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Expert Panel report on a study of Splenda in male rats

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ABSTRACT

A recent study in rats investigated the retail sweetener product, Granulated SLENDA[®] No Calorie Sweetener (Splenda) (Abou-Donia et al., 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), which is composed of (by dry weight) maltodextrin (~99%) and sucralose (~1%). The investigators reported that Splenda increased body weight, decreased beneficial intestinal bacteria, and increased the expression of certain cytochrome P450 (CYP450) enzymes and the transporter protein, P-glycoprotein (P-gp), the latter of which was considered evidence that Splenda or sucralose might interfere with the absorption of nutrients and drugs. The investigators indicated that the reported changes were attributable to the sucralose present in the product tested. An Expert Panel conducted a rigorous evaluation of this study. In arriving at its conclusions, the Expert Panel considered the design and conduct of the study, its outcomes and the outcomes reported in other data available publicly. The Expert Panel found that the study was deficient in several critical areas and that its results cannot be interpreted as evidence that either Splenda, or sucralose, produced adverse effects in male rats, including effects on gastrointestinal microflora, body weight, CYP450 and P-gp activity, and nutrient and drug absorption. The study conclusions are not consistent with published literature and not supported by the data presented.

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1. Background

Non-nutritive sweeteners are found in a wide range of foods and beverages. They enable production of lower-sugar foods and beverages that can be a means to reduce sugar intake, which can, in turn, be useful in carbohydrate and calorie management strategies (Rolls, 1991; Blackburn et al., 1997; de la Hunty et al., 2006; Rodemmel et al., 2007). In the US and elsewhere, several non-nutritive sweeteners have been confirmed as safe and are permitted for use in the general food supply (e.g., US FDA, 1984, 1998a, 1999, 2002, 2003). Although they are not all compositionally related, permitted non-nutritive sweeteners all have in common a high-sweetness intensity and are approximately 200–13,000 times as sweet as sucrose on a weight-to-weight basis (Am Diet Assoc,

2004). This high-sweetness intensity means that very little is needed to achieve sweetness, and amounts needed for usual consumer uses, e.g., addition to beverages or cereal or use in recipes made at home, are exceedingly small. For example, less than 1/100 teaspoon of any approved non-nutritive sweetener is needed to replace the sweetness of 1 teaspoon of sugar. Retail formulations of non-nutritive sweeteners intended for consumer use (e.g., packets and granulated products) therefore include other ingredients that add volume, so that consumers can use them more like sugar on a volume-for-volume basis. The ingredients chosen must also allow the resulting retail sweetener product to have few calories per serving. The US Food and Drug Administration (US FDA) has determined that a food or beverage with less than five calories per serving may bear a no calorie claim (21 CFR 101.60(b)).

A recent study investigated the effects of a popular retail sweetener, Granulated SLENDA[®] No Calorie Sweetener (Splenda), in male rats when administered by gavage in amounts up to 1000 mg/kg/day (Abou-Donia et al., 2008). The tested product is

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a mixture of sucralose and maltodextrin (1% and 99%, respectively, on a dry weight basis).

The safety of sucralose as a food ingredient has been affirmed by the Joint (World Health Organization and United Nations' Food and Agricultural Organization's) Expert Committee on Food Additives (JECFA, 1989, 1991) and all regulatory agencies that evaluated the extensive safety data in animals and humans (e.g., Canada Gazette, 1991; US FDA, 1998a, 1999; JMHW, 1999; SCF, 2000; EU, 2004; FSANZ, 2008 [formerly ANFSC, approved 1993]). At least 100 studies of sucralose in humans and animals were conducted to assess the safety of sucralose (US FDA, 1998b). These studies included those required by health and regulatory agencies for food additive safety assessment and additional research, which helped to further describe sucralose safety. Research was conducted to investigate potential genotoxicity, carcinogenicity, neurotoxicity, immunotoxicity, reproductive and developmental toxicity, and general toxicity following acute, subchronic, and chronic exposures, and included studies on sucralose absorption, distribution, metabolism, elimination and pharmacokinetics. Studies were also conducted in both normoglycemic and diabetic subjects to investigate tolerance and effects on blood glucose homeostasis and control. Critical safety studies were conducted according to the standards required by the United States Food and Drug Administration (FDA; Red Book) and recommended by international organizations (e.g., Organisation for Economic Cooperation and Development [OECD]). Studies that investigated the safety of sucralose have been subjected to extensive safety reviews, conducted by internationally recognized experts who have unanimously concluded that sucralose is safe for its intended use (e.g., JECFA, 1989, 1991; Canada Gazette, 1991; US FDA, 1998a, 1999; JMHW, 1999; SCF, 2000; EU, 2004; FSANZ, 2008 [formerly ANFSC, approved 1993]; Grice and Goldsmith, 2000).

Similarly, maltodextrin, a readily digestible partially-hydrolyzed starch, generally derived from corn and used in a wide array of food products internationally, is Generally Recognized as Safe (GRAS) by the FDA for use in food (21 CFR 184.1444) (US FDA, 2008). No safety concerns are expected with exposure to maltodextrin.

The stated objective of the study by Abou-Donia et al. (2008) "was to determine the effects of orally administered Splenda on the composition and number of the major microbial population groups of fecal microflora in the GIT [gastrointestinal tract] of male Sprague–Dawley rats. The subsequent effects of Splenda treatment were also investigated on body weight, fecal pH, the integrity of the epithelium of the colon, the expression of intestinal membrane P-gp, and the expression of two members of the CYP protein family (CYP3A4 and CYP2D1)." In this study, 50 male Sprague–Dawley rats (10/group) were administered Splenda by gavage, at doses of 0, 100, 300, 500, or 1000 mg/kg body weight/day, for 12 consecutive weeks. Half of the animals (5/group) were euthanized at the end of 12 weeks and the remaining animals were kept alive for an additional 12 weeks to assess recovery. Control rats were administered water.

Abou-Donia et al., concluded "the findings of this study indicate that Splenda suppresses beneficial bacteria and directly affects the expression of the transporter P-gp and cytochrome P450 isozymes that are known to interfere with the bioavailability of drugs and nutrients. Furthermore, these effects occur at Splenda doses that contain sucralose levels that are approved by the FDA for use in the food supply." The reported findings included reduction in beneficial fecal microflora, increased fecal pH, histologic changes in the colon, increased body weight and enhanced protein expression levels of P-glycoprotein (P-gp) and cytochrome P450s 3A4 (CYP3A4) and 2D1 (CYP2D1). In the discussion, Abou-Donia et al., hypothesize that these effects are related to the sucralose present in the product tested, e.g., "The

effects on P-gp and CYP enzymes seen here cannot be due to the maltodextrin component of Splenda because it is hydrolyzed ... and then rapidly absorbed."

Following publication of this report, McNeil Nutritionals, a marketer of retail products that contain the non-nutritive sweetener, sucralose, requested an independent detailed review of the report by a panel of experts (Expert Panel) in areas of relevant expertise including general toxicology, food and chemical safety, reproduction and developmental toxicology, risk assessment, *in vitro* and *in situ* toxicology, toxicology study methodology and design, histopathology, nutrition, weight management, and clinical practice and research. Following its independent and rigorous review of the 2008 study by Abou-Donia et al., the Expert Panel prepared the following report.

2. Critique

2.1. Body weight gain measures

Abou-Donia et al., reported increased body weight gain to be an adverse effect of treatment with Splenda. Evaluation of the data does not support this conclusion. After 12 weeks' treatment, body weight gain, reported as percent change from baseline, in male rats receiving Splenda at doses of 100, 300, 500 and 1000 mg/kg/day was statistically significantly increased, not different, not different, and decreased, respectively, compared to body weight gain in control male rats. Body weight gain was also presented only in the unconventional manner of percent, and not actual, change from baseline. Percent weight gain after 12 weeks' treatment was reported as 93.1, 104.0, 100.7, 101.5 and 88.5% increased from baseline for rats receiving 0, 100, 300, 500, and 1000 mg/kg/day Splenda, respectively. There were no means or standard deviations reported for baseline weight, final body weight or actual change in body weight from baseline. The number of animals per group (10) was small and only one sex was studied. In light of the absence of statistical analysis of actual body weight data, particularly baseline and end-of treatment weights; minimal changes and no dose–response relationships in percent change in body weight gain; and the small number of animals studied, no biological significance can be attributed to the reported percent change in body weight gain. Similarly, the significance of changes in weight gain during the recovery period cannot be ascertained from this study.

The evaluation of any body weight change in the study by Abou-Donia et al., is confounded by the fact that no isocaloric solution was administered to control rats to ensure that effects on body weight gain were not due to differences in caloric intake. Without such isocaloric controls, the conclusions of increased weight gain are invalid. The authors also failed to report feed and water consumption levels during the study, and feed efficiency was not reported. Information regarding these nutritional parameters is absolutely essential to the proper assessment and interpretation of the reported changes in body weight gain.

These data contrast with data from larger published studies that demonstrate that sucralose, at doses as high as 1500 mg/kg/day, does not cause an increase in body weight (Goldsmith, 2000; Mann et al., 2000a,b). A recent clinical trial also showed improved weight management in overweight children in a family lifestyle study that introduced simple lifestyle changes including small increases in physical activity and instructions to reduce sugar intake by use of products containing sucralose (Rodearmel et al., 2007).

It is concluded that the body weight gain differences reported by Abou-Donia et al., are not evidence of a treatment-related effect.

2.2. Fecal microflora and pH measures

Abou-Donia et al., reported that “Splenda exerted numerous adverse effects, including... reduction in beneficial fecal microflora, [and] “increased fecal pH, ...” The reported changes in both fecal microflora and fecal pH cannot be defined as adverse effects based on the available data. Fecal microflora concentrations and pH are used as surrogate measures of gut (lower intestinal) microflora concentrations and pH. Significant variations in colon microflora concentrations have been reported as a result of diets differing in carbohydrate sources and intakes (Maczulak et al., 1993; Cresci et al., 1999). For example, diets containing high amounts of sugar appear to result in decreased concentrations of fecal bacteria and short chain fatty acids (SCFA). Bolus doses of carbohydrates can also have osmotic activity that can influence the gastrointestinal environment supporting gut microflora. The study by Abou-Donia, et al., did not include an isocaloric carbohydrate control, to control for the bolus dosing of carbohydrate, which is inherent with the gavage administration of Splenda that is predominantly composed of maltodextrin. Inclusion of an isocaloric carbohydrate control would have provided information on the effects resulting from common carbohydrate consumption. Thus, the significance of the reported changes in fecal microflora is unknown. Another issue is that the changes in fecal microflora reported by Abou-Donia, et al., were based on counts of colony forming units (CFU)/gram of wet-, and not dry-weight feces. No correction for the water content of stools was performed, despite the reports of between-group differences in fecal appearance in the early stage of the study. Since fecal moisture can influence total fecal weight, fecal moisture data are needed to standardize the fecal bacterial counts in terms of dry fecal weight. Without this information, it is not possible to interpret the reported fecal microflora concentration values, and it is not clear whether the reported results truly reflect the *in vivo* condition. In evaluating fecal microflora, bacterial counts are also typically and appropriately presented on a logarithmic scale (Best, 1970; Alber and Shaffner, 1992; Schaffner, 1998), since fecal bacterial counts can range exponentially. In the report by Abou-Donia et al., however, bacterial counts were presented on a linear scale. It is not known whether the statistical analysis of fecal microflora reported was performed on the raw data or on log transformed data; however, on an absolute basis, the bacterial counts were all very high. For all groups, including the control group, reported weekly values during the 12-week treatment phase ranged from >5 to <25 CFU/g $\times 10^5$ for enterobacteria; >0 to <5 CFU/g $\times 10^8$ for bifidobacteria; approximately 2 to <12 CFU/g $\times 10^9$ for total anaerobes; >1 to <7 CFU/g $\times 10^9$ for total aerobes; approximately 1 to <6 CFU/g $\times 10^9$ for lactobacilli; >0 to <4 CFU/g $\times 10^8$ for bacteroides; and >1 to <5 CFU/g $\times 10^6$ for clostridia. It can be seen from these data that, while the bacterial counts were very high, possible differences between treated and control groups are comparatively small. Based on the data presented graphically in the report by Abou-Donia, et al., reductions in bacterial counts between treatment and control groups were all less than 10-fold. As such, these are minor reductions that are not meaningful in relation to the total amount of bacteria present in each category. Also, the Expert Panel noted that, in the control rats of the study by Abou-Donia, et al., absolute values for fecal bacterial counts were lower in the recovery phase of the study than they were in the treatment phase of the study, and that the differences were similar to the differences seen between control and Splenda treatment groups during the treatment phase. This suggests that the decreases reported for Splenda-treated animals during the treatment phase were changes reflective of normal biological variation. The data were not compared with historic control data from the laboratory nor with literature-

based data. It is concluded that the data on fecal bacterial counts reported by Abou-Donia et al., cannot be interpreted as evidence of an adverse change. The results likely represent the range of normal variation, given the small magnitude of the changes reported, the observed decreases in the bacterial counts in the controls during the recovery period, and the known effects of dietary components, such as starches, on gut and fecal microflora populations. The data do not represent evidence of any toxic effect, particularly in light of the published literature on the safety of both sucralose and maltodextrin and on the finding that all Splenda-treated rats in the study by Abou-Donia et al., were reported to be similar to control rats in general clinical condition over the entire study duration.

Similarly, the reported increased fecal pH in male rats receiving Splenda by gavage compared to control male rats receiving water by gavage cannot be considered an adverse effect. Abou-Donia et al., reported increased fecal pH in all Splenda-treated groups compared to the control group throughout the course of the treatment period. Actual pH values were only presented in graphical form in the publication with no evidence of statistical analysis. Again, the absence of an isocaloric carbohydrate control group precludes meaningful interpretation of the results. As Splenda is mostly maltodextrin, a carbohydrate and metabolizable source of energy, a carbohydrate isocaloric control should have been included to reflect the impact of sugar or starch on fecal pH, particularly since it is known that the carbohydrate composition of the diet can affect the fecal pH (Mallett et al., 1988; Caderni et al., 1993; Licht et al., 2006). Diets high in simple polysaccharides, such as sucrose, may result in higher fecal pH values compared to more complex polysaccharides, such as starch. Moreover, the fecal pH levels reported in all the Splenda-treated groups were within the range of fecal pH levels reported with many commonly consumed carbohydrates, both simple and complex (Mallett et al., 1988; Caderni et al., 1993; Licht et al., 2006). No safety concerns can be drawn from these observations.

Additionally, Abou-Donia et al., reported “no visual differences ... in general condition between Splenda-treated animals and controls.” This is consistent with expectations from the expert safety assessments of both sucralose and maltodextrin, previously cited.

The Expert Panel also noted that, during the recovery phase of the study by Abou-Donia et al., fecal pH level rose to a level that was similar to the levels reported in Splenda-treated rats during the treatment phase of the study. This brings into question the health significance of the fecal pH levels reported in Splenda-treated rats.

It is concluded that, in the absence of appropriate calorie and carbohydrate controls in the study by Abou-Donia et al., the data on fecal pH cannot be interpreted as evidence of any adverse change. The results likely represent the range of normal variation or normal response to the presence of carbohydrates in the diet.

2.3. P-gp measures

P-gp is a transporter protein that has been implicated in multidrug resistance to certain cancer chemotherapies in some, but not all, patients. Abou-Donia et al., report that “Splenda directly affects the expression of the transporter P-gp... known to interfere with the bioavailability of drugs and nutrients,” and that “Splenda over time might lead to extrusion of high doses of drugs.” As previously discussed, the authors hypothesize that changes in P-gp are related to the sucralose present in the product tested. The literature does not associate changes in P-gp activity or expression with altered nutrient absorption. Subchronic gavage and long-term dietary studies in rats also show that daily intake of sucralose, at doses hundreds of times greater than expected human intakes, has no effect on growth or development (Goldsmith, 2000; Mann et al.,

2000a,b). This provides significant evidence that sucralose does not adversely affect nutrient absorption.

Effects on drug absorption are also not expected with sucralose, because it is a simple molecule and has a low potential for chemical reactivity. For example, in studies of baked goods made with radiolabeled sucralose, 100% of the radioactivity is recovered as unchanged sucralose (Barnrdt and Jackson, 1990). Pharmacokinetic studies in humans using radiolabeled sucralose show that it is stable *in vivo* (Roberts et al., 2000). Sucralose is not digested nor otherwise broken down for energy. Most sucralose is not absorbed, and all is relatively rapidly excreted. Maximum estimated intake is also low (<3 mg/kg/day) (US FDA, 1998a, 1999), further limiting potential for interactions. Protein binding of sucralose has not been observed in humans or laboratory animals (John et al., 2000b; Roberts et al., 2000; Sims et al., 2000; Wood et al., 2000). In a study of persons with type 2 diabetes, high doses of sucralose were also not associated with any changes in diabetic therapeutic regimens (Grotz et al., 2003). Maltodextrin, which represents the majority of the product tested in the study by Abou-Donia et al. (Splenda), is a common food ingredient that is Generally Recognized As Safe for use in food (US FDA, 2008). Readily digested carbohydrates, such as maltodextrin, have not been reported to be associated with drug interactions or reduced bioavailability of drugs. It may be concluded that Splenda is not expected to interfere with drug absorption.

The changes in P-gp expression levels reported by Abou-Donia et al., in the distal region of the rat small intestine were not dose-dependent. Increased expression at a level of approximately 2-fold over that observed in the control animals was seen only at the mid-dose levels (300 mg/kg body weight/day and 500 mg/kg body weight/day). Among all groups, including the control group, P-gp expression levels were lowest in the high-dose group.

The increased level of P-gp expression detected in the mid-dose treatment groups is likely within range of normal biological variation. A 2-fold increase in intestinal P-gp protein expression is within the inter-individual, as well as intra-individual, variability observed within the human population (Lin and Yamazaki, 2003). Furthermore, experts agree that P-gp expression, alone, is not considered a reliable marker of increased P-gp efflux activity, since P-gp protein levels do not always correlate with P-gp efflux activity or with chemotherapeutic response (Bates et al., 1989; Mickley et al., 1989; Leith et al., 1995; Beck et al., 1996; Hege-wisch-Becker et al., 1998; Lin and Yamazaki, 2003). For instance, data from *in vitro* studies show that P-gp protein induction values of up to 20–25 times control values were not associated with significant increases in efflux activity (Bates et al., 1989; Mickley et al., 1989). There is a consensus among experts that the use of multiparameter assays, which consist of both gene and protein expression assays, functional activity assays, as well as the use of positive control standards, is essential when evaluating the biological relevance of a change in P-gp expression (Beck et al., 1996). The authors should have provided data demonstrating an induction of P-gp-mediated drug efflux activity before concluding that their findings represent a potentially adverse effect, but they did not do so. Additionally, no conclusions can be drawn about the effect of sucralose on P-gp expression or activity, since the product tested was a mixture, predominantly maltodextrin, and the study included no evaluation of rats given an isocaloric carbohydrate control substance to assess the potential nutrient and/or physiologic effects of bolus (gavage) dosing of carbohydrate on normal P-gp expression.

It is concluded that the minimal and non-dose-dependent changes in P-gp expression reported in the study by Abou-Donia et al., cannot be considered evidence of an effect of Splenda, or sucralose, on either P-gp efflux activity or the absorption and bioavailability of therapeutic drugs.

2.4. Cytochrome P450 measures

Abou-Donia et al., reported dose-related increases in colonic CYP3A4 and CYP2D1 protein expression. Based on their findings, they hypothesized that sucralose induces the expression of CYP3A4 and CYP2D1, which, in turn, may lead to altered bioavailability of drugs that are usually subject to metabolism by these enzymes. The authors also hypothesized that the increase in CYP3A4 and CYP2D1 protein levels results in the metabolism of sucralose, thereby decreasing its bioavailability.

Although the authors hypothesized intestinal sucralose metabolism, their study did not investigate the metabolism of sucralose. Published studies, including studies using radiolabeled sucralose, demonstrate that gastrointestinal metabolism of sucralose does not occur in experimental animals or humans; but rather that sucralose is excreted unchanged in feces. There is also no evidence of any cytochrome P450 monooxygenase-mediated metabolism of sucralose.

Studies in both experimental animals and humans have shown that most of an oral dose (~85%) is unabsorbed. Of the limited amount absorbed, most is excreted unchanged in the urine and only a small amount (about 2% of an ingested dose) undergoes phase II metabolism via glucuronidation (John et al., 2000a,b; Roberts et al., 2000; Sims et al., 2000; Wood et al., 2000). Glucuronidation is not CYP450-mediated, and oxidized metabolites of sucralose that are indicative of cytochrome P450 metabolism have not been detected. Therefore, sucralose is not a substrate for cytochrome P450s. Finally, specific testing of sucralose demonstrated that sucralose is not an inducer of the cytochrome P450 family of drug metabolizing enzymes or of parameters associated with enzyme induction (Hawkins et al., 1987; JECFA, 1989). Consequently, the reported changes in CYP3A4 and CYP2D1 expression levels associated with Splenda ingestion and the proposed hypothesis of P450-mediated metabolism of sucralose by Abou-Donia et al., are not consistent with, nor supported by, the published literature.

In discussing their CYP450 enzyme data, Abou-Donia et al., state that in “Prior studies [that] examined the metabolic profile of sucralose in rats treated with chronic, non-physiological ‘mega’ doses,” “high dose[s] of sucralose may have saturated. CYP metabolism enzymes, thus impeding the body’s ability to metabolize sucralose.” The authors infer that the dose levels used in their study would not result in such saturation, and, therefore, sucralose, at low doses, could be metabolized by CYP450 enzymes, thus explaining the increased expression of CYP450 enzymes reported in their study.

While it is true that enzyme “saturation” can occur, one would not expect a complete absence of metabolism with high doses, but simply a lower level of metabolism than what might have been projected with a linear extrapolation of the dose–response curve. The detection of only unchanged sucralose in the feces following both lower intakes (consistent with expected human exposure of approximately 1 mg/kg body weight/day) and high intakes (e.g., up to 1500 mg/kg body weight/day in studies in rats) is evidence that gastrointestinal metabolism of sucralose does not occur. Importantly, sucralose is not a substrate for the CYP450 family of Phase I metabolizing enzymes.

High inter-individual differences can occur in the expression of the family of CYP450 enzymes, despite use of an inbred rat strain, timed sample preparations, and controlled animal housing conditions (Mitschke et al., 2008). Inter-individual differences in expression of intestinal cytochrome P450 enzymes have also been observed in humans. For example, a 6- to 11-fold variation in the expression of various CYP3A intestinal enzymes has been detected in humans, the highest occurring with CYP3A4 (Lown et al., 1994). Thus, the observed treatment group differences in the study by Abou-Donia et al., could have been simply indicative of normal bio-

logical variation. Additionally, it is known that some dietary components can increase the expression of certain cytochrome P450 enzymes, including that of CYP3A4 (Evans, 2000). The effects that differential carbohydrate consumption, such as that which occurred in this study, could have on CYP450 expression are also not known.

The Expert Panel also questioned the methodology used for assessing P-gp and CYP450 expression levels. While Abou-Donia et al., reference Dürr et al. (2000), for the Western blot methodology used, they appear to not have used the methodology cited. The Dürr et al. (2000) methodology appropriately places test and control samples on a single blot. This is appropriate because of the inter-experimental variability always possible with running Western blots. Such variability can significantly affect the signal intensity that represents the expression of a particular protein. Sources of variability include the length of the chemiluminescence reaction, the exposure time to film, the shelf age of the reagents used, or a combination thereof. The loading of β -actin will not control for these inter-experimental variables. Thus, one can only reliably compare target protein expression/ β -actin expression ratios for different samples, when the different samples are run on the same gel. From Figure 5 in the report by Abou-Donia et al., however, it is apparent that samples from control rats were run on one gel, and samples from Splenda-treated rats were run on separate gels – one gel per group. Thus, one cannot reliably interpret differences between the groups in the reported CYP450 or P-gp expression/ β -actin expression ratios.

It is concluded that the differences reported in intestinal CYP450 enzyme content cannot be considered evidence of any untoward effect or metabolism of sucralose, both of which have been disproved in previously published well-designed studies. The study by Abou-Donia et al., provided no evidence to the contrary, and the reliability of the differences reported is questionable, in light of the analytical procedures used.

2.5. Histological evaluation of colon cells

Abou-Donia et al., reported increased alterations in colon cells based on histological analysis of colon tissue from 5 male animals per group. This number of animals is insufficient for accurate diagnosis or proper statistical analysis of reported changes.

The authors presented histopathology data using percentages rather than actual data. This leads to exaggerated appearances of differences. Without individual or actual incidence data for the number of animals used (5 per group), percentages are not reliable indicators of the relevance of the findings. For example, a report of 20% of animals with any particular histopathological finding is equal to 1 of the 5 samples assessed having that particular finding.

For four of the six alterations reported (lymphocytic infiltrates into epithelium, glandular disorganization [of different types], submucosal lymphoid aggregates or lymphoid follicles, and mild depletion of goblet cells/loss of superficial mucin), there is no corresponding dose–response relationship either during the treatment or recovery phases. Three of these four alterations were present in control animals during the recovery phase, with the percentage of control animals “affected” being at least as great, or greater, than the percentage of “affected” treated animals during the treatment phase. The authors characterize these changes during the recovery phase, however, as “apparently age-related” for control animals, while these same alterations are described as clinically meaningful adverse effects for animals treated with Splenda during the first 12 weeks of the study.

The remaining two alterations cited were ‘intravascular lymphocytes’ and ‘epithelial scarring.’ The presence of intravascular lymphocytes, described in the author’s textual comments as “focally dilated vessels stuffed with intravascular lymphocytes”, was

acknowledged by the authors to be “an artifact that was almost always related to large nodular lymphoid aggregates within the submucosa and apparently related to a procurement effect at autopsy that occurred when the microtome cut the colon into thin sections.” There is thus no relevance of this particular finding among the histopathological alterations noted. Epithelial scarring was noted as present in one high-dose animal during the treatment phase and in no other animals during either the treatment or recovery phase. Severity is not reported, and no meaning can be drawn from this singular observation. Importantly, none of these changes are pathognomic of gastrointestinal disease or malfunction.

It is concluded that the histopathology data reported by Abou-Donia et al., cannot be considered evidence of an adverse effect of Splenda on colonic tissue. Moreover, several studies with sufficient animal numbers to allow for meaningful statistical analyses clearly demonstrate that sucralose, at doses several 100-fold greater than those used in the study by Abou-Donia et al., with exposure from conception and then with dietary intake daily for essentially a lifetime, had no adverse effect on intestinal tissue, including the colon (Mann et al., 2000a,b).

2.6. General comments

The study by Abou-Donia et al., has significant deficiencies in study design and methodology. Notable is the size and likely power of the study, which are not adequate to define adverse effects in male rats that were in direct conflict with results of larger studies that were conducted in male and female rats for longer periods at higher doses. The use of 10 male animals per group with half that number assigned to the recovery portion of the study was clearly less than adequate for appropriate sampling and statistical analyses. The use of male animals only is inappropriate for safety assessments and in conflict with internationally recognized guidelines for the safety evaluation of food ingredients (e.g., US FDA, 1993 [Redbook II]; OECD, 1997).

The statistical analysis methodology reported by Abou-Donia et al., appears to be appropriate, but the data provided in the publication do not disclose information on standard deviations or standard errors both to confirm that statistical analyses were conducted and to allow for evaluation of the variability of the data generated. Error bars in the graphical representations of the data are generally absent (e.g., body weight changes, the fecal bacterial count data, and fecal pH data). There are no indications as to the variability of the data within each treatment group, including controls.

It seems that some of the references cited may be inappropriate. For example, in contrast to the authors’ comment that “The low absorption of sucralose from the GIT is surprising, because this sweetener is an organochlorine molecule with appreciable lipid solubility,” the low absorption of sucralose is not surprising in light of its lipid solubility. The octanol–water partition coefficient of sucralose, 0.32, is low (Jenner and Smithson, 1989; Molinary and Jenner, 1999), and confirms that sucralose has low lipid solubility and high water solubility (Jenner and Smithson, 1989; US FDA, 1998a). The demonstrated octanol–water partition coefficient of sucralose also indicates that sucralose is preferentially water soluble. This is entirely consistent with the fact that sucralose is a relatively small (MW ~ 400) polyhydroxylated substance. Such substances are typically hydrophilic. The same references cited by Abou-Donia et al., as supportive of appreciable fat solubility of sucralose, discuss the high water solubility of sucralose, and all discussion of lipid and water solubility is in the context of sucralose as a potential sweetener in various food matrixes (Miller, 1991; Wallis, 1993; Yatka et al., 1992), which is different than discussion of fat solubility in the context of human health effects and/or safety.

In contrast to highly water soluble substances like sucralose, highly fat soluble, bioaccumulative organochlorine molecules, to which Abou-Donia et al., liken sucralose, e.g., chlorinated hydrocarbons, have octanol:water partition coefficients as much as 10,000 times higher. Statements made by the authors from which it could be inferred that there are similarities between these types of substances, e.g., “The impact of Splenda (and particularly the chlorocarbon sucralose) ... is consistent with previous reports that chlorinated hydrocarbons. ... are not founded scientifically. Sucralose is not a chlorinated hydrocarbon, but a chlorinated carbohydrate, chemically and biochemically very different substances. Sucralose and chlorinated hydrocarbons are biologically and toxicologically dissimilar types of compounds.

The published literature also clearly demonstrates that sucralose is not metabolized to products that could be considered products of CYP450 metabolism, whether at low or high doses (McLean Baird et al., 2000; Grice and Goldsmith, 2000; Mann et al., 2000a,b; Sims et al., 2000; Roberts et al., 2000). There is no basis for hypothesizing that reported differences in CYP450 metabolism of sucralose could explain non-dose relationships in measured P-gp levels. There is similarly no basis for the hypothesis that sucralose treatment with low, and not high, doses could adversely affect health. The authors also infer that sucralose could cause adverse effects “...at Splenda doses that contain sucralose levels that are approved by the FDA for use in the food supply.” In contrast, the lack of dose relationships in the Abou-Donia et al. (2008) study is a clear signal of no adverse effects.

Because the general public continues to be concerned about the safety of food ingredients, including non-nutritive and nutritive sweeteners, it is important that all safety data regarding food ingredients be made publicly available, and the data should be critically evaluated to assure the public that the conclusions presented are supported by data from properly designed and executed studies. The extensive safety data of sucralose and maltodextrin have been rigorously evaluated by experts around the world, and the available evidence demonstrates that Splenda, sucralose, and maltodextrin are safe for their intended uses.

3. Conclusions

The study by Abou-Donia et al. (2008), is not scientifically rigorous and is deficient in several critical areas that preclude reliable interpretation of the study results with regard to the effects of either sucralose or Splenda in rats or humans. Therefore, the results do not provide acceptable evidence that Splenda or sucralose produced adverse effects in male rats exposed by gavage. Further, the data presented are not consistent with published data on the safety of sucralose. Therefore, the conclusions by Abou-Donia et al. (2008), that Splenda (or sucralose) “exerted numerous adverse effects, including”... “a decrease in beneficial intestinal bacteria, elevated fecal pH, histopathological changes in the colon, increased body weight, and enhanced intestinal expression of”... “the transporter P-gp and cytochrome P450 enzymes that are known to interfere with the bioavailability of drugs and nutrients,” are not scientifically valid, since they are not supported by the data presented.

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