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Letter to the editor

Revisited: Assessing the *in vivo* data on low/no-calorie sweeteners and the gut microbiota

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ABSTRACT

Over the last two decades, safety concerns about low/no-calorie sweeteners (LNCS) have been described in the archival scientific literature including elevated risk of metabolic syndrome, type 2 diabetes, excessive weight gain, cardiovascular disease, safety, and disruption of the gut microbiome. A recent review by Lobach, Roberts, and Roland in Food and Chemical Toxicology examined 17 research articles on modulation of gut bacteria by LNCS along with other selected publications. In the conclusions of their paper, they claim that LNCS 1) do not affect gut microbiota at use levels and 2) are safe at levels approved by regulatory agencies. *Both of these claims are incorrect.* The scientific literature on LNCS clearly indicates that it is inappropriate to draw generalized conclusions regarding effects on gut microbiota and safety issues for compounds that vary widely chemical structure and pharmacokinetics. Scientific studies on the sweetener sucralose, used here as a representative LNCS, indicate that this organochlorine compound unequivocally and irrefutably disrupts the gut microbiome *at doses relevant to human use.* Results of dozens of additional research publications added and reviewed here also raise significant and extensive concerns about the safety of sucralose for the human food supply.

1. Introduction

A recent publication in Food and Chemical Toxicology entitled "Assessing the *in vivo* data on low/no-calorie sweeteners and the gut microbiota" by A. R. Lobach, A. Roberts, and I. R. Rowland made two primary claims (Lobach et al., 2018). First, they claimed that low/no-calorie sweeteners (LNCS) as a group do not have "any adverse effect on the gut microbiota at doses relevant to human use." Secondly, they asserted that "low or no-calorie sweeteners as a group, or individually, pose no safety concerns at their currently approved levels." They dismiss an extensive scientific literature on LNCS, often with unwarranted and irrelevant criticisms. The scientific literature on microbiota and safety of structurally diverse LNCS does not support their claims. Furthermore, examination of the aggregate data from multiple independent studies of some specific sweetener types, e.g. sucralose, show that dysbiosis consistently and unfailingly occurs at levels approved by regulatory authorities and at doses relevant to human use. The publication by Lobach, Roberts, and Rowland was financially supported, and reviewed prior to journal submission, by the Calorie Control Council, a special-interest trade group funded by sweetener companies.

2. Evidence against the first claim regarding gut bacteria

Let us first examine in more detail the first claim by Lobach, Roberts, and Rowland that LNCS have no adverse effects on the gut microbiota at doses relevant to human use. Scientific research studies and systematic reviews have concluded that some LNCS cause significant alterations in the composition of the gut microbiome and that dysbiosis occurs at levels approved by regulatory agencies (Abou-Donia et al., 2008; Schiffman and Rother, 2013; Suez et al., 2014; Bian et al., 2017; Uebanso et al., 2017; Frankenfeld et al., 2015; Ruiz-Ojeda et al., 2019). Let us focus on one of the LNCS, sucralose, and show that it does adversely alter gut microbiota *at doses relevant to human use*. Sucralose is a sweet-tasting organochlorine compound that has a major share of the United States and global market for LNCS (Lerner, 2009; Hexa Research, 2018). Abou-Donia et al. (2008) administered sucralose (delivered in Splenda®) to male rats for 12 weeks at dosages approved for human use (1.1–11 mg/kg/day). In the United States, the Acceptable Daily Intake (ADI) level for sucralose is 5 mg/kg body weight per day (mg/kg/d) (US FDA, 1998), and in the European Union, the ADI is set at 15 mg/kg/d (European Union, 2004). The rat was selected by Abou-Donia et al. (2008) as the animal model for testing because the United States Food and Drug Administration (US FDA, 1998) determined that the rat was the appropriate animal model for establishing the ADI for sucralose in humans, and the manufacturer used the rat model to obtain FDA approval.

Five groups of rats (n = 10 per group) received water with or without sucralose by oral gavage for 12 weeks at the following dosages:

Group 1, Control: no sucralose.
Group 2, 1.1 mg/kg/d sucralose.
Group 3, 3.3 mg/kg/d sucralose.
Group 4, 5.5 mg/kg/d sucralose.
Group 5, 11 mg/kg/d sucralose.

Fecal samples were collected weekly for bacterial analysis of total anaerobes, bifidobacteria, lactobacilli, *Bacteroides*, clostridia, total aerobes, and enterobacteria in culture media resulting in a total of 4200 measurements of bacterial counts (50 animals x 12 weeks x 7 bacterial groups) from the 12-wk treatment period. The analysis showed an orderly pattern of progressive and monotonic reduction in bacterial counts relative to controls over the 12-week period, and the decreases for total anaerobes, bifidobacteria, lactobacilli, and *Bacteroides* were highly statistically significant. The decrements over 12 weeks relative to controls are shown in Fig. 1 and Table 1. The reductions in total

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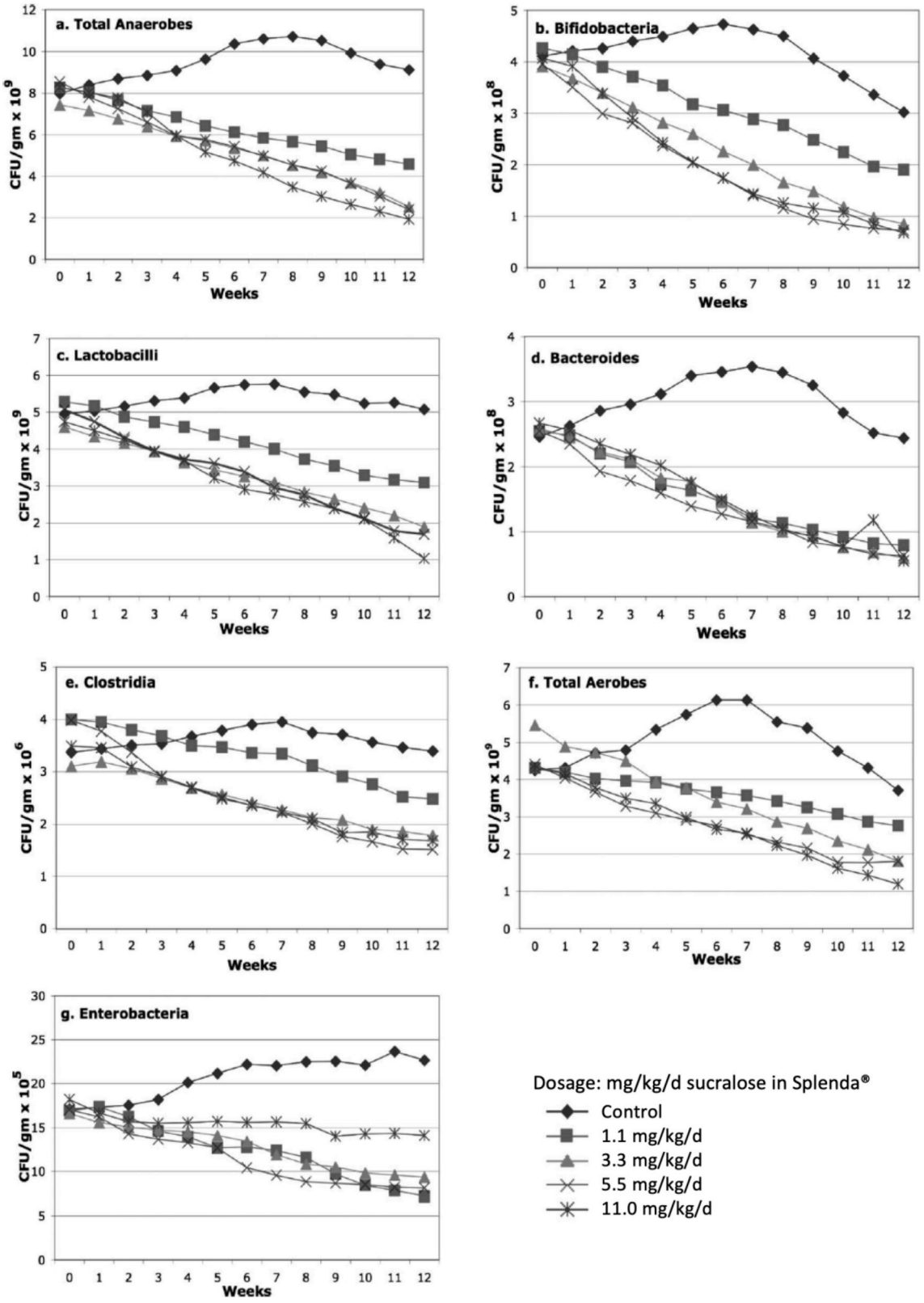

Fig. 1. Effect of sucralose delivered in Splenda® on bacterial counts in rat: (a) total anaerobes, (b) bifidobacteria, (c) lactobacilli, (d) *Bacteroides*, (e) clostridia, (f) total aerobes, and (g) enterobacteria, respectively. The viable counts are expressed as colony-forming units (CFU) per gram of wet weight feces. After Abou-Donia et al. (2008).

anaerobes, bifidobacteria, lactobacilli, and *Bacteroides* were statistically significant at the lowest dosage of 1.1 mg/kg/d (approximately one 12-ounce drink sweetened with sucralose for a 120-pound child, teen, or woman). The findings also indicate that sucralose altered the relative composition of microbiota with a greater reduction in beneficial bacteria (e.g. lactobacilli and bifidobacteria) relative to pathogenic

Table 1
Percent difference in bacterial counts for rats treated with sucralose relative to untreated controls for 12 weeks with p values given in parentheses (sucralose was delivered in Splenda®). After Abou-Donia et al. (2008).

	% Change at 1.1 mg/kg/d	% Change at 3.3 mg/kg/d	% Change at 5.5 mg/kg/d	% Change at 11 mg/kg/d
Total Anaerobes	−49.8 $p < 0.001$	−72.2 $p < 0.00001$	−73.7 $p < 0.00001$	−78.9 $p < 0.00001$
Bifidobacteria	−36.9 $p < 0.05$	−71.9 $p < 0.0001$	−76.0 $p < 0.0001$	−77.7 $p < 0.0001$
Lactobacilli	−39.1 $p < 0.01$	−62.8 $p < 0.00001$	−66.8 $p < 0.00001$	−79.7 $p < 0.00001$
Bacteroides	−67.5 $p < 0.00001$	−75.6 $p < 0.00001$	−74.1 $p < 0.00001$	−77.5 $p < 0.00001$
Clostridia	NS[a]	−47.4 $p < 0.05$	−55.3 $p < 0.05$	−50.5 $p < 0.05$
Total Aerobes	NS	−51.2 $p < 0.05$	−51.2 $p < 0.05$	−67.8 $p < 0.01$
Enterobacteria	NS	NS	NS	NS

[a] NS indicates not significantly different from the control group.

bacteria (e.g. enterobacteria). The disruptions of the microbiome were accompanied by histopathological changes in the intestine including lymphocytic infiltrates into epithelium, epithelial scarring, and glandular disorganization. Furthermore, the total anaerobes and bifidobacteria did not fully recover after a 12-wk depuration during which sucralose treatment was withdrawn.

The study by Abou-Donia et al. (2008) demonstrated that sucralose delivered in Splenda® significantly reduces the number and balance of gut microbiota at levels equivalent to a single sucralose-sweetened drink per day. However, Lobach, Roberts, and Rowland criticized the Abou-Donia et al. findings on three counts. First, they suggested that individual differences in food and fluid intake for the animals confound the results. This is false; a control group was included by Abou-Donia et al. to address individual differences in food and fluid intake across treatment groups. The minor variations in food and fluid intake across groups were dominated by the massive effects of sucralose/Splenda® on bacteria counts. As shown in Table 1, the decreases at Week 12 were for *Bacteroides* were statistically significant at $p < 0.00001$ for all four sucralose doses. Second, they suggest that maltodextrin in the Splenda® formulation may play a role in the decrements of bacterial counts. However, sucralose in the absence of maltodextrin also causes dysbiosis (Bian et al., 2017). Furthermore, the digestible end-product of maltodextrin is glucose (Hofman et al., 2016), and glucose does not reduce bacterial counts with repeated ingestion. Third, Lobach, Roberts, and Rowland invoke a scientifically flawed critique of the Abou-Donia et al. study by individuals who described themselves as an “independent” panel of “experts” (Brusick et al., 2009). The panel's self-characterization is unfounded. An easily conducted literature search on the Internet revealed that the senior author of the critique served as a consultant to the sucralose manufacturer and has no publications or demonstrable expertise on LNCS. The Internet search also revealed that other members of the self-acclaimed ‘independent panel of experts’ were long-time consultants to the sucralose manufacturer and/or served as co-authors on publications sponsored by the manufacturer. The issues raised by this panel are without scientific merit and were fully addressed by Schiffman and Abou-Donia (2012) and Schiffman and Rother (2013), neither of which were reviewed or cited in the Lobach, Roberts, and Rowland publication.

Additional studies in other laboratories with different animal models and measurement techniques have consistently and unvaryingly shown that sucralose affects bacterial counts. Bian et al. (2017) administered sucralose in drinking water to mice for 6 months at a dosage equivalent to the ADI in the US (5 mg/kg/d). They found that sucralose altered the composition of gut microbiota, enriched bacterial pro-inflammatory genes, and disrupted fecal metabolites at the human ADI. Uebanso et al. (2017) administered sucralose to mice at dosages approved by regulatory agencies in the US and EU; they found a decrease in the relative amount of *Clostridium cluster XIVa* in the fecal microbiome. In another study (Suez et al., 2014), mice, treated with elevated concentrations of a commercial formulation of sucralose for 11 weeks, developed glucose intolerance, and this effect was attributed to alterations in gut microbiota. Wang et al. (2018) found that sucralose had a direct effect on commensal bacteria on both *in vitro* and *in vivo* studies. *In vitro* sucralose inhibited the growth of the *E. coli* HB101 strain in both solid media and in liquid culture (Wang et al., 2018); *in vivo* studies in mice showed that sucralose altered the relative composition of gut microbiota. The growth of gut bacteria *E. coli* and *E. aerogenes* was inhibited by 150mM sucralose (Corder and Knobbe, 2018). In addition, sucralose increased antibacterial resistance and mutation frequency of *E. coli* (Qu et al., 2017). Rettig et al. (2014) reported that sucralose caused a concentration-dependent metabolic inhibition of the gut flora *Bacteroides, B. fragilis* and *B. uniformis* but with less effect on Firmicutes, *E. faecalis* and *C. sordellii*. Sucralose induced a toxicological pattern in a bioluminescent bacterial assay (Harpaz et al., 2018). The growth of oral bacteria (Prashant et al., 2012) as well as environmental bacteria (Omran et al., 2013a,b) is inhibited by sucralose. Furthermore, sucralose is metabolized by environmental bacteria to numerous metabolic by-products including 1,6-dichloro-1,6-dideoxyfructose, a mutagen (Labare and Alexander, 1994; Schiffman and Rother, 2013). These studies are consistent with the findings of Abou-Donia et al. (2008) described above and illustrate the unwavering conclusion that sucralose has a profound effect on bacteria.

3. Evidence against the second claim that there are no safety concerns

In addition to altering gut bacteria, other safety concerns about some LNCS have been described in the scientific literature including elevated risk of metabolic syndrome, type 2 diabetes, excessive weight gain, and cardiovascular disease (Swithers and Davidson, 2008; Swithers et al., 2010; Swithers, 2013; Pepino, 2015, 2018; Fagherazzi et al., 2013; Huang et al., 2017; Azad et al., 2017). Sucralose will again be used here as an example of potential safety concerns. First, independent researchers have shown that sucralose alters metabolic functions in some subjects. Pepino et al. (2013) found that acute ingestion of sucralose, at levels found in some soft drinks, by obese persons, who were non-habitual consumers of low-calorie drinks, potentiated glucose-stimulated insulin secretion and reduced insulin sensitivity. Romo-Romo et al. (2018) found that moderate ingestion of sucralose at 15% of the US ADI for fourteen days decreased insulin sensitivity in normal weight subjects. Lertrit et al. (2018) reported that daily administration of capsules containing 200 mg of sucralose for 4 weeks reduced insulin sensitivity as well as the acute insulin response in an intravenous glucose tolerance test. Veldhuizen et al. (2017) have also shown that sucralose disrupts normal physiological responses to carbohydrate ingestion. The effects of sucralose on glycemic control and insulin sensitivity have been linked to alteration in gut bacteria (Suez et al., 2014) and interaction of sucralose with the sweet taste receptor located in the gut and pancreas (Margolskee et al., 2007; Jang et al., 2007; Nakagawa et al., 2009; Mace et al., 2007; Shi et al., 2019). These findings by independent researchers do not support early studies by the manufacturer that reported no effects of sucralose consumption on blood glucose (Grotz et al., 2003, 2017). The Grotz et al. (2003) paper, however, has numerous methodological problems including, for

example: 1) patients with diabetes were instructed to self-administer capsules of sucralose, and there were no quantitative data given assuring that the capsules were reliably consumed as directed, 2) non-compliance with treatment regimens are high in this population (WHO , 2003), 3) reliance on glycated hemoglobin (HbA1c) has been reported to underestimate diabetes prevalence (Gupta, 2019), and 4) the location of dissolution of the capsules in the gastrointestinal tract is unknown. The Grotz et al. (2017) paper, published 21 years after the data collection, also utilized self-administration of capsules with no quantitative measurements of sucralose concentration in the urine or blood.

Other safety concerns regarding sucralose have emerged from *in vivo* studies in both humans and animals. In humans, sucralose has been found in breast milk of nursing mothers at levels above the human sweet taste threshold (Sylvetsky et al., 2015; Rother et al., 2015), and amplification of the sweetness in infancy can increase later preferences for elevated sweet taste (Pepino and Mennella, 2005; Mennella, 2014). Sucralose ingestion by infants in breast milk is also of concern because healthy gut microbiota play an important role in protection against chronic diseases, particularly during early infancy (Azad, 2019). Ingestion of sucralose by rats, at dosages relevant to human consumption, induces the expression of two intestinal proteins involved in first-pass metabolism, specifically P-glycoprotein (P-gp) and cytochrome P450 (CYP), and the magnitude of the increase in P-gp and CYP is sufficient to interfere with the bioavailability of therapeutic drugs (Abou-Donia et al., 2008). In rats, administration of sucralose at low to moderate levels (1.5 mg/kg/day) for 6 weeks induced neurobehavioral effects, exhibited by impaired passive avoidance learning; it also altered brain chemistry with increased glial fibrillary acidic protein (GFAP) expression and elevated brain lipid peroxides (Erbaş et al., 2018). Sucralose blunted thyroid function in rats at levels approved by the EU (Pałkowska-Goździk et al., 2018). In mice, sucralose elevated lymphocyte levels and carbonylated proteins that are markers of oxidative damage (Escoto-Herrera et al., 2017), increased the percentage of CD3 + T cells, CD19 + B cells, and IgA + plasma cells in Peyer's patches (Rosales-Gómez et al., 2018), and has been linked to neoplastic lesions including leukemia at elevated concentrations (Soffritti et al., 2016). Furthermore, new findings by Bornemann et al. (2018) indicate that sucralose, like organochlorine pesticides, bioaccumulates in body fat over time. Bornemann et al. also found that sucralose is metabolized in the gastrointestinal tract (GIT) to two metabolites that are more lipophilic than sucralose itself. The findings of bioaccumulation and metabolism of sucralose were not part of the original regulatory decision process for this tri-chlorinated chemical agent and indicate a need for reassessment of its appropriateness for the general food supply. The German Federal Institute for Risk Assessment (BfR, 2019) has also raised safety concerns about sucralose breakdown due to its instability at elevated temperatures and potential formation of harmful chemicals including chloropropanols generated when sucralose is heated with fats (Rahn and Yaylayan, 2010).

In vitro analyses also raise other biological issues. Sucralose interacted with reduced vitamin B_{12}, and this chemical reaction could occur *in vivo* (Motwani et al., 2011). Sucralose in solution destabilized the globular protein bovine serum albumin and reduced its melting temperature (Chen et al., 2015). In addition, sucralose has also been found to inhibit invertase and sucrose permease (Omran et al., 2013a), induce DNA fragmentation in Caco-2, HT-29 (colon) and HEK-293 (kidney) cell lines (van Eyk, 2015), and suppress the levels of the immunological parameters, interleukin-6 and interleukin-10 using whole blood culture assays (Rahiman and Pool, 2014).

4. Conclusion

In closing, the claims by Lobach, Roberts, and Rowland that low/no-calorie sweeteners 1) have no adverse effects on the gut microbiota at doses relevant to human use, and 2) pose no safety concerns as a group and individually at their currently approved levels, are *both incorrect*. 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. Sucralose, used here as an example, unequivocally and irrefutably disrupts the gut microbiome at levels approved by regulatory agencies and associated with human use. Furthermore, there are extensive safety concerns about sucralose including its impact on glycemic control, risk of metabolic syndrome, inflammation concomitant with alterations of gut bacteria, potential limitation of the absorption of therapeutic drugs, bioaccumulation with repeated intake, and toxicity when heated in food products. Going forward, further studies are needed to clearly delineate how specific LNCS, other than sucralose, impact biological processes when consumed alone and, importantly, in mixtures with other sweeteners. This will provide scientific information to regulatory agencies about the impacts of LNCS on biological systems and human health.

Competing interests

The authors have no competing financial interests or personal relationships that have any influence on the work reported in this paper.

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Susan S. Schiffman*, H. Troy Nagle
North Carolina State University, Raleigh, NC, 27695-7911, USA
E-mail address: schiffmansusan@gmail.com (S.S. Schiffman).

* Corresponding author. 18 Heath Place, Durham, NC, 27705, USA.