Fei Yao
Synaura Biotechnology (Shanghai) Co., Ltd.
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Shanghai, CHINA

Re: GRAS Notice No. GRN 001157

Dear Fei Yao:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001157. We received Synaura Biotechnology (Shanghai) Co., Ltd (Synaura)'s notice on June 8, 2023, and filed it on November 16, 2023. Synaura submitted amendments to the notice on February 14, 2024, April 17, 2024, and July 1, 2024, that revised the specifications and clarified the manufacturing process, production organism, minor impurities, analytical methods, intended uses, estimates of dietary exposure, and aspects of the safety narrative.

The subject of the notice is 2′-fucosyllactose (2′-FL) for use as an ingredient in milk- and soy-based, non-exempt infant formula for term infants at a maximum level of 2.4 g/L of formula as consumed[1] and in other food categories at the maximum levels shown in Table 1.[2] The notice informs us of Synaura's view that these uses of 2′-FL are GRAS through scientific procedures.

Table 1: Intended food categories and maximum use levels for 2′-FL

Food Categories	**Maximum use levels (g/kg or g/L)**
Enhanced or fortified waters	0.8
Sports, isotonic, and "energy" drinks	0.8
Hot breakfast cereals, prepared	4.8
Ready-to-eat (RTE) cereals, puffed	80
RTE cereals, high fiber	30
RTE cereals, biscuit-type	20
Imitation milks	1.2
Milk-based coffee drinks	1.2
Frozen dairy-based desserts	17

[1] Synaura states that 2′-FL is intended for use in in ready-to-feed or reconstituted infant formula prepared from powder.
[2] Synaura states that 2′-FL is not intended for use in products under the jurisdiction of the United States Department of Agriculture and in foods for which standards of identity do not permit its addition.

Food Categories	Maximum use levels (g/kg or g/L)
Puddings, custards, and mousses	17
Fruit pie filling	14.1
Fruit filling in bars, cookies, yogurt, cakes	30
Non-exempt infant formula for term infants[3]	2.4
Formula intended for young children (>12 months)	2.4
Hot cereals for infants and young children, prepared (from dry instant) and ready-to-serve	10.9
Baby food desserts including fruit desserts, cobblers, yogurt/fruit combinations	10.9
Drinks for infants and young children: juice and yogurt drinks	10
Baby snacks: crackers, pretzels, cookies, and other dry snack items	57
Jams, jellies, preserves, and fruit butters	60
Cereal bars including snack, granola, and breakfast bars	12
Meal replacement bars for general use and weight management	12
Milk-based meal replacement drinks (including nutritional drinks, smoothies) for general use and weight management	1.2
Milk-based meal replacement beverages for children (e.g., pediatric nutritional drinks)	2.4
Unflavored pasteurized and sterilized milk	1.2
Flavored and fermented milk drinks	1.2
Yogurt	5.3
Fruit juices and nectars	1.2
Fruit-flavored drinks and ades	1.2
Syrups used to flavor milk beverages	7
Nutritional drinks for pregnant women	6

Synaura describes the identity and composition of 2′-FL stating that 2′-FL is a white to off-white powder containing a minimum of 94% (dry basis) 2′-FL. Synaura notes that 2′-FL is a trisaccharide consisting of L-fucose, D-galactose, and D-glucose. The chemical name for 2′-FL is α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucose and the CAS registry number is 41263-94-9. Synaura states that 2′-FL is chemically and structurally identical to 2′-FL from human milk, as confirmed by high performance anion exchange chromatography-pulsed amperometric detection, liquid chromatography with tandem mass spectrometry, infrared spectroscopy, and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy.

Synaura describes the production organism used in the manufacture of 2′-FL. The production organism, *Escherichia coli* EB011065, is genetically engineered from the

[3] Although Synaura lists "infant formula (0-6 months)" and "follow-on formula (6-12 months)" among the intended uses, FDA subsumes these uses under one category of non-exempt infant formula for term infants.

host strain *E. coli* BL21 (DE3). Synaura constructed the production organism by knocking out six genes in the host strain genome and inserting six additional heterologous genes.[4] Synaura states that *E. coli* EB011065 is non-pathogenic and non-toxigenic and that all genetic modifications are confirmed using polymerase chain reaction-based methods.

Synaura states that 2′-FL is manufactured according to current good manufacturing practices, and that all raw materials and processing aids are food-grade and are used in accordance with applicable U.S. regulations or have previously been determined to be GRAS for their respective uses. In the first stage of the manufacturing process, 2′-FL is produced from D-lactose and D-glucose by fermentation with *E. coli* EB011065 under controlled conditions and the 2′-FL is secreted into the fermentation medium. After fermentation is complete, the production organism is inactivated by heat treatment. The microbial biomass is removed from the fermentation medium by flocculation, followed by centrifugation and membrane filtration. The second stage of the manufacturing process consists of a series of purification steps. The product is ultrafiltered to remove residual protein and nucleic acids and then subjected to a series of cationic and anionic resins to remove minerals, amino acids, and pigments. The solution is further decolorized using activated carbon. Finally, the solution is concentrated by evaporation and further purified by crystallization. The crystals are dissolved in water and then the product is subjected to germ filtration followed by spray drying under heat and vacuum. The final product is sieved to produce the 2′-FL powder.

Synaura provides specifications for 2′-FL, which include the minimum content of 2′-FL (≥94% on a dry weight basis (DW)), and limits on D-lactose (≤3% DW), 3,2′-difucosyl-D-lactose (≤1% DW), 2′-fucosyl-D-lactulose (≤1% DW), L-fucose (≤1% DW), D-glucose (≤1% DW), total impurities or sum of identified minor carbohydrates (≤5% DW), moisture (≤9%), residual proteins (≤0.01%), sulphated ash (≤0.2%), pH 3.2-7.0, heavy metals, including lead (≤0.02 mg/kg), and microorganisms, including *Salmonella* serovars (absent in 25 g) and *Cronobacter spp.* (absent in 100 g). Purity specifications are consistent with the monograph for 2′-FL in the 13th edition of the Food Chemicals Codex (FCC, 2023). Synaura provides the results from the analyses of three non-consecutive batches of 2′-FL to demonstrate that the ingredient can be manufactured to meet the specifications.

Synaura states that the intended uses of 2′-FL are substitutional for those described in GRNs 000735 and 000932[5] and incorporates the estimates of dietary exposure to 2′-FL from GRN 000932. Using food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES), Synaura states that the estimated dietary exposures to 2′-FL are 2.06 g/person (p)/d (0.33 g/kg body weight (bw)/d) at the mean and 3.17 g/p/d (0.53 g/kg bw/d) at the 90th percentile for infants 0-5 months

[4] Synaura describes construction of the 2′-FL production organism in GRN 001157; *E. coli* EB011065 contains inserted genes that encode for lactose permease, phosphomannomutase, mannose-1-phosphate guanylyltransferase, GDP-mannose-4,6-dehydratase, GDP-fucose synthase, and α-1,2-fucosyltransferase.
[5] 2′-FL was the subject of GRNs 000735 and 000932. We evaluated these notices and responded in letters dated April 6, 2018, and February 18, 2021, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

of age, and 2.60 g/p/d (0.29 g/kg bw/d) at the mean and 4.95 g/p/d (0.53 g/kg bw/d) at the 90th percentile for infants 6-11 months of age. For children 1-3 years of age, the estimated dietary exposures to 2′-FL are 1.45 g/p/d (0.12 g/kg bw/d) and 2.23 g/p/d (0.19 g/kg bw/d) at the mean and 90th percentile, respectively. For the total population (all ages), estimates of dietary exposure to 2′-FL are 1.77 g/p/d (0.034 g/kg bw/d) and 3.59 g/p/d (0.074 g/kg bw/d) at the mean and 90th percentile, respectively. Synaura acknowledges that the intended uses in GRN 001157 and the dietary exposure estimates described therein do not encompass all uses of 2′-FL previously determined to be GRAS.[6] Synaura notes that the intended uses described in GRN 001157 are substitutional for those in GRNs 000735 and 000932 and therefore, there is not an increase in the dietary exposure to 2′-FL.

Synaura discusses the data and information supporting the safety of 2′-FL and states that a literature search conducted through April 2023 did not identify any studies which would contradict its GRAS conclusion. Synaura states that 2′-FL is structurally and chemically identical to the 2′-FL in human milk and has a compositional similarity to other 2′-FL ingredients previously concluded to be GRAS. Synaura states that the safety of 2′-FL is supported by preclinical and clinical studies conducted with other 2′-FL ingredients. Synaura incorporates into the notice and provides summaries of published and unpublished information discussed in GRNs 000546, 000571, 000650, 00735, 000749, 000815, 000852, 000897, 000929, 000932, 001014, and 001034[7] including absorption, distribution, metabolism, and excretion data for 2′-FL as well as single dose acute and repeated dose subchronic studies in rats, tolerability studies in piglets, genotoxicity tests, and clinical studies in infants and adults. Synaura also provides summaries of published clinical studies demonstrating safe consumption of 2′-FL by adults with irritable bowel syndrome, ulcerative colitis, or celiac disease, and the safe consumption of 2′-FL by infants. Synaura states that these clinical studies demonstrate that 2′-FL is safe and well tolerated when consumed by adults and infants. Synaura also

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[6] The uses summarized in Table 1 do not include some intended uses evaluated in recent GRAS notices for 2′-FL. We note that a recent cumulative estimate of dietary exposure to 2′-FL was presented in GRN 001051, where the notifier evaluated all uses of 2′-FL expected to contribute to the overall dietary exposure to 2′-FL in the U.S. This cumulative dietary exposure incorporated additional uses not included in GRN 001157 and higher maximum use levels in specific food categories that were introduced in prior GRAS notices. We evaluated GRN 001051 and responded in a letter dated November 21, 2023, stating that we had no questions at that time regarding the notifier's GRAS conclusion. For reference, the following cumulative dietary exposures, based on food consumption data from the 2017-2018 NHANES, were presented in GRN 001051: mean and 90th percentile dietary exposures to 2′-FL for infants 0 to 6 months of age were estimated to be 2.4 and 4.4 g/p/d (360 and 578 mg/kg bw/d), respectively; mean and 90th percentile dietary exposures to 2′-FL for infants 7 to 12 months of age were estimated to be 4.3 and 7.7 g/p/d (474 and 812 mg/kg bw/d), respectively; mean and 90th percentile dietary exposures to 2′-FL for the U.S. population aged 2 years and older were estimated to be 4.2 and 9.1 g/p/d (65 and 146 mg/kg bw/d), respectively, and mean and 90th percentile dietary exposures to 2′-FL for children 1 to 2 years of age to be 2.9 and 5.7 g/p/d (237 and 477 mg/kg bw/d), respectively.

[7] 2′-FL (referred to as 2′-O-fucosyllactose (2′-O-FL) in earlier GRNs) for use in non-exempt infant formula for term infants, and in multiple additional food categories including infant and toddler foods, is the subject of GRNs 000546, 000571, 000650,000749, 000815, 000852, 000897, 000929, 001014, and 001034. We responded in letters dated September 16, 2015, November 6, 2015, November 23, 2016, April 23, 2018, August 20, 2019, November 15, 2019, June 12, 2020, February 26, 2021, July 15, 2022, and October 21, 2022, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

concludes that 2′-FL has low allergenic potential.

Based on the totality of the data and information, Synaura concludes that 2′-FL is GRAS for its intended use.

Standards of Identity

In the notice, Synaura states its intention to use 2′-FL in several food categories, including foods for which standards of identity exist located in Title 21 of the CFR. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

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 (OFAS) 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 nine foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, soybeans, and sesame) or a food ingredient that contains protein derived from one of those foods. 2′-FL derived from lactose may require labeling under the FD&C Act because it may contain protein derived from milk. Questions about petitions or notifications for exemptions from the food allergen labeling requirements should be directed to the Division of Food Ingredients in OFAS. Questions related to food labeling in general should be directed to ONFL in the Center for Food Safety and Applied Nutrition.

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 Synaura'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 in the Center for Food Safety and Applied Nutrition.

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 Synaura'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 Synaura provided, as well as other information available to FDA, we have no questions at this time regarding Synaura'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 001157 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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