used because of their technological advantages. Many are stable during heat processing and long-term storage. This property makes them suitable for baked products, beverages, tabletop sweeteners, and pharmaceutical formulations. Aspartame is a notable exception as it is less stable at high temperatures and under prolonged heating, which limits its use in some food applications (Shankar *et al*., 2013) [70]. From a safety standpoint, artificial HIS cannot be treated as a single group. Their chemical diversity leads to different metabolic and exposure profiles. Some are extensively metabolised, while others are largely excreted unchanged. These differences are important for interpreting toxicological data and for estimating systemic and local exposure.

Overall, Natural and artificial HIS both include compounds with distinct biological behaviours. Risk assessment therefore requires compound-specific evaluation rather than reliance on broad categories. This principle underpins later discussion of metabolism, health effects, and regulatory decision-making.

- **Aspartame:** Aspartame was discovered in 1965 and approved for food use in more than 100 countries by 1981. It is used in products such as breakfast cereals, chewing gum, gelatine desserts, puddings, and soft drinks. Aspartame is composed of two amino acids, L-aspartic acid and L-phenylalanine (Czarnecka *et al*., 2021) [10]. Due to its phenylalanine content, it is contraindicated for individuals with phenylketonuria (Keskin *et al*., 2022) [40]. Aspartame is commercially available as a white crystalline powder or granules. It is soluble in water and ethyl alcohol. The compound is unstable under high temperature and extreme pH conditions. It undergoes rapid hydrolysis when exposed to heat. Aspartame provides a clean and sucrose-like sweetness without strong off-flavours. Its relative sweetness ranges from 180 to 250 times that of sucrose, depending on the food matrix. Due to thermal instability, aspartame is unsuitable for baked or heat-processed foods and is mainly used in products with minimal thermal treatment (Sun *et al*., 2014; Dudure *et al*., 2023) [80, 15].
- **Saccharin:** Saccharin appears as a white, colourless crystalline powder. Its sweetness develops slowly and persists after reaching peak intensity (Sun *et al*., 2025) [81]. At higher concentrations, it produces a noticeable bitter or metallic aftertaste. Saccharin is also available

in tablet form and exhibits approximately 500 times the sweetness of sucrose (Wilk *et al*., 2022) [88]. Saccharin shows high solubility in water. It is stable under heat treatment, including exposure to temperatures of up to 150°C for one hour. It also remains stable across a wide pH range from 2 to 7. These properties make saccharin suitable for a variety of food applications (Kawakami *et al*., 2025; Redha *et al*., 2025) [39, 64].

- **Acesulfame K:** Acesulfame potassium exhibits approximately 200 times the sweetness of sucrose (Shankar *et al*., 2013) [70]. It produces a rapid onset of sweetness that closely resembles sucrose when used at low concentrations. At higher levels, a mild bitter note may appear. This bitterness is generally less intense than that of saccharin (Flad *et al*., 2025) [25]. Acesulfame potassium is frequently used in combination with other sweeteners. Such combinations enhance sweetness intensity and improve flavour balance. Synergistic effects are commonly observed when it is blended with aspartame or sucralose (Choi *et al*., 2024) [8].
- **Sucralose:** Sucralose is a synthetic sweetener produced by chemical modification of sucrose. The replacement of three hydroxyl groups with chlorine atoms yields a trichlorinated sucrose derivative. These substitutions occur at the 4, 1′, and 6′ positions of the sucrose molecule (Slade *et al*., 2021) [76]. Sucralose appears as a white, odourless crystalline powder. It shows moderate solubility in water and ethanol (Dhartiben & Aparnathi, 2017) [13]. It is highly stable under heat and across a wide pH range. Sucralose exhibits a sweetness intensity of approximately 450 to 700 times that of sucrose. A slight residual aftertaste has been reported at higher concentrations (Wang *et al*., 2022) [84].
- **Stevia & Rebaudioside A:** Leaves of *Stevia rebaudiana* contain more than 30 different steviol glycosides. Major compounds include stevioside and rebaudioside A (Reb A). These compounds often produce a bitter or liquorice-like aftertaste, which complicates product formulation (Hao *et al*., 2024) [33]. Rebaudioside A is the most abundant and well-characterised steviol glycoside. It is preferred for commercial use due to its higher sweetness intensity and relatively improved sensory profile. Reb A exhibits approximately 250 to 300 times the sweetness of sucrose. Its efficient extraction process supports widespread industrial application (Karakütük *et al*., 2023) [38].
- **Neotame:** Neotame is a non-caloric artificial sweetener and a structural derivative of aspartame. Its chemical name is N-[N-(3,3-dimethylbutyl)-L-α-asparty]-L-phenylalanine 1-methyl ester. Neotame shows a sweetness intensity 30 to 40 times greater than aspartame. It is approximately 7,000 to 13,000 times sweeter than sucrose. Neotame is stable under heat and can be used in hot beverages such as coffee. It received approval for food use in the European Union in 2010 (Otabe *et al*., 2011) [56]. Due to its high potency, it is used in very small quantities.
- **Advantame:** Advantame is a high-intensity artificial sweetener developed by Ajinomoto, Japan. It is produced using chemical technology that combines structural features of aspartame and vanillin (Dwivedi, 2022) [16]. Advantame was introduced commercially after 2008 and represents one of the most potent sweeteners currently approved. Advantame exhibits an exceptional sweetness intensity ranging from 20,000 to 37,000 times that of sucrose (Otabe *et al*., 2011) [56]. It is non-caloric and has a zero glycaemic index. Advantame is structurally similar to neotame but shows greater sweetness potency. Earlier records for sweetness intensity were held by neotame and monatin (Patel *et al*., 2025) [59].
- **Cyclamate:** Cyclamate exhibits a sweetness intensity of approximately 30 to 50 times that of sucrose (Lobach *et al*., 2019) [44]. It is commercially available as colourless crystals or a white crystalline powder. Due to its relatively low sweetness potency, cyclamate is rarely used alone. Cyclamate is commonly used as sodium or calcium salts. It is frequently combined with saccharin or aspartame to enhance sweetness and reduce aftertaste. Such combinations improve overall sensory quality in food and beverage products (Behrens *et al*., 2017) [4].

3. Regulatory Framework and Acceptable Daily Intake (ADI)

High-intensity sweeteners (HIS) are regulated through structured safety assessment systems intended to protect public health. Major regulatory authorities include the U.S. Food and Drug Administration (FDA), the European Food Safety Authority (EFSA), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). National agencies such as Health Canada and Food Standards Australia New Zealand (FSANZ) also play important roles. These bodies evaluate toxicological, metabolic, and exposure data before approving HIS for use in foods (Renwick & Nordmann, 2007; EFSA, 2013) [66] Some sweeteners are approved as food additives following extensive pre-market testing. Others are permitted through processes such as Generally Recognized as Safe (GRAS) notifications in the United States. Steviol glycosides have been authorised for food use through GRAS determinations in the United States and have also undergone independent safety evaluations by EFSA and JECFA (EFSA, 2010; WHO, 2006; FDA, 2008). In contrast, aspartame and sucralose were approved as food additives following extensive long-term toxicological studies, including assessments of carcinogenicity, reproductive toxicity, and neurological effects (EFSA, 2013; Magnuson *et al*., 2017) [46]. As a result, regulatory limits and usage conditions may vary between regions even for the same sweetener.

The Acceptable Daily Intake is defined as the amount of a substance that can be consumed daily over a lifetime without appreciable health risk. It is expressed in milligrams per kilogram of body weight per day (EFSA, 2013). Acceptable Daily Intakes are derived primarily from animal toxicology studies. The process begins with identification of a no observed adverse effect level. This level represents the highest tested dose that does not produce harmful effects in the most sensitive toxicological endpoint. An uncertainty factor is then applied, which is typically set at 100. This factor accounts for differences between experimental animals and humans, as well as variability within the human population (Renwick, 1991) [65]. For example, long-term studies in rodents identified a no observed adverse effect level of 1,500 mg/kg body weight per day for sucralose. Application of the standard uncertainty factor of 100

resulted in the establishment of an Acceptable Daily Intake of 15 mg/kg body weight per day (JECFA, 2002; EFSA, 2016). Similar approaches have been applied to other high-intensity sweeteners. ADIs are intended as conservative guidance values for chronic lifetime exposure. Occasional intake above the ADI does not necessarily imply health risk, provided that average long-term intake remains below the established limit (Renwick, 1991) [65].

Table 1: Acceptable Daily Intake (ADI) recommendations for high-intensity sweeteners by international regulatory agencies

Sweetener	Acceptable Daily Intake (ADI) milligram per kilogram body weight per day (mg/kg/bw/d)			
	FDA	JECFA	EU	
Acesulfame Potassium (ACE-K) - E950	15	15	0-9	JECFA (2002, 2016); EFSA (2010, 2013, 2016); FDA (2008)
Advantame (ADV)	32.8	NS	NS	
Aspartame (ASP)	50	40	0-40	
Neotame (NEO)	0.3	2	---	
Saccharin (SAC)	15	5	0-5	
Sucralose (SCL)	5	15	0-15	
Cyclamate (CYC)	NS	11	0-7	

Differences in ADI values reflect variation in regulatory interpretation and assessment timelines.

Aspartame provides a well-known example. The ADI for aspartame is 50 mg/kg body weight per day in the United States and 40 mg/kg body weight per day in the European Union as shown in Table 1. EFSA adopted the lower value after reviewing updated data on neurological and genotoxic endpoints (EFSA, 2013). Cyclamate represents a well-known case of regulatory divergence. It was banned in the United States following early reports of bladder tumours in rodent studies. Subsequent evaluations indicated that these effects were species-specific and related to microbial conversion to cyclohexylamine. Despite the U.S. ban, cyclamate remains approved in many countries, including those in the European Union and Canada, with Acceptable Daily Intake values typically ranging from 7 to 11 mg/kg body weight per day (JECFA, 1982; Baines and DiNovi, 2010; Kroger *et al*., 2006) [3, 41]. In recent years, regulatory agencies have faced increasing pressure to review ADI frameworks. Traditional evaluations focused on endpoints such as carcinogenicity, organ toxicity, and reproductive effects. Newer research has highlighted endpoints that were not central to earlier assessments. These include changes in gut microbiota, endocrine signalling, and metabolic regulation (Suez *et al*., 2014; Bian *et al*., 2017; Gopalakrishnan *et al*., 2024) [78, 5, 28].

4. Absorption, Distribution, Metabolism, and Excretion (ADME) of High-Intensity Sweeteners

Understanding absorption, distribution, metabolism, and excretion is essential for evaluating the safety of high-intensity sweeteners (HIS). ADME characteristics determine systemic exposure, tissue distribution, and duration of biological interaction. These factors strongly influence toxicological relevance and interpretation of animal and human studies. HIS differ widely in chemical structure. As a result, their pharmacokinetic behaviour also varies substantially (Magnuson *et al*., 2017) [46].

4.1 General ADME Characteristics of High-Intensity Sweeteners

Many artificial HIS show limited metabolic transformation in humans. After ingestion, they are either poorly absorbed or rapidly eliminated. This leads to low or transient systemic exposure. Sucralose is a well-studied example. Most ingested sucralose passes through the gastrointestinal tract unchanged and is excreted in faeces. A smaller fraction is absorbed and excreted unchanged in urine, indicating minimal metabolism (Magnuson *et al*., 2017 [46]

Saccharin and acesulfame potassium follow a different pattern. These compounds are readily absorbed from the gastrointestinal tract. However, they undergo little or no metabolic transformation. They are rapidly eliminated through renal excretion. This results in brief systemic exposure with limited accumulation (Renwick & Nordmann, 2007) [66].

Aspartame represents an important exception among artificial high-intensity sweeteners. It is completely hydrolysed in the gastrointestinal tract into phenylalanine, aspartic acid, and methanol. These breakdown products are absorbed and enter normal metabolic pathways. As a result, systemic exposure occurs to the metabolites rather than to intact aspartame (Oppermann and Ranney, 1979; EFSA, 2013). This metabolic profile clearly distinguishes aspartame from most other high-intensity sweeteners.

Natural high-intensity sweeteners, particularly steviol glycosides, follow a different metabolic pathway. These compounds are resistant to digestion in the upper gastrointestinal tract and are not absorbed in the small intestine. They reach the colon, where gut microbiota hydrolyse them to steviol. Steviol is then absorbed, conjugated in the liver to steviol glucuronide, and excreted mainly in urine (Renwick & Nordmann, 2007; EFSA, 2010) [66]. This pathway results in low systemic exposure to the parent compounds.

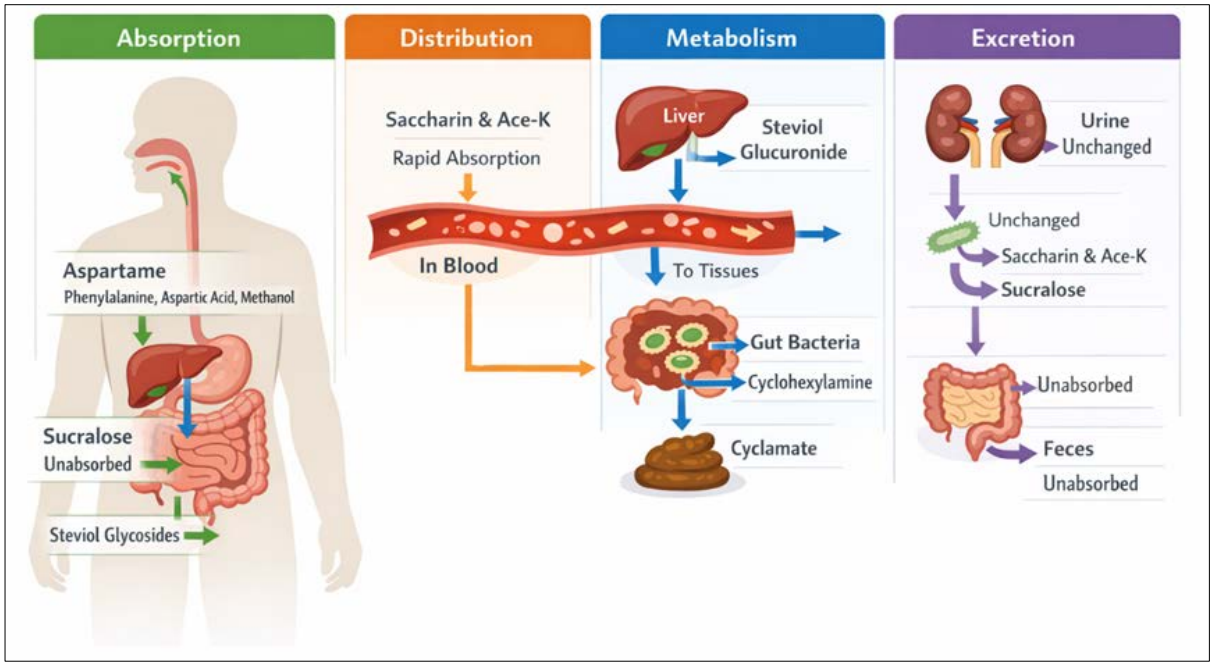

Fig 1: ADME pathways of high-intensity sweeteners

4.2 Comparative Systemic Exposure and Local Gastrointestinal Effects

ADME characteristics influence how toxicological data should be interpreted. Sweeteners that are extensively metabolised, such as aspartame, expose the body to common dietary metabolites. Comparative analysis of ADME profiles highlights substantial heterogeneity among HIS. Aspartame leads to rapid absorption of its metabolites and systemic exposure. This has raised concerns related to neurological and metabolic effects, particularly in susceptible individuals such as those with phenylketonuria (EFSA, 2013). In contrast, sucralose exhibits limited absorption and extensive excretion in unchanged form. Although systemic exposure is low, sucralose remains in the intestinal lumen for extended periods. This increases the potential for interaction with gut microbiota and intestinal epithelial cells (Magnuson *et al*., 2017) [46]. Saccharin and acesulfame potassium show moderate systemic exposure followed by rapid elimination. Their limited metabolism reduces the likelihood of bioaccumulation.

Neotame and advantame are consumed in extremely small amounts due to their very high sweetness potency. They are rapidly metabolised and eliminated. As a result, systemic exposure is minimal. Available toxicological data indicate low concern at approved intake levels (Magnuson *et al*., 2017) [46].

Cyclamate displays a more complex profile. A portion of ingested cyclamate can be converted by certain gut bacteria into cyclohexylamine as shown in Figure 1. This metabolite shows greater biological activity than the parent compound. Absorption, metabolism, and excretion profiles of some of high-intensity sweeteners are shown in Table 2.

Table 2: Absorption, metabolism, and excretion profiles of high-intensity sweeteners

Sweetener	Absorption	Metabolism	Excretion Route
Aspartame	Complete	Fully metabolised	Urine, breath
Sucralose	Limited	Minimal	Faeces, urine
Saccharin	High	None	Urine
Ace-K	High	None	Urine
Cyclamate	Partial	Microbial conversion	Urine
Steviol glycosides	None (parent)	Microbial hydrolysis	Urine

Sources: EFSA, 2013; Magnuson *et al*., 2017 [46]

5. Health and Toxicological Concerns of High-Intensity Sweeteners

High-intensity sweeteners (HIS) have undergone extensive toxicological testing before regulatory approval. Most have been considered safe when consumed within established Acceptable Daily Intake limits. Early evaluations focused on toxicity, organ damage, and carcinogenicity. Over time, assessment expanded to include genotoxicity, reproductive and developmental toxicity, neurotoxicity, and immunological effects (Roberts, 2015) [68].

5.1 Acute & Chronic Toxicity

High-intensity sweeteners (HIS) are widely used because of their low or negligible caloric contribution. However, concerns persist regarding their potential acute and chronic toxicological effects (Diniz *et al*., 2022) [14]. Acute toxicity refers to adverse effects that manifest shortly after short-term exposure, whereas chronic toxicity encompasses long-term health effects resulting from prolonged consumption, even at relatively low doses. The nature and magnitude of these effects are highly dependent on the specific sweetener involved (Palmnäs *et al*., 2014) [58]. For instance, studies investigating the acute toxicity of aspartame in rats have reported potential neurobehavioral alterations. Administration of aspartame at a dose of 3 mg/kg/day for six weeks was associated with impaired passive avoidance learning compared with control animals (Erbaş *et al*., 2018) [23]. Similarly, neurobehavioral changes have been documented in the aquatic organism *Daphnia magna*

following exposure to sucralose and acesulfame-K at environmentally relevant concentrations (Wiklund *et al*., 2023) [87]. Evidence of chronic effects has also been reported; a long-term study in Wistar rats demonstrated that continuous intake of acesulfame-K (0.015% in drinking water) significantly influenced body weight gain and glucose metabolism (Mendoza-Pérez *et al*., 2021 [50]

5.2 Carcinogenicity

Early concerns of carcinogenicity of HIS arose primarily from animal studies. For example, saccharin, in the 1970s, was linked to bladder cancer in male rats, leading to a ban in the US that was eventually lifted due to inconsistent epidemiological evidence in humans (Debras *et al*., 2022; Sharma *et al*., 2025) [12, 71]. Aspartame was classified as "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC). Despite the classification, results of carcinogenicity of aspartame are inconsistent. In a study using a mouse model revealed that dietary aspartame did not influence the development, progression, or mortality associated with pancreatic acinar carcinoma. The research showed no significant differences in the proportion of tumor onset or the age at which tumors appeared among mice consuming a standard diet, aspartame, or stevia (Lopes *et al*., 2023) [11]. Nonetheless, studies on its effect on breast and pancreatic cancer, need further investigation (Han *et al*., 2024). Acesulfame K has been linked to increased overall cancer risk in some epidemiological studies (Debras *et al*., 2022) [12]. In conclusion, although epidemiological studies indicate association between intake of specific sweeteners with an elevated risk of cancers, other studies and systematic reviews have not consistently confirmed these findings (Abu-Zaid *et al*., 2025; Marchitti *et al*., 2025 [1, 49]

5.3 Genotoxicity

Genotoxicity testing is a core component of HIS safety evaluation. It assesses the potential for DNA damage, mutations, and chromosomal alterations. Approved HIS have been tested using standard assay batteries. These include bacterial reverse mutation tests, in vitro chromosomal aberration assays, and in vivo micronucleus tests. Studies on human peripheral lymphocytes have indicated aspartame and Acesulfame K at high concentration can induces DNA damage. Mechanism of interaction of Acesulfame K with DNA was studied, suggesting formation of DNA-Ace-K complex in the minor groove (Hadidi *et al*., 2023) [30]. The mechanisms by which some artificial sweeteners might exert genotoxic effects are still being investigated. Some sweeteners can interact directly with DNA, as suggested for Acesulfame-K and saccharin (Hadidi *et al*., 2023; Mansourian *et al*., 2020 [30. 48]. Some factors may trigger oxidative stress or inflammatory reactions that indirectly result in DNA damage (Effenberger & Tilg, 2025).

5.4 Reproductive and Developmental Toxicity

Impact of HIS on reproductive health is another concerning area of research. Some studies suggest that they negatively impact fertility in both men and women (Atalay *et al*., 2025) [6]. One significant concern is the potential of these sweeteners to cross the placental barrier, leading to its exposure to fetus (Leth-Møller *et al*., 2023) [43]. Research has shown that HIS can appear in breast milk shortly after mothers consume diet soda, indicating a direct transfer route to infants (Halasa *et al*., 2020) [31]. For instance, sucralose and acesulfame K have been detected in infants following milk consumption, with their concentration influenced by the timing of milk intake after the beverage. Higher peak levels were observed when milk was consumed soon after the drink (Langevin *et al*., 2023) [42]. Prenatal exposure to artificial sweeteners has been linked to potential adverse effects on the growth and metabolic programming of offspring. Epidemiological studies have raised a significant concern such as a positive correlation between prenatal HIS exposure and an increased Body Mass Index (BMI) at one year of age (Halasa *et al*., 2020) [31]. Animal studies have provided further insights into these mechanisms. In rats, prenatal exposure to aspartame has been shown to induce metabolic and feeding behavior alterations in offspring (Toigo *et al*., 2015) [83]. These alterations may contribute to weight gain and an altered metabolic profile, a phenomenon that has also been observed with other non-illicit substances of abuse like high-fat diet, ethanol, and nicotine during gestation (Poon & Leibowitz, 2016) [62].

5.5 Neurotoxicity

Various sweeteners seem to have distinct effects on cognitive function and brain activity. Frequent consumption of sucralose has been linked to noticeable declines in memory performance and executive functioning, along with changes in brain electrical activity, such as abnormal EEG patterns. In contrast, sucrose primarily affected encoding memory but did not cause widespread neurological changes, as EEG activity remained stable. Meanwhile, steviol glycosides exhibited no significant metabolic or neurological impact during the short-term study period, indicating a relatively neutral profile. These findings underscore the need for larger, long-term studies to validate these preliminary results (López-Meza *et al*., 2022) [45].

5.6 Weight Management and Appetite

HIS are widely adopted as sugar substitutes in various food products and beverages due to their intense sweetness and negligible caloric content (Xue *et al*., 2025) [89]. The rationale behind their use is to give a sweet taste without the calories of sugar. This can help with weight loss and controlling blood sugar, especially for people with diabetes. However, the relationship between HIS consumption and obesity is not straightforward. Epidemiological data have often suggested a direct association between HIS intake and increased body weight, glycemic status, and adiposity (Mallu *et al*., 2020; Pearlman *et al*., 2017; Shearer & Swithers, 2016) [47, 60, 72]. Chronic saccharin consumption has been shown to increase food intake and body weight gain in rats (Aoyama & Nagano, 2020) [2]. A meta-analysis, for instance, indicated that both sugar-sweetened beverages (SSBs) and artificially sweetened beverages (ASBs) are linked to an increased risk of obesity (Ruanpeng *et al*., 2017) [69]. Despite strong associations in observational studies, establishing a causal link between HIS and metabolic syndrome risk factors remains challenging. Some studies suggest that while short-term replacement of sugar-sweetened beverages (SSBs) with NNS can lead to reduced caloric intake and modest weight loss, long term effects are less clear and sometimes contradictory (Nadolsky, 2021) [53]. Behavioural compensation refers to the phenomenon where individuals consuming HIS may inadvertently increase their

caloric intake from other sources, thereby negating the intended calorie reduction. This can occur due to several mechanisms. Artificial sweeteners may stimulate appetite, leading to increased caloric intake and a higher body mass index (Effenberger & Tilg, 2025) [17]. Another reason can be repeated consumption of artificially sweetened foods, which might alter the brain reward pathways, potentially increasing the preference for sweet tastes and driving increased consumption of other palatable, often calorie dense, foods (Roberts, 2015) [68]. The overall impact of HIS on weight and appetite is influenced by individual factors, dietary habits, and metabolic health (O'Connor *et al*., 2021) [54]. Therefore, it is crucial to cultivate a detailed understanding, recognizing that the long-term effectiveness and safety of HIS are still being actively investigated. Some research indicates possible negative impacts on metabolic health and cardiovascular risk.

5.7 Gut Microbiota Alteration

Although HIS were initially considered biologically inert due to limited gastrointestinal absorption and favored for their lack of calories, emerging studies now suggest they can induce dysbiosis (Hosseini *et al*., 2023) [34]. This has been linked to a range of adverse health outcomes, including inflammation, metabolic disturbances, and increased susceptibility to diseases such as inflammatory bowel disease (IBD) and type 2 diabetes (Conz *et al*., 2023) [9]. Studies suggests that even negligible amounts of artificial sweetener, that reaches the gut, can influence microbial diversity and activity, with potential negative effects on health (Plizga *et al*., 2024) [61]. Studies employ 16S RNA microbiota profiling and quantitative polymerase chain reaction (qPCR) analysis to dis cover alterations in the composition of gut microbiota. Studies on consumption of Aspartame, suggests that it significantly influenced the gut microbiota composition, with increase in total bacterial populations, particularly *Enterobacteriaceae* and *Clostridium leptum*. Additionally, an interaction was observed between aspartame and a high-fat diet, affecting *Roseburia ssp*. abundance and reducing the Firmicutes/ Bacteroidetes ratio (Palmnäs *et al*., 2014) [58]. In another study, it was found that consumption of sucralose at levels deemed acceptable for humans, over a period of 6 months, may disrupt gut microbial balance and lead to tissue inflammation. This is indicated by the presence of pro-inflammatory genes in bacteria and changes in fecal metabolites (Bian *et al*., 2017) [5].

6. Emerging Concerns and Research Directions

Traditional toxicological assessments have supported the safety of HIS within established ADI limits. However, advances in nutrition science and systems biology have revealed potential concerns beyond classical endpoints. These concerns relate to real-world exposure patterns and biological complexity.

One emerging issue in the safety evaluation of high-intensity sweeteners is cumulative exposure. Consumers rarely ingest a single sweetener in isolation. Multiple high-intensity sweeteners and other food additives are often consumed together within the same diet. Experimental and conceptual evidence suggests that combined exposure may influence gut microbiota composition and intestinal function more strongly than exposure to individual compounds alone (Gibney *et al*., 201) [27]. Current risk assessment frameworks rarely account for such mixture effects. Current regulatory frameworks usually assess additives individually. Combined exposure is not routinely evaluated. Technological advances in food formulation also raise new questions. Techniques such as encapsulation are used to improve stability and sensory quality of sweeteners. These approaches may alter absorption or interaction with biological tissues. The toxicological relevance of such modifications is not fully understood.

Endocrine-related effects represent another area of scientific interest in the safety evaluation of high-intensity sweeteners. Experimental studies have explored potential interactions of certain sweeteners with hormone-related pathways under in vitro conditions. Regulatory evaluations of steviol glycosides have reported no evidence of biologically relevant endocrine effects in vivo at dietary exposure levels (EFSA, 2010). More broadly, endocrine-active compounds have been shown to exhibit non-linear dose-response relationships in experimental systems. Traditional Acceptable Daily Intake frameworks are based on threshold assumptions and monotonic dose-response models. These assumptions may not fully capture subtle biological effects that occur at low exposure levels.

Vulnerable populations require special attention in assessments of high-intensity sweeteners. Infants, children, pregnant women, and individuals with metabolic disorders may differ in exposure patterns and physiological sensitivity. Evidence from human cohort studies suggests that maternal consumption of artificially sweetened beverages during pregnancy is associated with differences in infant gut microbiome composition and early growth outcomes, highlighting potential early-life programming effects (Richardson *et al*., 2022; Palatnik *et al*., 2020) [67-57]. Individual variability in response to non-nutritive sweeteners is increasingly recognised. In a controlled human study, consumption of saccharin and sucralose produced person-specific alterations in microbiome signatures and glycemic response, suggesting that genetic background and baseline microbiome composition influence metabolic outcomes (Suez *et al*., 2022) [78]. Reviews of clinical trials also indicate that baseline microbiota profiles may predict how individuals respond to sweetener exposure, with some showing impairment in glucose tolerance and others showing no significant effect (Gauthier *et al*., 2024) [26]. This supports the need for personalised approaches in nutrition and toxicology research.

7. Conclusion and Future Outlook

High-intensity sweeteners play an important role in modern food systems. Regulatory evaluations have generally concluded that approved HIS are safe within established ADI limits. These conclusions are supported by extensive toxicological testing. At the same time, new evidence highlights biological effects that extend beyond traditional endpoints. Gut microbiota modulation, metabolic signalling, and individual variability are increasingly recognised. These findings do not invalidate current safety conclusions. They highlight uncertainty and knowledge gaps. Future research should prioritise long-term human studies. These studies should integrate dietary assessment with clinical, microbiome, and molecular endpoints. Vulnerable populations should receive greater attention. Regulatory frameworks should continue to evolve. Incorporating cumulative exposure, emerging endpoints, and population

variability will strengthen confidence in safety assessments. In conclusion, HIS can be used safely within current regulatory limits. Ongoing scientific scrutiny remains essential. A compound-specific and evidence-based approach is required to guide future policy, research, and consumer guidance.

References

1. Abu-Zaid A, Kutbi E, Alshammari N, AlJurayyan AN, Adly HM, Saleh SAK, *et al*. The association of artificial sweeteners intake and risk of cancer: an umbrella meta-analysis. Frontiers in Medicine. 2025;12:1647178. DOI:10.3389/fmed.2025.1647178.
2. Aoyama K, Nagano A. Effects of saccharin consumption on operant responding for sugar reward and incubation of sugar craving in rats. Foods. 2020;9(12):1823. DOI:10.3390/foods9121823.
3. Baines MJ, DiNovi M. Cyclamic acid and its salts: dietary exposure assessment. Safety evaluation of certain food additives. 2010;29:1-44.
4. Behrens M, Blank K, Meyerhof W. Blends of non-caloric sweeteners saccharin and cyclamate show reduced off-taste due to TAS2R bitter receptor inhibition. Cell Chemical Biology. 2017;24(10):1199-1204.
5. Bian X, Chi L, Gao B, Tu P, Ru H, Lu K. The artificial sweetener acesulfame potassium affects the gut microbiome and body weight gain in CD-1 mice. PLOS ONE. 2017;12(6):e0178426. DOI:10.1371/journal.pone.0178426.
6. Celik Atalay E, Er Demirhan B, Sagdıcoglu Celep AG. Low-calorie sweeteners and reproductive health: evidence and debates. Current Nutrition & Food Science. 2025;21(3):309-332. DOI:10.2174/0115734013315621240802055207.
7. Chattopadhyay S, Raychaudhuri U, Chakraborty R. Artificial sweeteners - a review. Journal of Food Science and Technology. 2014;51(4):611-621. DOI:10.1007/s13197-011-0571-1.
8. Choi Y, Wong RR, Cha YK, Park TH, Kim Y, Chung SJ. Sweet-bitter taste interactions in binary mixtures of sweeteners: relationship between taste receptor activities and sensory perception. Food Chemistry. 2024;459:140343.
9. Conz A, Salmona M, Diomede L. Effect of non-nutritive sweeteners on the gut microbiota. Nutrients. 2023;15(8):1869. DOI:10.3390/nu15081869.
10. Czarnecka K, Pilarz A, Rogut A, Maj P, Szymańska J, Olejnik Ł, *et al*. Aspartame—true or false? Narrative review of safety analysis of general use in products. Nutrients. 2021;13(6):1957.
11. De Souza Lopes A, Antunes ECA, Machado IÁ, Sartoratto A, Duarte CT. The impact of antimicrobial food additives and sweeteners on the growth and metabolite production of gut bacteria. Folia Microbiologica. 2023;68(5):813-821. DOI:10.1007/s12223-023-01076-6.
12. Debras C, Chazelas E, Srour B, Druesne-Pecollo N, Esseddik Y, Szabo de Edelenyi F, *et al*. Artificial sweeteners and cancer risk: results from the NutriNet-Santé population-based cohort study. PLOS Medicine. 2022;19(3):e1003950. DOI:10.1371/journal.pmed.1003950.
13. Dhartiben BK, Aparnathi KD. Chemistry and use of artificial intense sweeteners. International Journal of Current Microbiology and Applied Sciences. 2017;6(6):1283-1296.
14. Diniz JAD, Pedreira MEDO, Moore SR, Dala-Paula BM. Edulcorantes artificiais: regulamentação no Brasil, implicações tecnológicas na produção de alimentos e na saúde. Revista Uningá. 2022;59:eUJ4280. DOI:10.46311/2318-0579.59.eUJ4280.
15. Dudure R, Ganorkar K, Beldar V, Ghosh SK, Panda AK, Jadhao M. Effect of artificial sweetener saccharin on lysozyme aggregation: a combined spectroscopic and in silico approach. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2023;290:122269.
16. Dwivedi RS. Saccharide sweet (SS) principles, classification and structural and functional details of SS sweeteners and plants. In: Dwivedi RS, editor. Alternative Sweet and Supersweet Principles. Singapore: Springer Nature; 2022. p. 113-223. DOI:10.1007/978-981-33-6350-2_4.
17. Effenberger M, Tilg H. Potential health risks of artificial sweeteners. Annual Review of Medicine. 2025;76:1-15. DOI:10.1146/annurev-med-043024-012626.
18. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Scientific opinion on the safety of steviol glycosides for the proposed uses as a food additive. EFSA Journal. 2010;8(4):1537. DOI:10.2903/j.efsa.2010.1537.
19. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Safety of the proposed extension of use of sucralose (E 955) in foods for special medical purposes in young children. EFSA Journal. 2016;14(1):4361.
20. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Scientific opinion on the re-evaluation of aspartame (E 951) as a food additive. EFSA Journal. 2013;11(12):3496. DOI:10.2903/j.efsa.2013.3496.
21. EFSA Scientific Committee; More SJ, Bampidis V, Benford D, Bennekou SH, Bragard C, *et al*. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal. 2019;17(3):e05634. DOI:10.2903/j.efsa.2019.5634.
22. EFSA Scientific Committee. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal. 2012;10(3):2579. DOI:10.2903/j.efsa.2012.2579.
23. Erbaş O, Erdoğan MA, Khalilnezhad A, Solmaz V, Gürkan FT, Yiğittürk G, *et al*. Evaluation of long-term effects of artificial sweeteners on rat brain: a biochemical, behavioral, and histological study. Journal of Biochemical and Molecular Toxicology. 2018;32(6):e22053. DOI:10.1002/jbt.22053.
24. US Food and Drug Administration. Agency response letter GRAS Notice No. GRN 253: Rebaudioside A purified from *Stevia rebaudiana* (Bertoni) Bertoni. Silver Spring (MD): FDA; 2008.
25. Flad E, Altstädt A, Beglinger C, Rehfeld JF, Van Oudenhove L, Wölnerhanssen BK, *et al*. Effects of oral xylitol, sucrose, and acesulfame potassium on total

energy intake during a subsequent ad libitum test meal: a randomized, controlled, crossover trial in healthy humans. Nutrients. 2025;17(3):484.
26. Gauthier E, Milagro FI, Navas-Carretero S. Effect of low- and non-calorie sweeteners on the gut microbiota: a review of clinical trials and cross-sectional studies. Nutrition. 2024;117:112237. DOI:10.1016/j.nut.2023.112237.
27. Gibney MJ, Forde CG, Mullally D, Gibney ER. Ultra-processed foods in human health: a critical appraisal. American Journal of Clinical Nutrition. 2017;106(3):717-724. DOI:10.3945/ajcn.117.160440.
28. Gopalakrishnan NK, Balasubramanian B, Kundapur R, Chaudhary A, Meyyazhagnan A, Pappuswamy M. Unraveling connections with artificial sweeteners and their impact on human health: a comprehensive review. eFood. 2024;5(5):e184. DOI:10.1002/efd2.184.
29. Gwak MJ, Chung SJ, Kim YJ, Lim CS. Relative sweetness and sensory characteristics of bulk and intense sweeteners. Food Science and Biotechnology. 2012;21(3):889-894. DOI:10.1007/s10068-012-0115-0.
30. Hadidi S, Varmira K, Soltani L. Evaluation of DNA damage induced by acesulfame potassium: spectroscopic, molecular modeling simulations and toxicity studies. Journal of Biomolecular Structure and Dynamics. 2023;41(13):6262-6271. DOI:10.1080/07391102.2022.2105955.
31. Halasa BC, Sylvetsky A, Conway EM, Walter PJ, Cai H, Walter MF, *et al*. Prenatal exposure to artificial sweeteners. Journal of the Endocrine Society. 2020;4(Suppl 1):SUN-055. DOI:10.1210/jendso/bvaa046.1281.
32. Han S, Yang J, Park JE, Kim JH. Artificial sweeteners and pancreatic cancer: is aspartame a culprit or a coincidence? Pakistan Journal of Medical Sciences. 2024;40(4):1-6. DOI:10.12669/pjms.40.4.8490.
33. Hao S, Guthrie B, Kim SK, Balanda S, Kubicek J, Murtaza B, *et al*. Steviol rebaudiosides bind to four different sites of the human sweet taste receptor (T1R2/T1R3) complex explaining confusing experiments. Communications Chemistry. 2024;7(1):236.
34. Hosseini A, Barlow GM, Leite G, Rashid M, Parodi G, Wang J, *et al*. Consuming artificial sweeteners may alter the structure and function of duodenal microbial communities. iScience. 2023;26(12):108530. DOI:10.1016/j.isci.2023.108530.
35. Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants: fifty-seventh report. Geneva: World Health Organization; 2002.
36. Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants: eightieth report. Geneva: World Health Organization; 2016.
37. Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants: twenty-sixth report. Rome: FAO/WHO; 1982.
38. Karakütük İA, Şengül M, Zor M, Aksoy S. The effects of using different plant species and sweeteners (stevia and sucrose) in sherbet production on chemical and sensory quality of sherbet. Journal of Food Measurement and Characterization. 2023;17(5):5308-5321.
39. Kawakami CA, Selani MM, Saldana E, Pimentel-Filho NJ, Domingues MAF. Sensory dynamic profile and consumer acceptance of short-dough biscuits with reduced sucrose and thaumatin addition. Food Research International. 2025;200:115524.
40. Keskin FN, Şahin TÖ, Capasso R, Ağagündüz D. Protein substitutions as new-generation pharmanutrition approach to managing phenylketonuria. Clinical and Experimental Pediatrics. 2023;66(8):320-328.
41. Kroger M, Meister K, Kava R. Low-calorie sweeteners and other sugar substitutes: a review of the safety issues. Comprehensive Reviews in Food Science and Food Safety. 2006;5(2):35-47. DOI:10.1111/j.1541-4337.2006.tb00081.x.
42. Langevin B, Gopalakrishnan M, Kuttamperoor J, Van Den Anker J, Murphy J, Arcaro KF, *et al*. The MILK study: investigating intergenerational transmission of low-calorie sweeteners in breast milk. Contemporary Clinical Trials Communications. 2023;36:101212. DOI:10.1016/j.conctc.2023.101212.
43. Leth-Møller M, Duvald CS, Stampe S, Greibe E, Hoffmann-Lücke E, Pedersen M, *et al*. Transplacental transport of artificial sweeteners. Nutrients. 2023;15(9):2063. DOI:10.3390/nu15092063.
44. Lobach AR, Roberts A, Rowland IR. Assessing the in vivo data on low/no-calorie sweeteners and the gut microbiota. Food and Chemical Toxicology. 2019;124:385-399.
45. López-Meza MS, Otero-Ojeda G, Estrada JA, Esquivel-Hernández FJ, Contreras I. The impact of nutritive and non-nutritive sweeteners on the central nervous system: preliminary study. Nutritional Neuroscience. 2022;25(8):1623-1632. DOI:10.1080/1028415X.2021.1885239.
46. Magnuson BA, Roberts A, Nestmann ER. Critical review of the current literature on the safety of sucralose. Food and Chemical Toxicology. 2017;106:324-355.
47. Mallu MR, Naredla K, Meesala ML, Karyamsetty KV, Dudekula S, Boppa H, *et al*. Artificial sweeteners and metabolic syndrome: paradox of physiological behavior or neuroendocrine mechanisms. Journal of Pharmaceutical Research International. 2020;32(33):85-93. DOI:10.9734/jpri/2020/v32i3330953.
48. Mansourian M, Mahnam K, Rajabi HR, Roushani M, Doustimotlagh AH. Exploring the binding mechanism of saccharin and sodium saccharin to promoter of human p53 gene by theoretical and experimental methods. Journal of Biomolecular Structure and Dynamics. 2020;38(2):548-564. DOI:10.1080/07391102.2019.1582438.
49. Marchitti SA, Boon D, Jack M, Goodman JE. Lack of genotoxic and carcinogenic potential for nonsugar sweeteners: a review of animal and mechanistic evidence. Advances in Nutrition. 2025;16(12):100552. DOI:10.1016/j.advnut.2025.100552.
50. Mendoza-Pérez S, Guzmán-Gómez MB, García-Gómez RS, Ordaz-Nava G, Gracia-Mora MI, Macías-Rosales L, *et al*. Effects on weaned male Wistar rats after 104, 197, and 288 days of chronic consumption of nutritive and non-nutritive additives in water. Journal of Food Science and Technology. 2021;58(6):2349-2359. DOI:10.1007/s13197-020-04746-2.

51. Mooradian AD, Smith M, Tokuda M. The role of artificial and natural sweeteners in reducing the consumption of table sugar: a narrative review. Clinical Nutrition ESPEN. 2017;18:1-8. DOI:10.1016/j.clnesp.2017.01.004.
52. Mora M, Wijaya F, Jiang G, Gibney P, Dando R. Sensory profiling of natural sweeteners and sucrose-sweetener binary mixtures. Journal of Food Science. 2023;88(7):2984-2995. DOI:10.1111/1750-3841.16610.
53. Nadolsky KZ. Artificial sweeteners for obesity—better than sugary alternatives; potentially a solution. Endocrine Practice. 2021;27(10):1056-1061. DOI:10.1016/j.eprac.2021.06.013.
54. O'Connor D, Pang M, Castelnuovo G, Finlayson G, Blaak E, Gibbons C, *et al*. A rational review on the effects of sweeteners and sweetness enhancers on appetite, food reward and metabolic/adiposity outcomes in adults. Food & Function. 2021;12(2):442-465. DOI:10.1039/D0FO02424D.
55. Oppermann JA, Ranney RE. The metabolism of aspartate in infant and adult mice. Journal of Environmental Pathology and Toxicology. 1979;2(4):987-998.
56. Otabe A, Fujieda T, Masuyama T, Ubukata K, Lee C. Advantame—an overview of the toxicity data. Food and Chemical Toxicology. 2011;49(Suppl 1):S2-S7.
57. Palatnik A, Moosreiner A, Olivier-Van Stichelen S. Consumption of non-nutritive sweeteners during pregnancy. American Journal of Obstetrics and Gynecology. 2020;223(2):211-218. DOI:10.1016/j.ajog.2020.03.034.
58. Palmnäs MSA, Cowan TE, Bomhof MR, Su J, Reimer RA, Vogel HJ, *et al*. Low-dose aspartame consumption differentially affects gut microbiota-host metabolic interactions in the diet-induced obese rat. PLOS ONE. 2014;9(10):e109841. DOI:10.1371/journal.pone.0109841.
59. Patel Y, Elfadil OM, Patel S, Ghanem OM, Hurt RT, Mundi MS. Rediscovering sweetness: the evolution and impact of non-nutritive and natural sweeteners. Current Nutrition Reports. 2025;14(1):54-65.
60. Pearlman M, Obert J, Casey L. The association between artificial sweeteners and obesity. Current Gastroenterology Reports. 2017;19(12):64. DOI:10.1007/s11894-017-0602-9.
61. Plizga J, Głuszczyk A, Surma A, Cecot J, Parfianowicz A, Grajnert F, *et al*. Understanding the relationship between artificial sweeteners and gut microbiota: literature review. Archiv Euromedica. 2024;14(4):1-10. DOI:10.35630/2024/14/4.411.
62. Poon K, Leibowitz SF. Consumption of substances of abuse during pregnancy increases consumption in offspring: possible underlying mechanisms. Frontiers in Nutrition. 2016;3:11. DOI:10.3389/fnut.2016.00011.
63. Prakash I, Markosyan A, Bunders C. Development of next generation stevia sweetener: rebaudioside M. Foods. 2014;3(1):162-175. DOI:10.3390/foods3010162.
64. Redha AA, Torquati L, Bows JR, Gidley MJ, Cozzolino D. Microencapsulation of broccoli sulforaphane using whey and pea protein: in vitro dynamic gastrointestinal digestion and intestinal absorption by Caco-2-HT29-MTX-E12 cells. Food & Function. 2025;16(1):71-86.
65. Renwick AG. Safety factors and establishment of acceptable daily intakes. Food Additives & Contaminants. 1991;8(2):135-149. DOI:10.1080/02652039109373964.
66. Renwick AG, Nordmann H. First European conference on aspartame: putting safety and benefits into perspective. Food and Chemical Toxicology. 2007;45(7):1308-1313. DOI:10.1016/j.fct.2007.02.019.
67. Richardson IL, Frese SA. Non-nutritive sweeteners and their impacts on the gut microbiome and host physiology. Frontiers in Nutrition. 2022;9:988144. DOI:10.3389/fnut.2022.988144.
68. Roberts JR. The paradox of artificial sweeteners in managing obesity. Current Gastroenterology Reports. 2015;17(1):1. DOI:10.1007/s11894-014-0423-z.
69. Ruanpeng D, Thongprayoon C, Cheungpasitporn W, Harindhanavudhi T. Sugar and artificially sweetened beverages linked to obesity: a systematic review and meta-analysis. QJM. 2017;110(8):513-520. DOI:10.1093/qjmed/hcx068.
70. Shankar P, Ahuja S, Sriram K. Non-nutritive sweeteners: review and update. Nutrition. 2013;29(11-12):1293-1299.
71. Sharma H, Hilal A, Aseri GK, Jain N. Sweet or sour? A review of the aspartame market landscape, carcinogenicity, and its socioeconomic impact. Journal of Food Science and Technology. 2025;62(1):24-37. DOI:10.1007/s13197-024-06077-y.
72. Shearer J, Swithers SE. Artificial sweeteners and metabolic dysregulation: lessons learned from agriculture and the laboratory. Reviews in Endocrine and Metabolic Disorders. 2016;17(2):179-186. DOI:10.1007/s11154-016-9372-1.
73. Silva MM, Reboredo FH, Lidon FC. Sweetener food additives: a synoptical overview on their chemical properties, applications in food products and side effects. Emirates Journal of Food and Agriculture. 2023;35:1-16.
74. Silva MR, Moya CA, Samano Leon AG, Velasco RR, Castro Flores AM. Genotoxic activity of saccharin, acesulfame-K, stevia and aspartame-acesulfame-K in commercial form. Journal of Clinical Toxicology. 2018;8(3):1-6. DOI:10.4172/2161-0495.1000385.
75. Silva PD, Cruz R, Casal S. Sugars and artificial sweeteners in soft drinks: a decade of evolution in Portugal. Food Control. 2021;120:107481.
76. Slade L, Kweon M, Levine H. Exploration of the functionality of sugars in cake-baking, and effects on cake quality. Critical Reviews in Food Science and Nutrition. 2021;61(2):283-311. DOI:10.1080/10408398.2020.1729694.
77. Sousa RCSD, Gloria MBA. Sweeteners in Brazilian processed foods and beverages: prevalence, profile and concomitant addition of sugars and nutritional claims. Food Additives & Contaminants Part A. 2023;40(10):1285-1297. DOI:10.1080/19440049.2023.2255291.
78. Suez J, Cohen Y, Valdés-Mas R, Mor U, Dori-Bachash M, Federici S, *et al*. Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. Cell. 2022;185(18):3307-3328.
79. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, *et al*. Artificial sweeteners induce

glucose intolerance by altering the gut microbiota. Nature. 2014;514(7521):181-186. DOI:10.1038/nature13793.
80. Sun JP, Han Q, Zhang XQ, Ding MY. Investigations on the degradation of aspartame using high-performance liquid chromatography/tandem mass spectrometry. Chinese Chemical Letters. 2014;25(9):1259-1264.
81. Sun Y, Zhang S, Bao T, Jiang Z, Huang W, Xu X, *et al*. Comprehensive new insights into sweet taste transmission mechanisms and detection methods. Foods. 2025;14(13):2397.
82. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, *et al*. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocrine Reviews. 2012;33(3):378-455. DOI:10.1210/er.2011-1050.
83. Von Poser Toigo E, Huffell AP, Mota CS, Bertolini D, Pettenuzzo LF, Dalmaz C. Metabolic and feeding behavior alterations provoked by prenatal exposure to aspartame. Appetite. 2015;87:168-174. DOI:10.1016/j.appet.2014.12.213.
84. Wang C, Liu Y, Zhao X, Liu B. Current advances and future aspects of sweetener synergy: properties, evaluation methods and molecular mechanisms. Applied Sciences. 2022;12(10):5096.
85. Whitehouse CR, Boullata J, McCauley LA. The potential toxicity of artificial sweeteners. AAOHN Journal. 2008;56(6):251-261. DOI:10.1177/216507990805600604.
86. World Health Organization. Safety evaluation of certain food additives. Geneva: WHO; 2006.
87. Wiklund AKE, Guo X, Gorokhova E. Cardiotoxic and neurobehavioral effects of sucralose and acesulfame in *Daphnia*: toward understanding ecological impacts of artificial sweeteners. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2023;273:109733. DOI:10.1016/j.cbpc.2023.109733.
88. Wilk K, Korytek W, Pelczyńska M, Moszak M, Bogdański P. The effect of artificial sweeteners use on sweet taste perception and weight loss efficacy: a review. Nutrients. 2022;14(6):1261. DOI:10.3390/nu14061261.
89. Xue H, Kang X, Hong K, Gao Y, Tang Y, Lin Y, *et al*. Effects of artificial and natural sweeteners on host metabolic health: a double-edged sword. Food Research International. 2025;220:117158. DOI:10.1016/j.foodres.2025.117158.
90. Zeynep F, Sifa T. Determination of the effects of some artificial sweeteners on human peripheral lymphocytes using the comet assay. Journal of Toxicology and Environmental Health Sciences. 2014;6(8):147-153. DOI:10.5897/JTEHS2014.0313.