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Letter to the editor

Response to the Letter to the Editor by S. Schiffman and H. Nagle: Revisiting the data and information that has collectively established the safety of low/no-calorie sweeteners, including sucralose

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Dear Editors,

The Letter to the Editor by Drs. Susan S. Schiffman and H. Troy Nagle, which was submitted in response to our publication that reviewed the published data on low/no-calorie sweeteners (LNCS) and the gut microbiota (Lobach et al., 2019), primarily challenges 2 statements made within our article. The first statement, as written in the conclusion of our article, is that "studies of LNCS establish no clear evidence of any adverse effect on the gut microbiota at doses relevant to human use". The second statement relates to the overall safety of LNCS: "The safety databases that have been developed over decades for acesulfame K, aspartame, saccharin, sucralose, and steviol glycosides, which are structurally unrelated, indicate that these low or no-calorie sweeteners as a group, or individually, pose no safety concerns at their currently approved levels". We have critically reviewed the comments related to these 2 statements in the Letter to the Editor from Drs. Schiffman and Nagle and provide a response to their remarks in the paragraphs that follow.

1. Response to statement 1: No clear evidence of adverse effects of LNCS on the gut microbiota at exposures relevant to human use

The purpose of our review article (Lobach et al., 2019) was to assess the data and information present in the scientific literature reporting evaluation of the effects on the gut microbiota and the potential for associated adverse health outcomes following exposure to the majority of currently permitted LNCS. Since we were primarily interested in the relevance of such reports to human dietary exposures, we focused on evaluating studies of a nonclinical or clinical nature, whereby doses of the sweeteners could be related to the acceptable daily intakes (ADIs) established by regulatory authorities such as the United States Food and Drug Administration (U.S. FDA). *In vitro* studies were excluded due to the limitations in directly extrapolating concentrations used in benchtop experiments to human exposure levels, which in general provide non-validated techniques both from a scientific and regulatory perspective. The search strategy employed identified relevant publications on several approved LNCS including acesulfame potassium (acesulfame K), aspartame, cyclamate, neotame, saccharin, sucralose, and rebaudioside A. The data for each LNCS were evaluated independent from the others, as these compounds are structurally diverse and behave very differently from a pharmacokinetic perspective. This point was highlighted within the Schiffman and Nagle Letter to the Editor but then focused only on the LNCS sucralose, while failing to highlight how the differences in pharmacokinetics and metabolism could impact the gut microbiome. As clearly identified in our original article, some LNCS are digested to metabolites that are absorbed prior to reaching the lower gastrointestinal tract (*e.g.,* aspartame), some are absorbed unchanged in the upper gastrointestinal tract (*e.g.,* acesulfame K and saccharin), whereas others can reach the lower gastrointestinal tract unchanged (*e.g.,* sucralose, steviol glycosides, and cyclamate) (Magnuson et al., 2016). Thus, the opportunity for different LNCS to directly interact with the gut microbiota varies greatly and a sweetener class effect is not scientifically supported. Contrary to this understanding (based upon differences in pharmacokinetics), Suez et al. (2014) concluded that all LNCS induce glucose intolerance through modulation of the gut microbiota, when in fact saccharin was the only LNCS for which a direct measure of the gut microbiome was obtained, and this was done in a very small clinical study with only 7 subjects. Furthermore, no control group was included in this study and evaluation of the gut microbial data did not adhere to standard practices. Glycemic responses were utilized to separate the 7 subjects into a group of "responders" and "nonresponders" and the authors reported that after 7 days of saccharin exposure the microbiome distribution between the 2 groups was different. Prior to saccharin exposure, however, the

Abbreviations: acesulfame K, acesulfame potassium; ADI, acceptable daily intake; EFSA, European Food Safety Authority; FSANZ, Food Standards Australia New Zealand; GLP-1, glucagon-like peptide-1; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LNCS, low/no-calorie sweeteners; U.S. FDA, United States Food and Drug Administration

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"responders" and "nonresponders" already had significantly different gut microbial distributions, so the significance of the authors association between saccharin exposure and gut microbiota modulation is questionable, let alone extending this conclusion to encompass all LNCS. Following comprehensive evaluation of the Suez et al. (2014) study, as well as 16 others deemed relevant to our assessment, we identified several common critical shortfalls in the design of both the nonclinical and clinical studies reviewed, which directly impacted the ability to relate study outcomes on the gut microbiota to humans in the context of expected daily exposure through the food supply. These common shortcomings included confounding factors such as the lack of isocaloric control groups, as dietary factors are key drivers of the composition of the gut microbiota (Muegge et al., 2011; Wu et al., 2011; David et al., 2014; Graf et al., 2015), and factors limiting extrapolation of findings to humans, such as the use of LNCS doses that were in excess of the currently established ADIs. Of the 17 relevant publications identified in our literature search, only 1 publication reporting studies on sucralose and acesulfame K in mice was determined to be free of both confounding and dose-extrapolation limiting factors. This study was conducted by Uebanso et al. (2017) and reported (a) a dose dependent decrease in fecal *Clostridium IVXa* in mice that consumed 1.5 or 15 mg sucralose/kg body weight/day for 8 weeks; and (b) no changes in the gut microbiota of mice that consumed 15 mg acesulfame K/kg body weight/day for 8 weeks. Although this study reports a change in one microbial population in the mouse gut following exposure to sucralose at a dose equivalent to the human ADI, there is no evidence that this change is associated with an actual adverse effect on human health, particularly when considering that only 4% of the bacterial genes in the human and murine intestinal tracts share considerable identity (Hugenholtz and de Vos, 2018). A change in the gut microbiota cannot automatically be considered synonymous with an adverse health effect. Several other studies in rodents were also found to report changes in the gut microbiota following sucralose exposure (Abou-Donia et al., 2008; Suez et al., 2014; Bian et al., 2017; Farzi et al., 2017; Olivier-Van Stichelen et al., 2017; Rodriguez-Palacios et al., 2018), as pointed out in the Letter to the Editor from Drs. Schiffman and Nagle, however, we determined that none of these findings could be attributed to sucralose alone due to the presence of at least one confounding factor in each study (see Table 1 in Lobach et al., 2019). Briefly, doses in excess of the ADI limit the relevance to human sucralose exposure in the mouse studies conducted by Suez et al. (2014) [doses ~100× the sucralose ADI] and Wang et al. (2018) [~300 to 600×] and possibly Bian et al. (2017) [at a minimum of 2×]. Although not included in our review since it was published following submission of our manuscript, Wang et al. (2018) administered about 1.5 and 3.3 g/kg body weight/day to C57BL/6 mice (n = 8/group) in the diet for 8 weeks. These doses were calculated by the authors to be "roughly 300 to 600 times higher than the recommended average daily intake (5 mg/kg/d) for humans". As such, the reported changes in the gut microbiota by Wang et al. (2018) cannot be extrapolated to the human scenario. Common confounding factors in the sucralose studies, as well as studies with other LNCS, included the lack of food consumption data to confirm an isocaloric diet. In fact, in Drs. Schiffman and Nagle's Letter to the Editor, as part of the detailed discussion on the Abou-Donia et al. (2008) Splenda® study in rats, it is stated that "The minor variations in food and fluid intake across groups were dominated by the massive effects of sucralose/Splenda® on bacteria counts". However as reported in the study publication, body weight gain in 3 of the 4 Splenda® groups (*i.e.,* the 100, 300, and 500 mg/kg/day dose groups, but not the 1000 mg/kg/day group) was not equivalent to the water control group (Abou-Donia et al., 2008). Differences in body weight between groups implies differences in food intake, which will impact the microbiota since diet alone is known to be the biggest single contributor to microbiome changes in the gut (Muegge et al., 2011; Wu et al., 2011; David et al., 2014; Graf et al., 2015). Differences in food intake would therefore significantly confound any measures of the gut microbiota. Furthermore, given that Splenda® is a mixture of primarily maltodextrin (93.59%) and glucose (1.08%) with a small percentage of sucralose (1.10%), in the absence of data from control groups that were exposed to each component of Splenda®, it is not possible to attribute the reported changes in gut microbiota to sucralose alone since the single control group consumed water only. This comment also applies to the Splenda® study in mice by Rodriguez-Palacios et al. (2018). Changes in the amount and/or type of carbohydrate consumed in the human diet (Clarke et al., 2012; Singh et al., 2017; Gentile and Weir, 2018) and also in the rodent diet (Noble et al., 2017; Kovatcheva-Datchary et al., 2019) has been shown to influence the composition of the gut microbiota thereby reinforcing the necessity for maltodextrin control groups in both the Abou-Donia et al. (2008) and Rodriguez-Palacios et al. (2018) studies. With respect to the sucralose data, for these reasons combined, it was not deemed scientifically appropriate to extrapolate to humans any of the gut microbiota changes reported by Abou-Donia et al. (2008) as well as the other sucralose animal studies. Only the one sucralose study in mice by Uebanso et al. (2017) reported a change in the gut microbiome at a relevant dose that could be directly attributed to sucralose exposure, but there is no evidence that this change in mice is translatable to an actual adverse effect in humans. To date, there have been no human data published on sucralose that report any direct effects on the gut microbiota. This is supported by the opinion published by the European Commission's Scientific Committee on Food on sucralose, in which the stability, metabolism, and effects on gut microflora were evaluated. It was concluded by the Committee that "the structure of the molecule is such that it is extremely resistant to hydrolysis" and "metabolic adaptation in humans was highly unlikely" (SCF, 2000a). With respect to the other regularly accepted LNCS that we reviewed, each compound was evaluated independently as per the sucralose example above, and the data collectively support our conclusion that there is no clear evidence suggesting that acesulfame K, aspartame, cyclamate, neotame, saccharin, sucralose, or rebaudioside A adversely impact the gut microbiota when consumed by humans at approved levels in the diet.

2. Response to statement 2: acesulfame K, aspartame, saccharin, sucralose, and steviol glycosides pose no safety concerns at their currently approved levels

Based on our review of the scientific literature reporting evaluation of the *in vivo* effects on the gut microbiota following consumption of several approved LNCS, we did not find any evidence of adverse health effects in humans. This is in line with the approvals issued by the international regulatory authorities, including for example the U.S. FDA, the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Food Safety Authority (EFSA), Health Canada, Food Standards Australia New Zealand (FSANZ), and the Japan Ministry of Health, Labour & Welfare, that permit the addition of various LNCS in human food and beverage products at use levels that have been determined to be safe. LNCS, such as acesulfame K, aspartame, saccharin, sucralose and steviol glycosides, have been safely consumed as low or no-calorie sweetener alternatives to sugar for decades worldwide. Drs. Schiffman and Nagle in their Letter to the Editor, however, have challenged the safety of all LNCS and proclaim that these sweeteners are not safe at levels approved by regulatory agencies. Where our publication comprehensively looked at the data for all the currently approved LNCS, Drs. Schiffman and Nagle support their generalized safety challenge of all LNCS by focusing on only one sweetener, sucralose, providing no data for any other LNCS. This is in direct contrast with the statement in their Letter that "LNCS are structurally diverse and vary widely in pharmacokinetics. Therefore, it is inappropriate to draw generalized conclusions regarding effects on gut microbiota and safety for this diverse group of chemicals." Despite this, Drs. Schiffman and Nagle only reported on and referenced one sweetener to support their position that all LNCS are unsafe.

The consumer safety of sucralose was specifically challenged in Drs. Schiffman and Nagle's Letter by reference to several specific reports in the public domain but lacked a comprehensive approach by excluding consideration of the extensive safety database that has been established for sucralose. The safety of sucralose was first reviewed 30 years ago by JECFA (JECFA, 1989). The sucralose safety database at that time included numerous pharmacokinetic and metabolic studies in animals and humans, extensive toxicological evaluations encompassing mutagenicity, acute, subchronic, chronic, carcinogenicity, reproduction, teratogenicity, neurological and mineral utilization studies, and 2 human studies, one of which was a randomized controlled trial conducted for 13 weeks in healthy subjects. Based on these data and additional studies that were provided to the Committee in 1991 (*e.g.,* additional toxicological studies), JECFA established an ADI for sucralose of 0–15 mg/kg body weight (JECFA, 1991). Subsequent to this, several other regulatory authorities conducted their own independent reviews of sucralose safety and approved the use of the sweetener in various food and beverage products, including the U.S. FDA (1998, 1999), the Japan Ministry of Health, Labour & Welfare (JFCRF, 1999), EFSA (SCF, 2000a), FSANZ in 1993 (FSANZ, 2019), and Health Canada in 1991 (2019). Pharmacokinetic and metabolic studies in both animals and humans have consistently demonstrated that following oral ingestion sucralose is poorly absorbed and excreted primarily unmetabolized in the feces, with small amounts excreted in the urine as glucuronide conjugates (JECFA, 1989, 1991; John et al., 2000; Roberts et al., 2000; Sims et al., 2000; Wood et al., 2000). The uniformity of sucralose pharmacokinetics and metabolism across several species confirm that the safety studies that have been conducted in animals are representative models for sucralose exposure in humans.

Sucralose continues to be the subject of several preclinical and clinical investigations. The current literature on sucralose safety has been critically reviewed in a recent publication by Magnuson et al. (2017), which includes several of the studies that were referenced in the Letter to the Editor from Drs. Schiffman and Nagle challenging sucralose safety. In contrast to the 4 citations in their Letter reporting alteration of metabolic function in humans following sucralose consumption, the review by Magnuson et al. (2017) identified a total of 19 clinical studies that investigated metabolic function, including effects on blood glucose, insulin, and/or incretin levels. These included 12 single dose studies in healthy adults (Ma et al., 2009, 2010; Brown et al., 2009, 2011; Steinert et al., 2011; Ford et al., 2011; Wu et al., 2012, 2013; Stellingwerff et al., 2013; Temizkan et al., 2015; Ibero-Baraibar et al., 2014; Sylvetsky et al., 2016), 4 single dose studies in diabetic and prediabetic obese adults (Mezitis et al., 1996; Brown et al., 2012; Temizkan et al., 2015; Pepino et al., 2013), and 3 repeated dose studies in diabetic subjects (SCF, 2000b; Grotz et al. (2003); Reyna et al. (2003). Following ingestion of a glucose load, only 1 of the 19 clinical studies reported an increase in plasma glucagon-like peptide-1 (GLP-1) area under the curve that was directly attributable to sucralose, however, in this same study no statistically significant concomitant increase in absolute GLP-1 blood levels was reported (*i.e.,* GLP-1 blood levels were unchanged) (Temizkan et al., 2015). The totality of the clinical evidence reviewed by Magnuson et al. (2017) supports the lack of any adverse effect of sucralose on blood glucose control in both normoglycemic and hyperglycemic human populations. With respect to the carcinogenic potential of sucralose, in contrast to the 1 study cited in the Letter to the Editor from Drs. Schiffman and Nagle, which reported neoplastic lesion formation in mice exposed to sucralose using a lifetime study design, the lack of genotoxicity of sucralose is strongly supported by over 10 *in vitro* genotoxic toxicology investigations (see Table 4, Magnuson et al., 2017) and two 104-week carcinogenicity studies in mice and rats that were conducted according to Good Laboratory Practices using validated U.S. FDA Redbook protocols (Mann et al., 2000a,b). Likewise, with respect to neurotoxicity, Drs. Schiffman and Nagle's Letter to the Editor cited 1 study reporting neurobehavioral effects in rats exposed to sucralose, whereas Magnuson et al. (2017) identified a total of 4 neurotoxicity investigations, all of which reported a lack of neurotoxic effects in the following: adult mice exposed to sucralose for 21 days, adult monkeys exposed for 28 days, young mice exposed to sucralose on Postnatal Days 8–12, and adult mice exposed to sucralose on Postnatal Days 8–12 (Finn and Lord, 2000; Viberg and Fredriksson, 2011). With regard to the immunotoxic concerns raised, the potential for immunological effects associated with sucralose exposure has been previously evaluated in a 28-day study in rats that adhered to the Tier-I US National Toxicology Guidelines. No effects of sucralose on the lymphoid organs or immune function were reported at up to the highest dose tested (3000 mg/kg body weight/day), which included evaluation of immunoglobulin levels in the serum, immune cells present in the spleen (lymphocytes and natural killer cells), immune organ weight and histology (spleen and thymus), and bone marrow cell counts and pathology (SCF, 2000b). In response to the citations in Drs. Schiffman and Nagle's Letter to the Editor related to sucralose metabolism and breakdown, as discussed above, sucralose is a poorly absorbed substance that is primarily excreted through the feces unmetabolized, with small portions excreted in the urine as glucuronide conjugates (JECFA, 1989, 1991; John et al., 2000; Roberts et al., 2000; Sims et al., 2000; Wood et al., 2000). No metabolites consistent with cytochrome P450-mediated metabolism of sucralose have been identified, supporting that sucralose is not subjected to biotransformation throughout the gastrointestinal tract. The biological inertness of sucralose along with its lack of bioaccumulation are supported by long term studies, such as an 18-month study in rats, which showed that percent excretion of [^{14}C] sucralose in the feces and urine was unchanged over 18 months of oral exposure (Sims et al., 2000). Studies in various food matrices, one of which has been published (Barndt and Jackson, 1990), have shown sucralose to be thermally stable under intended conditions of use. Reports of degradation of sucralose (Rahn and Yaylayan, 2010; BfR, 2019), as cited by Drs. Schiffman and Nagle, either used temperatures or experimental systems of little to no relevance to the use of low levels of sucralose in food.

When the totality of the data on sucralose is critically assessed as a comprehensive package, it is evident that sucralose is safe for human consumption at levels currently approved by scientific and regulatory agencies across the globe, such as JECFA, the U.S. FDA, EFSA, Health Canada, FSANZ, and the Japan Ministry of Health, Labour & Welfare, and all these regulatory groups continue to support the safety of this LNCS.

3. Conclusion

We conclude that the Letter to the Editor authored by Drs. Schiffman and Nagle does not provide sufficient evidence to challenge 2 statements made within our review titled “Assessing the *in vivo* data on low/no-calorie sweeteners and the gut microbiota”, namely, that (i) review of the current literature provided no clear evidence of any adverse effects of any LNCS, including sucralose, on the gut microbiota at doses relevant to human exposure; and (ii) acesulfame K, aspartame, saccharin, sucralose, and steviol glycosides pose no safety concerns at their currently approved levels. While Drs. Schiffman and Nagle indicate that both of these statements are incorrect, it is clear that this is not a viewpoint that is accepted by any of the international regulatory agencies, all of whom continue to support the safety of the ingredients both individually and as a class of food additive.

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References

Abou-Donia, M.B., El-Masry, E.M., Abdel-Rahman, A.A., McLendon, R.E., Schiffman, S.S., 2008. Splenda alters gut microflora and increases intestinal P-glycoprotein and cytochrome P-450 in male rats. J. Toxicol. Environ. Health. A 71, 1415–1429. https://doi.org/10.1080/15287390802328630.

Bardnt, R.L., Jackson, G., 1990. Stability of sucralose in baked goods. Radiolabeling study using thin-layer chromatography confirms the stability of high-intensity sweetener during baking. Food Technol. 44 (1), 62–66.

BfR, Bundesinstitut für Risikobewertung, 2019. Harmful Compounds Might Be Formed when Foods Containing the Sweetener Sucralose Are Heated. Bundesinstitut für Risikobewertung [Federal Institute for Risk Assessment], Berlin, Germany BfR opinion No 012/2019 of 9 April 2019. https://mobil.bfr.bund.de/cm/349/harmful-compounds-might-be-formed-when-foods-containing-the-sweetener-sucralose-are-heated.pdf.

Bian, X., Chi, L., Gao, B., Tu, P., Ru, H., Lu, K., 2017. Gut microbiome response to sucralose and its potential role in inducing liver inflammation in mice. Front. Physiol. 8, 487. (13pp, plus supplementary data). https://doi.org/10.3389/fphys.2017.00487.

Brown, R.J., Walter, M., Rother, K.I., 2009. Ingestion of diet soda before a glucose load augments glucagon-like peptide-1 secretion. Diabetes Care 32, 2184–2186. https://doi.org/10.2337/dc09-1185.

Brown, A., Brown, M.M.B., Onken, K.L., Beitz, D.C., 2011. Short term consumption of sucralose, a nonnutritive sweetener, is similar to water with regard to select markers of hunger signalling and short term glucose homeostasis in women. Nutr. Res. 31, 882–888. https://doi.org/10.1016/j.nutres.2011.10.004.

Brown, R.J., Walter, M., Rother, K.I., 2012. Effects of diet soda on gut hormones in youths with diabetes. Diabetes Care 35, 959–964. https://doi.org/10.2337/dc11-2424.

Clarke, S.F., Murphy, E.F., Nilaweera, K., Ross, P.R., Shanahan, F., O'Toole, P.W., Cotter, P.D., 2012. The gut microbiota and its relationship to diet and obesity: new insights. Gut Microb. 3, 186–202. https://doi.org/10.4161/gmic.20168.

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., et al., 2014. Diet rapidly and reproducibly alters the human gut microbiome. Nature 505 (7484), 559–563. https://doi.org/10.1038/nature12820.

Farzi, A., Reed, F., Zhang, L., Holzer, P., Herzog, H., 2017. Peptide YY is a critical regulator of gut microbiota composition specifically under conditions of sucralose or high fat diet exposure. Neuro Gastroenterol. Motil. 29 (Suppl. 2), 86. (abstract 171). https://doi.org/10.1111/nmo.13180.

Finn, J.P., Lord, G.H., 2000. Neurotoxicity studies on sucralose and its hydrolysis products with special reference to histopathologic and ultrastructural changes. Food Chem. Toxicol. 38 (Suppl. 2), S7–S17. https://doi.org/10.1016/S0278-6915(00)00024-7.

Ford, H.E., Peters, V., Martin, N.M., Sleeth, M.L., Ghatei, M.A., Frost, G.S., Bloom, S.R., 2011. Effects of oral ingestion of sucralose on gut hormone response and appetite in healthy normal-weight subjects. Eur. J. Clin. Nutr. 65, 508–513. https://doi.org/10.1038/ejcn.2010.291.

FSANZ, Food Standards Australia New Zealand, 2019. Schedule 15: substances that may be used as food additives (F2019C00226). In: Australia New Zealand Food Standards Code. Food Standards Australia New Zealand (FSANZ), Australian Government, Canberra, Australia/Wellington, New Zealand. https://www.legislation.gov.au/Details/F2019C00226.

Gentile, C.L., Weir, T.L., 2018. The gut microbiota at the intersection of diet and human health. Science 362, 776–780. https://doi.org/10.1126/science.aau5812.

Graf, D., Di Cagno, R., Fåk, F., Flint, H.J., Nyman, M., Saarela, M., Watzl, B., 2015. Contribution of diet to the composition of the human gut microbiota. Microb. Ecol. Health Dis. 26, 26164. (11pp). https://doi.org/10.3402/mehd.v26.26164.

Grotz, V.L., Henry, R.R., McGill, J.B., Prince, M.J., Shamoon, H., Trout, J.R., Pi-Sunyer, F.X., 2003. Lack of effect of sucralose on glucose homeostasis in subjects with type 2 diabetes. J. Am. Diet. Assoc. 103, 1607–1612. https://doi.org/10.1016/j.jada.2003.09.021.

Health Canada, 2019. List of Permitted Sweeteners. Health Canada, Ottawa, ON Lists of Permitted Food Additives, No. 9. https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/lists-permitted/9-sweeteners.html (Date Modified: 2019-05-14).

Hugenholtz, F., de Vos, W.M., 2018. Mouse models for human intestinal microbiota research: a critical evaluation. Cell. Mol. Life Sci. 75, 149–160. https://doi.org/10.1007/s00018-017-2693-8.

Ibero-Baraibar, I., Cuervo, M., Navas-Carretero, S., Abete, I., Zulet, M.A., Martinez, J.A., 2014. Different postprandial acute response in healthy subjects to three strawberry jams varying in carbohydrate and antioxidant content: a randomized, crossover trial. Eur. J. Nutr. 53, 201–210. https://doi.org/10.1007/s00394-013-0517-7.

JECFA, Joint FAO/WHO Expert Committee on Food Additives, 1989. Trichorogalactosucrose [sucralose]. In: Toxicological Evaluation of Certain Food Additives and Contaminants. 33rd Meeting of JECFA, Mar. 21-30, 1989, Geneva. World Health Organization (WHO), Geneva, Switz, pp. 45–94 WHO Food Additives Series, no 24.

JECFA, Joint FAO/WHO Expert Committee on Food Additives, 1991. Trichlorogalactosucrose (TGS) [sucralose]. In: Toxicological Evaluation of Certain Food Additives and Contaminants. Thirty-Seventh Meeting of JECFA, June 5-14, 1990, Rome, Italy. World Health Organization (WHO), International Programme on Chemical Safety (IPCS), Geneva, Switz WHO Food Additives Series, no 26. http://www.inchem.org/documents/jecfa/jecmono/v28je14.htm.

JFCRF, Japan Food Chemical Research Foundation, 1999. A Report on Sucralose from the Food Sanitation Council. Ministry of Health, Labour and Welfare, Japan (MHLW) and Japan Food Chemical Research Foundation (JFCRF), Tokyo, Japan Available from: http://www.ffcr.or.jp/zaidan/ffcrhome.nsf/pages/e-kousei-sucra (January 6, 1999]).

John, B.A., Wood, S.G., Hawkins, D.R., 2000. The pharmacokinetics and metabolism of sucralose in the mouse. Food Chem. Toxicol. 38 (Suppl. 2), S107–S110. https://doi.org/10.1016/S0278-6915(00)00032-6.

Kovatcheva-Datchary, P., Shoaie, S., Lee, S., Wahlström, A., Nookaew, I., Hallen, A., Perkins, R., Nielsen, J., Bäckhed, F., 2019. Simplified intestinal microbiota to study microbe-diet-host interactions in a mouse model. Cell Rep. 26 (13), 3772–3783. https://doi.org/10.1016/j.celrep.2019.02.090.

Lobach, A.R., Roberts, A., Rowland, I.R., 2019. Assessing the *in vivo* data on low/no-calorie sweeteners and the gut microbiota. Food Chem. Toxicol. 124, 385–399. https://doi.org/10.1016/j.fct.2018.12.005.

Ma, J., Bellon, M., Wishart, J.M., Young, R., Blackshaw, L.A., Jones, K.L., Horowitz, M., Rayner, C.K., 2009. Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects. Am. J. Physiol. Gastrointest. Liver Physiol. 296, G735–G739. https://doi.org/10.1152/ajpgi.90708.2008.

Ma, J., Chang, J., Checklin, H.L., Young, R.L., Jones, K.L., Horowitz, M., Rayner, C.K., 2010. Effect of the artificial sweetener, sucralose, on small intestinal glucose absorption in healthy human subjects. Br. J. Nutr. 104, 803–806. https://doi.org/10.1017/S0007114510001327.

Magnuson, B.A., Carakostas, M.C., Moore, N.H., Poulos, S.P., Renwick, A.G., 2016. Biological fate of low-calorie sweeteners. Nutr. Rev. 74, 670–689. https://doi.org/10.1093/nutrit/nuw032.

Magnuson, B.A., Roberts, A., Nestmann, E.R., 2017. Critical review of the current literature on the safety of sucralose. Food Chem. Toxicol. 106, 324–355. https://doi.org/10.1016/j.fct.2017.05.047.

Mann, S.W., Yuschak, M.M., Amyes, S.J., Aughton, P., Finn, J.P., 2000a. A combined chronic toxicity/carcinogenicity study of sucralose in Sprague-Dawley rats. Food Chem. Toxicol. 38 (Suppl. 2), S71–S89. https://doi.org/10.1016/S0278-6915(00)00029-6.

Mann, S.W., Yuschak, M.M., Amyes, S.J., Aughton, P., Finn, J.P., 2000b. A carcinogenicity study of sucralose in the CD-1 mouse. Food Chem. Toxicol. 38 (Suppl. 2), S91–S98. https://doi.org/10.1016/S0278-6915(00)00030-2.

Mezitis, N.H., Maggio, C.A., Koch, P., Quddoos, A., Allison, D.B., Pi-Sunyer, F.X., 1996. Glycemic effect of a single high oral dose of the novel sweetener sucralose in patients with diabetes. Diabetes Care 19, 1004–1005. https://doi.org/10.2337/diacare.19.9.1004.

Muegge, B.D., Kuczynski, J., Knights, D., Clemente, J.C., Gonzalez, A., Fontana, L., Henrissat, B., Knight, R., Gordon, J.I., 2011. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 332 (6032), 970–974. https://doi.org/10.1126/science.1198719.

Noble, E.E., Hsu, T.M., Jones, R.B., Fodor, A.A., Goran, M., Kanoski, S.E., 2017. Early-life sugar consumption affects the gut microbiome independently of obesity. J. Nutr. 147 (1), 20–28. https://doi.org/10.3945/jn.116.238816.

Olivier-Van Stichelen, S., Rother, K.I., Hanover, J.A., 2017. Nascent microbiome and early metabolism are perturbed by pre-and post-natal exposure to artificial sweeteners. Glycobiology 27, 1185–1186. (abstract 38). https://doi.org/10.1093/glycob/cwx086.

Pepino, M.Y., Tiemann, C.D., Patterson, B.W., Wice, B.M., Klein, S., 2013. Sucralose affects glycemic and hormonal responses to an oral glucose load. Diabetes Care 36, 2530–2535. https://doi.org/10.2337/dc14-0268.

Rahn, A., Yaylayan, V.A., 2010. Thermal degradation of sucralose and its potential in generating chloropropanols in the presence of glycerol. Food Chem. 118, 56–61.

Reyna, N.Y., Cano, C., Bermúdez, V.J., Medina, M.T., Souki, A.J., Ambard, M., Nuñez, M., Ferrer, M.A., Inglett, G.E., 2003. Sweeteners and beta-glucans improve metabolic and anthropometrics variables in well controlled type 2 diabetic patients. Am. J. Therapeut. 10, 438–443. https://doi.org/10.1097/00045391-200311000-00010.

Roberts, A., Renwick, A.G., Sims, J., Snodin, D.J., 2000. Sucralose metabolism and pharmacokinetics in man. Food Chem. Toxicol. 38 (Suppl. 2), S31–S41. https://doi.org/10.1016/S0278-6915(00)00026-0.

Rodriguez-Palacios, A., Harding, A., Menghini, P., Himmelman, C., Retuerto, M., Nickerson, K.P., Lam, M., Croniger, C.M., McLean, M.H., Durum, S.K., et al., 2018. The artificial sweetener Splenda promotes gut proteobacteria, dysbiosis, and myeloperoxidase reactivity in Crohn's disease-like Ileitis. Inflamm. Bowel Dis. 24, 1005–1020. (plus supplementary data). https://doi.org/10.1093/ibd/izy060.

SCF, Scientific Committee on Food, 2000a. Opinion of the Scientific Committee on Food on Sucralose (Opinion Expressed by the SCF on 7 September 2000). European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food (SCF), Brussels, Belgium SCF/CS/ADD/EDUL/190 Final. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out68_en.pdf.

SCF, Scientific Committee on Food, 2000b. Annex I. Studies on glucose homeostasis in healthy humans and diabetic volunteers. In: Opinion of the Scientific Committee on Food on Sucralose (Opinion Expressed by the SCF on 7 September 2000). European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food (SCF), Brussels (Belgium), pp. 21–22. SCF/CS/ADD/EDUL/190 Final. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out68_en.pdf.

Sims, J., Roberts, A., Daniel, J.W., Renwick, A.G., 2000. The metabolic fate of sucralose in rats. Food Chem. Toxicol. 38 (Suppl. 2), S115–S121. https://doi.org/10.1016/S0278-6915(00)00034-X.

Singh, R.K., Chang, H.W., Yan, D., Lee, K.M., Ucmak, D., Wong, K., Abrouk, M., Farahnik, B., Nakamura, M., Zhu, T.H., et al., 2017. Influence of diet on the gut microbiome and implications for human health. J. Transl. Med. 15, 73. (17pp). https://doi.org/10.1186/s12967-017-1175-y.

Steinert, R.E., Frey, F., Töpfer, A., Drewe, J., Beglinger, C., 2011. Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal

satiety peptides. Br. J. Nutr. 105, 1320–1328. https://doi.org/10.1017/s000711451000512x.
Stellingwerff, T., Godin, J.P., Beaumont, M., Tavenard, A., Grathwohl, D., van Bladeren, P.J., Kapp, A.F., le Coutre, J., Damak, S., 2013. Effects of pre-exercise sucralose ingestion on carbohydrate oxidation during exercise. Int. J. Sport Nutr. Exerc. Metab. 23, 584–592. https://doi.org/10.1123/ijsnem.23.6.584.
Suez, J., Korem, T., Zeevi, D., Zilberman-Schapira, G., Thaiss, C.A., Maza, O., Israeli, D., Zmora, N., Gilad, S., Weinberger, A., et al., 2014. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature 514 (7521), 181–186. https://doi.org/10.1038/nature13793.
Sylvetsky, A.C., Brown, R.J., Blau, J.E., Walter, M., Rother, K.I., 2016. Hormonal responses to non-nutritive sweeteners in water and diet soda. Nutr. Metab. (Lond.) 13 (8pp), 71. https://doi.org/10.1186/s12986-016-0129-3.
Temizkan, S., Deyneli, O., Yasar, M., Arpa, M., Gunes, M., Yazici, D., Sirikci, O., Haklar, G., Imeryuz, N., Yavuz, D.G., 2015. Sucralose enhances GLP-1 release and lowers blood glucose in the presence of carbohydrate in healthy subjects but not in patients with type 2 diabetes. Eur. J. Clin. Nutr. 69, 162–166. https://doi.org/10.1038/ejcn.2014.208.
U.S. FDA, U.S. Food and Drug Administration, 1998. Food additives permitted for direct addition to food for human consumption: sucralose; final rule [21 CFR Part 172; Docket No. 87F-0086]. Fed. Regist. (US) 63, 16417–16433. https://www.govinfo.gov/app/details/FR-1998-04-03/98-8750.
U.S. FDA, U.S. Food and Drug Administration, 1999. Food additives permitted for direct addition to food for human consumption: sucralose; final rule [21 CFR Part 172; Docket No. 99F-0001]. Fed. Regist. 64, 43908–43909. https://www.govinfo.gov/app/details/FR-1999-08-12/99-20888.
Uebanso, T., Ohnishi, A., Kitayama, R., Yoshimoto, A., Nakahashi, M., Shimohata, T., Mawatari, K., Takahashi, A., 2017. Effects of low-dose non-caloric sweetener consumption on gut microbiota in mice. Nutrients 9, 560. (11pp). https://doi.org/10.3390/nu9060560.
Viberg, H., Fredriksson, A., 2011. Neonatal exposure to sucralose does not alter biochemical markers of neuronal development or adult behavior. Nutrition 2011 (27), 81–85. https://doi.org/10.1016/j.nut.2009.10.007.
Wang, Q.P., Browman, D., Herzog, H., Neely, G.G., 2018. Non-nutritive sweeteners possess a bacteriostatic effect and alter gut microbiota in mice. PLoS One 13 (7) e0199080 (13pp). https://doi.org/10.1371/journal.pone.0199080.
Wood, S.G., John, B.A., Hawkins, D.R., 2000. The pharmacokinetics and metabolism of sucralose in the dog. Food Chem. Toxicol. 38 (Suppl. 2), S99–S106. https://doi.org/10.1016/S0278-6915(00)00031-4.
Wu, T., Zhao, B.R., Bound, M.J., Checklin, H.L., Bellon, M., Little, T.J., Young, R.L., Jones, K.L., Horowitz, M., Rayner, C.K., 2012. Effects of different sweet preloads on incretin hormone secretion, gastric emptying, and postprandial glycemia in healthy humans. Am. J. Clin. Nutr. 95, 78–83. https://doi.org/10.3945/ajcn.111.021543.
Wu, T., Bound, M.J., Standfielde, S.D., Bellon, M., Young, R.L., Jones, K.L., Horowitz, M., Rayner, C.K., 2013. Artificial sweeteners have no effect on gastric emptying, glucagon-like peptide-1, or glycemia after oral glucose in healthy humans. Diabetes Care 36, e202–e203. https://doi.org/10.2337/dc13-0958.
Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.Y., Keilbaugh, S.A., Bewtra, M., Knights, D., Walters, W.A., Knight, R., et al., 2011. Linking long-term dietary patterns with gut microbial enterotypes. Science 334 (6052), 105–108. https://doi.org/10.1126/science.1208344.

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