James Heimbach, Ph.D.
JHeimbach LLC
923 Water Street
#66
Port Royal, VA 22535

Re: GRAS Notice No. GRN 000897

Dear Dr. Heimbach:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000897. We received the notice that you submitted on behalf of DuPont Nutrition and Health (DuPont) on December 2, 2019 and filed it on January 29, 2020. DuPont submitted amendments to the notice on April 2, 2020, April 6, 2020, and April 21, 2020 providing additional manufacturing specifications, safety information, and clarification on the intended uses.

The subject of the notice is 2′-O-fucosyllactose produced by *Escherichia coli* K12 strain INB000846 (2′-FL) for use as an ingredient in non-exempt milk or soy-based infant formula for term infants; toddler formulas; infant and toddler foods and toddler drinks at levels ranging from 1.2 to 12 g/kg (solids) or g/L (liquids), and in baked goods and baking mixes; non-alcoholic beverages and beverage bases; breakfast cereals; milk products; dairy product analogs; processed fruits and fruit juices; processed vegetables and vegetable juices and oral and enteral tube-feeding formulas at levels ranging from 1.2 to 40 g/kg. The notice informs us of DuPont's view that these uses of 2′-FL are GRAS through scientific procedures.

DuPont describes 2′-FL as a white to off-white powder. The chemical name for 2′-FL is α-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose. DuPont provides the CAS Reg. No. (41263-94-9), molecular weight (488.44 Da) and empirical formula ($C_{18}H_{32}O_{15}$) for 2′-FL.

DuPont states that 2′-FL is produced by the fermentation of lactose from cow's milk, and sucrose, by the production strain *Escherichia coli* K-12, referred to as INB000846. DuPont states that the production strain, *E. coli* K-12 strain INB000846, is deposited in the Inbiose culture collection and is a modification of *E. coli* K-12 strain MG1655.[1] The parental strain, *E. coli* K-12 strain MG1655, is non-pathogenic, non-toxigenic, and is available from the American Type Culture Collection (ATCC) as ATCC 700926 and from the *Coli* Genetic Stock Center (CGSC) as CGSC 7740. DuPont constructed the

[1] DuPont states that the production strain is derived from *E. coli* K-12 strain MG1655 using methods similar to those used to derive the production strain identified in GRN 000749.

production strain *E. coli* K-12 strain INB000846 after making multiple gene deletions[2] in the host strain, MG1655. Following the deletions, DuPont made five insertions of synthetic genes encoding functions for sugar metabolism and transport from four donor species to optimize the production of 2′-FL. DuPont states that the production strain was assessed to be stable through 61 generations of fermentation, based on whole genome sequencing.

DuPont states that the production method is the same as that described for 2′-FL in GRN 000749 and incorporates this information into GRN 000897.[3] The fermentation is conducted under controlled conditions to optimize 2′-FL production and secretion into the medium. Following fermentation, cell biomass, endotoxins, and large molecules are removed using microfiltration or ultrafiltration. The filtrate is concentrated, treated with ion exchange resins and activated carbon, and then filtered. The resulting product is concentrated into crystallized 2′-FL which may be used as is or dissolved in water and spray dried. DuPont states that the raw materials used are food-grade or conform to specifications in the Food Chemicals Codex (FCC, 10th ed, 2016) and comply with FDA regulations for such use under current good manufacturing practices.

DuPont provides the following specifications for 2′-FL: the minimum content of 2′-FL (> 96%) and limits on lactose (< 5%), difucosyllactose (< 5%), total other carbohydrates (< 5%), moisture (≤ 5%) and protein (≤ 100 mg/kg); heavy metals; lead (≤ 0.05 mg/kg); and limits for microorganisms, including *Salmonella* serovars (not detected in 25 g) and *Cronobacter sakazakii* (not detected in 10 g). DuPont provides the results from batch analyses of three non-consecutive lots to demonstrate that the ingredient can be manufactured to conform with the provided specifications.

DuPont provides estimates of dietary exposures to 2′-FL for use in foods (listed on Page 1 of this letter), and in oral and enteral and tube-feeding formulas using consumption data based on What We Eat in America (WWEIA) and the National Health and Nutrition Examination Survey (NHANES) (WWEIA/NHANES 2013-2016). DuPont reports the mean and 90th percentile dietary exposure estimates for the U.S. population (3+ y) on a per user basis from consumption of 2′-FL from the intended uses in foods to be 2.2 g/day (d) (40 mg/kg body weight (bw)/d) and 5.0 g/d (100 mg/kg bw/d) respectively. DuPont also reports mean and 90th percentile dietary exposure estimates of 2′-FL from consumption of non-exempt milk or soy-based infant formula for term infants and toddler foods to be 2.9 g/d (450 mg/kg bw/d) and 5.3 g/d (712 mg/kg bw/d) respectively, for infants up to 6 months of age; 4.6 g/d (520 mg/kg bw/d) and 8.4 g/d (987 mg/kg bw/d) respectively, for infants 7 to 12 months of age; and 1 g/d (85 mg/kg bw/d) and 2 g/d (146 mg/kg bw/d) respectively, for toddlers 13 to 36 months of age. DuPont notes that the uses of 2′-FL in infant formula and toddler foods are

[2] DuPont states that deletions made include *yhcE* (partial), *yhcG*, *yhcF*, and *yegH* (full) in addition to the deletions described in GRN 000749.

[3] We evaluated GRN 000749 and responded in a letter dated April 23, 2018, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

substitutional to those described in GRNs 000546, 000571, and 000650,[4] and will not result in an increase in dietary exposure to 2′-FL.

DuPont incorporates the safety information discussed in GRN 000749. DuPont reviews and discusses the publicly available safety information since the submission of GRN 000749 and includes a discussion on two new published studies with 2′-FL produced by microbial fermentation, similar to their article of commerce. DuPont notes that these published toxicological studies support the safety of their 2′-FL. DuPont also provides a discussion on several new tolerance or efficacy studies in rodents and humans that support the safety of 2′-FL for their intended uses. In addition, DuPont concludes that their intended uses of 2′-FL are safe based on data and information to establish safety for its use in infant formula, dietary exposure estimates, and the absence of adverse effects in tolerance and efficacy studies in humans.

DuPont includes the opinion of a panel of individuals (DuPont's GRAS panel). Based on its review, DuPont's GRAS panel concluded that 2′-FL is safe based on scientific procedures under the conditions of its intended uses.

Based on the data and information provided in the submission, DuPont concludes that 2′-FL is GRAS for its intended uses.

Potential Labeling Issues

Under section 403(a) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), a food is misbranded if its labeling is false or misleading in any way. Section 403(r) of the FD&C Act lays out the statutory framework for labeling claims characterizing a nutrient level in a food or the relationship of a nutrient to a disease or health-related condition (also referred to as nutrient content claims and health claims). If products containing 2′-FL bear any nutrient content or health claims on the label or in labeling, such claims are subject to the applicable requirements and are under the purview of the Office of Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition. The Office of Food Additive Safety did not consult with ONFL on this issue or evaluate any information in terms of labeling claims. Questions related to food labeling should be directed to ONFL.

Allergen Labeling

The FD&C Act requires that the label of a food that is or contains an ingredient that contains a "major food allergen" declare the allergen's presence (section 403(w)). The FD&C Act defines a "major food allergen" as one of eight foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. 2′-FL derived from lactose requires labeling under the FD&C Act because it contains protein derived from cow's milk.

[4] We evaluated GRNs 000546, 000571, and 000650 and responded in letters dated September 16, 2015, November 6, 2015, and November 23, 2016, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

Intended Use in Infant Formulas

Under section 412 of the FD&C Act, a manufacturer of a new infant formula must make a submission to FDA providing required assurances about the formula at least 90 days before the formula is marketed. Our response to DuPont's GRAS notice does not alleviate the responsibility of any infant formula manufacturer that intends to market an infant formula containing 2′-FL to make the submission required by section 412. Infant formulas are the purview of ONFL.

Section 301(ll) of the FD&C Act

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of DuPont's notice concluding that 2′-FL is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing 2′-FL. Accordingly, our response should not be construed to be a statement that foods containing 2′-FL, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that DuPont provided, as well as other information available to FDA, we have no questions at this time regarding DuPont's conclusion that 2′-FL is GRAS under its intended conditions of use. This letter is not an affirmation that 2′-FL is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 000897 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2020.06.12 17:40:20 -04'00'

Susan Carlson, Ph.D.
Director
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