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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 SPLENDA® 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.

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; Rodearmel 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 SPLENDA® 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.