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The potential application of a biomarker approach for the investigation of low-calorie sweetener exposure

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Low-calorie sweeteners (LCS) are commonly used as sugar substitutes in the diet to provide a desired sweet taste without increased energy intake. The number of LCS available on the market has increased considerably over the years and despite extensive evaluation of their safety prior to approval, debate continues around the effects of consumption on health. In Europe, Member States are obligated to monitor exposure to LCS and methods currently used tend to rely on self-reported dietary intake data alongside LCS concentrations in products. However, the acquisition of accurate data can be costly in terms of resources and time and are inherently imprecise. Although LCS are intensely sweet, they are chemically diverse and a limitation of many studies investigating the health effects of consumption is that they often fail to discern intakes of individual LCS. An approach which objectively assesses intakes of individual LCS would therefore allow robust investigations of their possible effects on health. Biomarker approaches have been utilised for the objective investigation of intakes of a range of dietary components and the feasibility of any such approach depends upon its validity as well as its applicability within the target population. This review aims to provide an overview of current understanding of LCS intake and explore the possibility of implementing a biomarker approach to enhance such understanding. Several commonly used LCS, once absorbed into the body, are excreted via the kidneys; therefore a urinary biomarker approach may be possible for the investigation of short-term exposure to these compounds.

Low-calorie sweeteners: Intense sweeteners: Biomarkers: Exposure assessment

Since the 1980s, the prevalence of obesity has more than doubled globally[1] with significant implications in terms of the development of chronic conditions such as CVD, type 2 diabetes mellitus and hypertension[2]. Given that the overall pattern of weight gain has been attributed to a culmination of a host of factors[2], it is of no surprise that a multi-faceted approach has been proposed to address the issue. One factor implicated in the development of weight gain, as well as a range of adverse health outcomes, is the over-consumption of non-milk extrinsic sugars from foodstuffs, particularly from sugar-sweetened beverages[3–8]. A logical strategy to address these issues would be to reduce the intakes of these beverages. However in practice, this approach may be difficult given the apparent innate preference for sweet taste among human subjects[9]. To satisfy the desire for sweet-tasting products without exacerbating the problem of overconsumption of non-milk extrinsic sugars, low-calorie sweeteners (LCS) have become more commonly used as substitutes in a wide range of products[10].

This review will provide an overview of current understanding in relation to intakes of LCS and will consider existing methods for monitoring exposure to these compounds. It will also explore the possibility of implementing a novel biomarker approach for investigating LCS exposure, which could be used to gain a better understanding of LCS consumption and health.

Abbreviations: ADI, acceptable daily intake; EU, European Union; LCS, low-calorie sweeteners.

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Low-calorie sweeteners

LCS can be broadly divided into two groups; bulk sweeteners (e.g. polyols), which have a similar sweetness to sucrose and are also commonly used for other functional purposes in products[11,12], and intense sweeteners, which are many times sweeter than sucrose and are mainly used only for their sweetening properties[11]. The focus of the remainder of this review will be on intense sweeteners. Intense sweeteners which are currently approved for use in the European Union (EU), together with their general characteristics, are listed in Table 1. The use of LCS has increased considerably over recent years and they can now be found in a wide variety of food and non-food products[10]. The level of sweetness that they impart, relative to sucrose, ranges from thirty times sweeter (cyclamates) to 37 000 times sweeter (advantame) and despite possessing the common characteristic of being intensely sweet, they represent a chemically diverse group of compounds. The overall contribution of LCS to energy intake is negligible and it is upon this basis, along with the fact that they are also non-cariogenic, that they are commonly used. Prior to approval, the safety of LCS consumption in human subjects is established through extensive evaluation of existing safety and toxicological data, usually culminating in the assignment of acceptable daily intake (ADI) and maximum permitted use levels[13–15].

Acceptable daily intake

An ADI, which is expressed as mg/kg body weight, has been defined as the amount of a chemical that can be consumed daily over the period of a lifetime with no appreciable risk to health[16]. To assign an ADI, long-term, multiple-dose animal studies are typically used to initially establish the no observed adverse effect level by identifying the highest level of exposure which causes no adverse effects in the most sensitive species of animals[13–15]. To account for variation between species and within human subjects, safety factors are then applied (Fig. 1). In the absence of serious adverse effects (e.g. teratogenicity) in animal studies, an overall safety factor of 100 is usually applied to the no observed adverse effect level[14], although higher or lower safety factors can also be applied.

Health effects of low-calorie sweeteners intake

Despite extensive evaluation of the safety of LCS, the potential health effects of consumption has remained topical within the area of nutrition research[11,17,18]. Moreover, the long-term efficacy of using LCS in place of non-milk extrinsic sugars as a weight management tool has yet to be conclusively established[19–25]. A recent meta-analysis of randomised controlled trials and prospective cohort studies into the effects of LCS consumption on weight status, reported that in prospective cohort studies, a small positive association was observed between LCS consumption and increased BMI (0·03 kg/m^2) but not body weight or fat mass. However in randomised controlled trials, LCS consumption was associated with modest, albeit significant reductions in body weight (−0·80 kg), BMI (−0·24 kg/m^2), fat mass (−1·10 kg) and waist circumference (−0·83 cm)[26]. The potential mechanisms by which LCS consumption might influence appetite and food intake were reviewed by Mattes and Popkin[27] and they concluded that, although the evidence was lacking for many putative mechanisms (such as cephalic phase stimulation, gut peptide response and increased palatability of products), further research in the free living population via long-term randomised controlled trials was warranted. In addition to body weight status, LCS consumption in relation to a range of adverse health outcomes including cancer[28,29], CVD[30,31], diabetes mellitus[32,33] and preterm deliveries[34] has also been investigated. No convincing evidence of a risk in the development of any adverse effects as a result of LCS consumption has been presented to date. The French Agency for Food, Environmental and Occupational Health and Safety recently undertook a review of the evidence with regard to many of these outcomes and concluded that, although the data do not demonstrate a risk, further research is required to establish the long-term beneficial effects of LCS consumption on health[35]. Furthermore, the French Agency for Food, Environmental and Occupational Health and Safety also recommended that future cohort studies should aim to distinguish the intakes of individual LCS, so that the effects of single and multiple LCS use can be investigated more effectively[35]. The ongoing debate around the safety of LCS consumption has served to fuel a somewhat negative perception within the lay media and the population in general. A recent study by Harricharan *et al.*[36] investigated the attitudes of dietitians from several European countries towards LCS and highlighted a diversity of opinions ranging from negative, ambivalent to positive; they suggested the provision of guidance similar to that which has been undertaken in the USA[37].

Assessment of exposure to low-calorie sweeteners

In accordance with EU Regulation 1333/2008, EU Member States are required to monitor levels of LCS intake within the population to ensure that the ADI is not being exceeded[38]. Assessment of exposure to LCS requires the consideration of food intake data along with LCS concentrations within products and can be expressed, according to the International Programme on Chemical Safety[39], as:

$$\frac{\Sigma(\text{Food LCS concentration} \times \text{Food consumption})}{\text{Body weight}}$$

Given that currently there are almost 400 food additives approved for use in the EU, the potential costs associated with collecting accurate intake data at the level of the individual are considerable. However, as the primary aim of monitoring is to ensure that the ADI is not being exceeded[40], a tiered approach is usually adopted, beginning with a conservative screening step and progressing to more refined, and thus costly assessments, if indicated[39,40].

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Table 1. Intense sweeteners approved for use in Europe

	E-number	Sweetness*	ADI (mg/kg BW)	Year of approval
Intense sweeteners				
Saccharin and its salts	E954	300–500	0–5	1977
Aspartame	E951	180–200	0–40	1984
Acesulfame-K	E950	200	0–9	1984
Cyclamates	E952	30	0–7	1984
Thaumatin	E957	2000–3000	No ADI	1984
NHDC	E959	1900	0–5	1988
Aspartame-acesulfame salt	E962	350	See aspartame and acesulfame-K	2000
Sucralose	E955	600	0–15	2000
Steviol glycosides	E960	300	0–4†	2011
Advantame	E969	37000	0–5	2014

NHDC, neohesperidine dihydrochalcone; ADI, acceptable daily intake; BW, body weight.
* Relative to sucrose.
† Expressed as steviol equivalents.

Tiered approaches for assessment of low-calorie sweeteners exposure

An example of a tiered approach for assessing exposure to LCS is illustrated in Fig. 2. Tier 1, which is carried out at European wide level, will usually consist of an initial screening method designed to generate a highly conservative estimate so that potentially at risk groups can be easily identified. Examples of such screening methods include the Sweetener Substitution Method[41] and the Danish Budget Method[42]. In the Danish Budget Method, conservative assumptions are made about the occurrence of LCS in food and beverages and an individual's energy and fluid requirements; this will result in an overestimate of exposure. If, following the initial screening step, the ADI is deemed to be exceeded, further more refined assessments would then be indicated. Tier 2 involves the assessment of actual consumption of foods and beverages known to contain LCS along with the maximum permitted use levels. A Tier 3 assessment would be indicated if the ADI is estimated to be exceeded following the Tier 2 assessment and this will consist of a more refined calculation using actual levels of consumption and actual concentrations in products in order to further elucidate the risk.

Methods of assessing exposure to low-calorie sweeteners

Dietary intake is traditionally assessed using tools such as food diaries, 24-h recalls and FFQ[43]. These assessment tools rely on self-reported data and a number of inherent limitations exist which often results in inaccuracies[44–47]. The optimal methodology for assessing habitual LCS exposure is a 1–2-week prospective, brand level diary, including information on portion sizes along with brand-specific information on LCS concentrations in products[48]. Given that obtaining such refined individual level data can be time and resource intensive for investigators, as well as labour intensive for participants, a variety of dietary assessment tools have been utilised in the past including 24-h recalls[49–53], FFQ covering various durations[54–57] and food diaries lasting 2 d[58], 5 d[59], 7 d and 14 d[60]. Some studies used a combination of retrospective and prospective intake data in order to first identify potential high consumers and then to further investigate these high consumers[61,62]. It is apparent therefore that many of the published studies do not satisfy these criteria, potentially making it difficult to make direct comparisons.

Data on LCS concentrations in products can be obtained from a number of sources; the least resource intensive, yet least accurate, is to use maximum permitted use levels. This source of information is commonly used in initial, conservative estimates of exposure, or when it is not possible to obtain more refined data[48]. However, the actual levels used in products are unlikely to meet these values, particularly when a blend of two or more LCS is used within a particular product, as is frequently done. Therefore using maximum permitted use levels in the calculation will result in an overestimate of exposure. Concentrations in specific products have been obtained from manufacturers[49,56,60–64], providing a more accurate measure; however, difficulties in obtaining such information in the past have been highlighted[65,66]. Furthermore, with product innovations and changing tastes among consumers, the concentrations of LCS within products are likely to evolve over time, potentially introducing error into subsequent estimates of exposure unless ongoing data are received from manufacturers. Another method for determining LCS concentrations in products is to directly measure them analytically and to this end, numerous methodologies for the determination of LCS in foods and beverages have been published[10]. Recent studies investigating LCS exposure have adopted this approach[50–53,57,58] and, although it allows for a more accurate and objective measure of LCS concentrations in products, it is also likely to be costly. Furthermore, with the ubiquity of LCS in today's market and the trend towards more widespread use, adopting such an approach as part of future assessments may prove unfeasible.

Recent developments in low-calorie sweeteners exposure assessment

A desire to harmonise food additive exposure assessment across the EU led to guidelines on how Member States should collect intake data for exposure assessments[40] and this was further enhanced through the recent implementation of the Flavourings, additives and food contact

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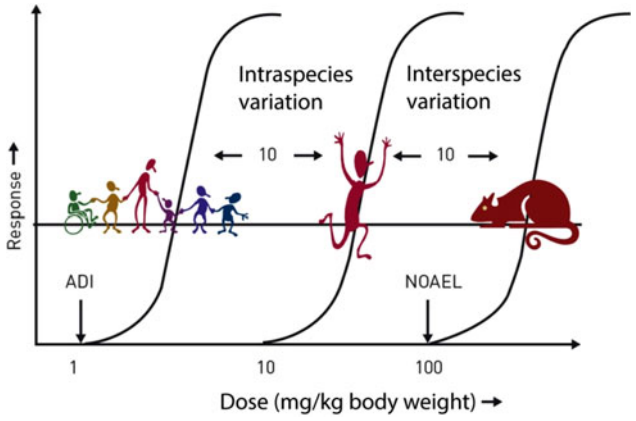

Fig. 1. (Colour online) Safety factors applied to the no observed adverse effect level to establish the acceptable daily intake (Source: Logue *et al.*[15]). ADI, acceptable daily intake; NOAEL, no observed adverse effect level.

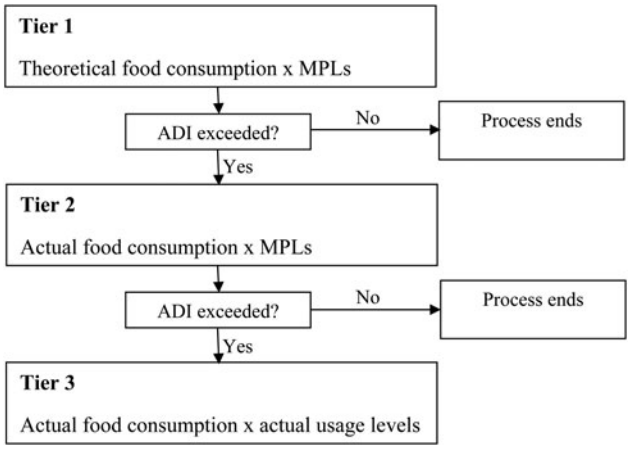

Fig. 2. Tiered approach for food additive exposure estimates (adapted from EC[40]). MPLs, maximum permitted levels; ADI, acceptable daily intake.

exposure tool (FACET)[66]. As a result of the FACET project, a publicly available exposure software package has been released (available at: http://expofacts.jrc.ec.europa.eu/facet/login.php). One of the strengths of the FACET project is that cooperation was obtained from FoodDrinkEurope, an alliance of national food and drink industries in the EU, for the provision of information on the concentrations of targeted food additives in products, including aspartame and acesulfame-K and this will result in a more sustainable system of monitoring exposure over time.

Alternatively, the Monte Carlo risk assessment software tool, developed as part of the EU-wide ACROPOLIS (Aggregate and cumulative risk of pesticides: an on-line integrated strategy) project, may also be used for the assessment of exposure to chemicals in foods (available at: https://mcra.rivm.nl/Account/Login). This tool is designed to allow a cumulative assessment of exposure to multiple chemicals from multiple sources[67] and has been successfully applied for the assessment of acute and chronic exposure to a group of pesticides in a number of European countries[68]. Like the FACET tool, the ACROPOLIS tool is freely accessible.

Actual low-calorie sweeteners exposure

European based studies carried out over the last 20 years have largely reported that the intakes of LCS fall well within the ADI (Table 2), with only the intake of cyclamate potentially exceeding the ADI in some population subgroups. Similar results were reported in studies conducted in Korea, Australia and New Zealand and Brazil[50–52,60]. A review by Renwick[48] reported that the overall intakes of LCS had not increased significantly during the preceding decade, although it has been reported elsewhere that the numbers of people consuming LCS were increasing[69].

Nutritional biomarkers: concepts and considerations

The focus of this review will be on nutritional biomarkers of exposure and within this specific context, biomarkers will be defined as cellular, biochemical, analytical, or molecular measures that are obtained from biological media such as tissues, cells or fluids and are indicative of exposure to an agent[39]. As such, nutritional biomarkers of exposure, by their very nature, are independent of the sources of bias associated with self-reported dietary intake data and can therefore provide a more objective measure of intake[70,71]. Such biomarkers can be used as measures of intake, for the assessment of nutritional status or in order to validate more traditional dietary assessment tools[72]. Although the application of a biomarker approach is not new in nutrition research, it has been suggested that many existing nutritional biomarkers have not been properly validated and the field of biomarkers is yet to be fully exploited[73]. A number of considerations are important for properly implementing such an approach; the target biomarker must be specific to the food or food component, be reproducible and be sensitive to changes in intakes over time[74]. Furthermore, the relevant biomarker should be obtained in a minimally invasive way[75] and the biological sample should be collected, processed and stored in an appropriate manner, so that a true reflection of intake can be obtained[76]. Factors that may affect the validity of a biomarker include genetic variability, physiologic factors, dietary factors, the biological sample of choice and the analytical method used to measure it[73].

Four classes of nutritional biomarkers, namely recovery, concentration, replacement and predictive biomarkers, have been described according to the relationship between the biomarker and intake of the component of interest[73]. Recovery biomarkers exhibit a strong time defined relationship between intake and excretion and can therefore be used to estimate absolute intakes. Examples of such biomarkers are doubly labelled water for energy intake[77] and urinary nitrogen[78,79] and potassium[78] for protein and potassium intakes, respectively. Concentration and replacement biomarkers differ from recovery biomarkers in that they exhibit a lower correlation with absolute intake[73]; however, they are

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Table 2. Exposure estimates of low-calorie sweeteners in Europe over the past 20 years

Country	Year of study	Population studied	Sample size	Average sweetener exposure (% ADI)*					Author
				Ace-k	Asp	Cyc	Sac	Suc	
UK	Not stated	Aged 3–74 years	188	10·3	–	–	6·5	–	Wilson *et al.*[63]
Italy	1996	Aged 13–19 years	212	0·1 (1·5)	0·1 (1·0)	2·2 (5·6)	4·2 (10·6)	–	Leclercq *et al.*[60]
France	1997	Insulin dependent diabetes mellitus, aged Aged 2–20 years	227	7 (27)	6 (20)	–	8 (26)	–	Garnier-Sagne *et al.*[59]
Netherlands	1997–1998	Aged 1–97 years	6250	<0·5 (0·7)	<0·3 (1·3)	0·9 (3·6)	0·4 (0·4)	–	Van Rooij-van den Bos *et al.*[58]
UK	2001	Aged 1·5–4·5 years	1110	6 (25)	8 (30)	41 (128)	23 (77)	–	FSA[64]
Italy	2000–2001	Aged 14–17 years	362	0·3 (0·7)	0·2 (0·4)	4·5 (4·5)	0·7 (0·7)	–	Arcella *et al.*[61]
Belgium	2004	Aged 15 years and over	3083	10	4	5	6	5	Huvaere *et al.*[51]
Europe	1995–2005	Adults aged 18–65 years	Not clear	2–6	0–3	–	–	–	Vin *et al.*[102]
Europe	1992–2005	Children aged 1–18 years	Not clear	7–31	2–8	–	–	–	Vin *et al.*[102]
Portugal	2006–2007	Aged 13–15 years	65	2·6	0·8	–	–	–	Lino *et al.*[57]

No data reported for other LCS. '–' indicates no data reported.
Ace-k, acesulfame-K; ADI, acceptable daily intake; Asp, aspartame; Cyc, cyclamate; Sac, saccharin; Suc, sucralose; FSA, Food Standards Agency.
* Mean % ADI presented with high consumers (% ADI) where available.

useful for the purposes of ranking individuals according to intake and therefore can be used in the investigation of the relationship between the food or food component and disease[72]. Examples of such biomarkers are carotenoids and aflatoxins[73]. The class of predictive biomarkers was first proposed by Tasevska *et al.*[80] when describing the use of urinary fructose and sucrose as markers for sugar intake. Although relatively small amounts of a dose were recovered in the form of urinary sucrose and fructose, it was demonstrated that a higher level of correlation ($R > 0{\cdot}6$) with dietary intake existed than with concentration or replacement biomarkers and therefore this class of biomarker would fall between recovery and concentration biomarkers. As part of the validation process, it is important to characterise the relationship between the target biomarker and intake of the food or food component of interest[81], as such information will inform the application of the biomarker (e.g. to estimate absolute intakes, rank individuals or monitor compliance).

Two broad strategies for the development of a biomarker approach have been described; discovery- and hypothesis-driven[81]. Discovery-driven approaches have become more prominent recently with the use of metabolomics to identify previously unknown biomarkers or panels of biomarkers that are associated with dietary patterns or the consumption of specific foods or food components[82]. Hypothesis-driven approaches differ in that prior knowledge of the component of interest and its metabolic fate are required and this information subsequently informs a more targeted approach to biomarker development[81].

Potential application of a biomarker approach for investigating low-calorie sweeteners intakes

The metabolic fates of LCS are well known (Table 3) and therefore a hypothesis-driven approach would appear to be the most appropriate for the implementation of a biomarker approach for investigating exposure. Following ingestion, aspartame is hydrolysed to aspartic acid, phenylalanine and methanol, each of which commonly occur in a normal diet[83,84], while thaumatin undergoes normal protein digestion[85]. Neohesperidine dihydrochalcone, although not known to exist in nature, is structurally similar to naturally occurring flavonoid glycosides and undergoes a similar metabolic fate to these analogues with the same or similar metabolites[86]. This finding would indicate that no obvious specific candidate biomarkers exist for these compounds. Acesulfame-K[87] and saccharin are almost completely absorbed and excreted unchanged via the urine,[88–90] while cyclamate (30–50 %)[91] and sucralose (10–15 %)[92,93] undergo partial absorption and the absorbed proportion is excreted unchanged via the urine with the unabsorbed proportions excreted via the faeces. In about 20 % of the population, cyclamate can be converted to cyclohexylamine via bacterial hydrolysis in the gut, which is absorbed and also excreted via the urine[94]. Furthermore, the extent of cyclamate conversion to cyclohexylamine can be variable during chronic exposure[95]. Steviol glycosides also undergo bacterial hydrolysis to steviol which is then absorbed and excreted via the urine as steviol glucuronide[96–98]. Advantame is converted to advantame acid and a small proportion is absorbed (about 6 %) and excreted via the urine while about 90 % of a dose is excreted via the faeces[99].

Saccharin, acesulfame-K, cyclamate and sucralose undergo no or limited metabolism following absorption into the body; therefore candidate biomarkers, in the form of the parent compounds, exist for these LCS. For steviol glycosides and advantame, the excretory products may act as suitable biomarkers for intakes. A high level of specificity of the candidate biomarkers exists as they are not found elsewhere in the diet or formed endogenously and given that at least a proportion of each of these compounds is excreted via the urine, a urinary biomarker approach may be feasible. Indeed, such an

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Table 3. Metabolic fates and routes of excretion of low-calorie sweeteners approved in Europe

Sweetener (CAS Registry No.)	Applications	Metabolic fate	Route(s) of excretion*	Author
Saccharin (81-07-2)	Oral hygiene products, table-top sweetener, soft drinks	Not metabolised, excreted unchanged	Urine	Byard *et al.*[88], Ball *et al.*[89]
Acesulfame-K (55589-62-3)	Chewing gum, soft drinks, dairy products	Not metabolised, excreted unchanged	Urine	Christ & Rupp[87]
Aspartame (22839-47-0)	Soft drinks, chewing gum, confectionery	Hydrolysed to aspartic acid, phenylalanine and methanol	N/A	Butchko *et al.*[83], Magnuson *et al.*[84]
Cyclamate (139-05-9)	Table-top sweetener, soft drinks, confectionery	80 % of the population do not metabolise cyclamate. In 20 %, it undergoes partial hydrolysis in the gut to cyclohexylamine. Extent of hydrolysis can vary between and within individuals	Faeces, urine	Renwick[94]
Thaumatin (53850-34-3)	Mainly used as a flavour enhancer	Undergoes normal protein digestion	N/A	JECFA[85]
NHDC (20702-77-6)	Chewing gum, soft drinks, pharmaceuticals	Metabolised by gut microflora to similar metabolites to naturally occurring flavonoids	Urine	Borrego & Montijano[86]
Salt of aspartame–acesulfame (106372-55-8)	Soft drinks, chewing gum, confectionery	Dissociates to individual sweeteners in digestive fluids and undergoes same metabolic fates	See information for acesulfame-K and aspartame	European Commission[103]
Sucralose (56038-13-2)	Table-top sweetener, soft drinks, chewing gum	Not metabolised, excreted mainly unchanged but 2 % of absorbed portion excreted as conjugates	Faeces, urine	Grice & Goldsmith[92], Roberts *et al.*[93]
Steviol glycosides†	Table-top sweetener, soft drinks, juices	Bacterial hydrolysis in the gut to steviol which is then absorbed and excreted as steviol glucuronide	Urine	Geuns *et al.*[96], Geuns *et al.*[97], *Wheeler et al.*[98]
Advantame (714229-20-6)	Milk products, frozen dairy, chewing gum	Converted to advantame acid and mainly excreted as such with two minor metabolites	Faeces, urine	Ubukata *et al.*[99]

CAS, Chemical Abstract Service; NHDC, neohesperidine dihydrochalcone; N/A, not applicable as broken down to normal dietary components; JECFA, Joint FAO/WHO Expert Committee on Food Additives.
* No CAS Number.
† Principal route of excretion listed first.

approach for assessing exposure to acesulfame-K and saccharin was previously described by Wilson *et al.*[63] who measured levels of excretion in 24-h urine samples and found excellent levels of correlation in an intake/excretion study ($R^2 > 0{\cdot}99$ for both compounds), demonstrating a clear dose–response relationship for both compounds. Slightly lower correlations were observed when validated against an FFQ which is probably more indicative of a limitation with the FFQ rather than the biomarker as the FFQ did not account for non-dietary sources of the LCS. The dose–response relationships for cyclamate, sucralose, steviol glycosides and advantame, however, are less clear so future work will be required to elucidate these relationships before the usefulness of measuring urinary concentrations for investigating intakes is established.

To fully characterise the relationship between urinary excretion and intakes of these LCS, a suitable and validated analytical method is first required[76]. To this end, a liquid chromatography, tandem-MS/MS method of simultaneously determining urinary levels of acesulfame-K, cyclamate, saccharin, steviol glucuronide and sucralose has recently been developed and validated[100] and allows for investigations of the feasibility of implementing a biomarker approach for assessing intakes of these compounds.

Potential limitations of a urinary biomarker approach for assessing low-calorie sweetener intakes

Although a biomarker approach would potentially offer many advantages over more traditional methods of investigating exposure to LCS, potential limitations should also be acknowledged. As urinary biomarkers, only short-term exposure (previous 24–48 h) can be investigated[81]. The mode of sampling is also an important factor to consider for the implementation of a biomarker approach and if 24-h urine samples are required, a lack of compliance could also represent a limitation. To mitigate this limitation, however, methods of assessing compliance have been developed such as the paraminobenzoic acid method[101]. It must also be acknowledged that a biomarker approach, utilised on its own, will not provide information on the source of exposure. Therefore in the event that the ADI of a particular LCS is being exceeded, more traditional methods would be required to identify the main sources of exposure[39]. As such, for the purposes of monitoring exposure

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in the population, a biomarker approach would likely be most useful when used as an adjunct to more traditional methods.

In summary, knowledge of the intakes of LCS is a legislative requirement in the EU and although the recent development and implementation of the FACET project will help harmonise methods among EU Member States and improve knowledge with regard to exposure to food additives, a successfully implemented biomarker approach for investigating LCS intake would undoubtedly be a useful adjunct to such monitoring activities. Furthermore, with ongoing interest and debate around the efficacy, as well as safety, of long-term LCS use, a biomarker approach would help elucidate the intakes of specific and combinations of LCS, and thereby address a limitation in the evidence to date, as highlighted by the French Agency for Food, Environmental and Occupational Health and Safety.

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Conflicts of Interest

None.

Authorship

C. L. conducted a literature search and drafted the manuscript. A. M. G., L. C. D., J. J. S. and H. V. reviewed and approved the final version.

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