U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Wing Yu
CIRS Group USA, Inc.
4250 Fairfax Drive
Suite 600
Arlington, VA 22203

Re: GRAS Notice No. GRN 001274

Dear Ms. Yu:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001274. We received the notice you submitted on behalf of Tianjin Hesheng Biotechnology Co., Ltd. (HS SynBio) (Tianjin) on April 30, 2025, and filed it on August 11, 2025. Tianjin submitted an amendment to the notice on October 3, 2025, that clarified the manufacturing, specifications, and aspects of the safety narrative.

The subject of the notice is 2′-fucosyllactose (2′-FL) for use as an ingredient in non-exempt infant formula[1] for term infants and formula for young children (>12 months) at a maximum use level of 2.4 g/L as consumed, and in other food categories at the maximum levels shown in Table 1. Tianjin states that 2′-FL is not intended for use in products under the U.S. Department of Agriculture's jurisdiction, alcoholic beverages, and in foods for which standards of identity do not permit its addition. The notice informs us of Tianjin's view that these uses of 2′-FL are GRAS through scientific procedures.

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

Food Categories	Maximum Use Levels (g/kg or g/L)
Breads and baked goods, gluten-free	48
Carbonated beverages	1.2
Enhanced or fortified waters	1.2
Sports, isotonic, and "energy" drinks	6
Hot breakfast cereals, prepared	31
Ready-to-eat (RTE) cereals, puffed	80
RTE cereals, high fiber	40
RTE cereals, biscuit-type	40

[1] Tianjin states that the use of 2′-FL in non-exempt infant formula is not restricted to any specific protein base (e.g., cow milk-based, soy-based).

Coffee and tea[2]	10
Milk substitutes	1.2
Beverage whiteners (powdered)	600
Beverage whiteners (liquid)	80
Non-dairy yogurt	12
Frozen dairy-based desserts	17
Puddings, custards, and mousses	17
Fruit pie filling	14.1
Fruit filling in bars, cookies, yogurt, cakes	30
Hot cereals for infants and young children, prepared (from dry instant) and ready-to-serve	12
Other foods for infants and young children (yogurt, fruits, vegetables, "toddler" meals, desserts)	12
Other drinks for infants and young children (juice and yogurt drinks)	10
Infant snacks: crackers, pretzels, cookies, and other dry snack items	57
Jams, jellies, preserves, and fruit butters	60
Meal replacement bars, general use	30
Cereal bars, including snack, granola, and breakfast bars	30
Meal replacement bars for weight management	40
Meal replacement drinks (including nutritional drinks, smoothies) for general use, milk and non-milk based	5
Meal replacement drinks for weight management, milk and non-milk based	12
Milk-based meal replacement beverages for children (e.g., pediatric nutritional drinks)	12
Unflavored pasteurized and sterilized milk	1.2
Buttermilk	1.2
Flavored and fermented milks	1.2
Yogurt	12
Fruit juices and nectars	1.2
Fruit-flavored drinks and ades	1.2
Vegetable juices and nectars	1.2
Sugar substitutes: table-top sweeteners	300
Syrups used to flavor milk beverages	7
Nutritional drinks for pregnant women	12
Oral and enteral tube feeding formulas for ages ≥11 years	20

Tianjin provides information on the identity and composition of 2′-FL. Tianjin describes 2′-FL as a white to off-white powder containing a minimum of 94% 2′-FL (dry-weight basis (DW)). Tianjin notes that 2′-FL is a trisaccharide consisting of L-fucose, D-

[2] The category of coffee and tea includes ready-to-drink (e.g., bottled, flavored, presweetened) coffee and tea and powder mixes used to prepare coffee and tea. For dietary exposure estimates, it is assumed that the intended uses of 2′-FL do not include plain brewed coffee or tea.

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. Tianjin states that 2′-FL is chemically and structurally identical to 2′-FL in human milk, as confirmed by nuclear magnetic resonance spectroscopy, low-resolution mass spectrometry, and high-performance liquid chromatography.

Tianjin describes the *Escherichia coli* BL21 (DE3) H3768 production organism used in the manufacturing of 2′-FL. The production organism is constructed through genetic engineering of the *E. coli* BL21 (DE3) host strain by inserting eight genes, a promoter, and completely or partially deleting 12 genes to enable efficient production of 2′-FL from lactose. Tianjin states that all genetic modifications are verified by whole genome sequencing, and the strain is non-pathogenic and non-toxigenic.

Tianjin describes the two-stage manufacturing process, including fermentation and purification. In the first stage, 2′-FL is produced from D-lactose and D-glucose during fermentation with *E. coli* BL21 (DE3) H3768 under controlled conditions. After fermentation is complete, the production organism is inactivated and removed using a plate-and-frame filtration method. The clarified fermentation liquid is subjected to a series of purification steps, such as sequential membrane filtration, membrane concentration, and activated carbon filtration to remove proteins, lipopolysaccharides, macromolecular polysaccharides, nucleic acids, and other impurities. The resulting liquid is concentrated by evaporation to yield crude 2′-FL. The crude 2′-FL is dissolved in water and concentrated under vacuum to obtain a crystalline product that is further purified by recrystallizing in water, followed by washing with ethanol solutions, air drying, and sieving to produce the final 2′-FL powder. Tianjin states that 2′-FL is manufactured according to current good manufacturing practices, and all raw materials and processing aids are food-grade and are used in accordance with applicable U.S. regulations, are GRAS for their intended uses, or are the subject of an effective food contact notification.

Tianjin provides specifications for 2′-FL, which include the minimum content of 2′-FL (≥94% DW) and limits on D-lactose (≤3% DW); 3,2′-difucosyl-D-lactose (≤2% DW); L-fucose (≤1% DW); 2′-fucosyl-D-lactulose (≤1% DW); D-glucose (≤3% DW); D-galactose (≤3% DW); moisture (≤5%); residual proteins (≤100 mg/kg); residual ethanol (≤500 mg/kg); ash (≤0.5%); pH (3.0-7.5); heavy metals, including lead (≤0.05 mg/kg); and microorganisms, including *Salmonella* serovars (absent in 25 g), *Listeria monocytogenes* (absent in 25 g) and *Cronobacter sakazakii* (absent in 100 g). Tianjin provides the results from three non-consecutive batch analyses to demonstrate that 2′-FL can be manufactured to meet the specifications.

Tianjin states that the intended uses of 2′-FL are substitutional for those described in GRN 001051[3] and incorporates into the notice those dietary exposure estimates. Tianjin states that the estimated dietary exposure to 2′-FL is 2.4 g/person (p)/d (360 mg/kg body weight (bw)/d) at the mean and 4.4 g/p/d (578 mg/kg bw/d) at the 90th percentile

[3] 2′-FL was the subject of GRN 001051. We evaluated this notice 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 infants aged 0-6 months, and 4.3 g/p/d (474 mg/kg bw/d) at the mean and 7.7 g/p/d (812 mg/kg bw/d) at the 90th percentile for infants aged 6-12 months. For children aged 1-2 years, the estimated dietary exposure to 2′-FL is 2.9 g/p/d (237 mg/kg bw/d) and 5.7 g/p/d (477 mg/kg bw/d) at the mean and 90th percentile, respectively. For the U.S. population aged 2 years and older, dietary exposure to 2′-FL is 4.2 g/p/d (65 mg/kg bw/d) and 9.1 g/p/d (146 mg/kg bw/d) at the mean and 90th percentile, respectively. Tianjin notes that the intended uses are substitutional for the current uses of other sources of 2′-FL, and an increase in the cumulative dietary exposure to 2′-FL is not expected.

Tianjin provides data and information supporting the safe use of 2′-FL and states that an updated literature search through February 2025, did not identify information that would contradict a safety conclusion. Tianjin incorporates into the notice information relevant to the absorption, distribution, metabolism, and excretion (ADME) of 2′-FL discussed in prior notices, including GRNs 001091 and 001060.[4] Tianjin concludes that 2′-FL produced by microbial fermentation is identical to 2′-FL present in human milk, and that there is reasonable certainty that its digestion and ADME profile will align with the physiological behavior of 2′-FL found in human milk. Tianjin also states that the safety of 2′-FL is supported by two bacterial reverse mutation tests, an *in vitro* mammalian micronucleus tests, and an *in vitro* mammalian cell gene mutation test that establish the lack of genotoxic potential of similar 2′-FL ingredients. Tianjin further maintains that a 90-day, repeated-dose oral toxicity study in Wistar rats is pivotal in establishing a lack of adverse health effects from repeated exposure to 2′-FL in the diet at levels up to 10% (equivalent to 7250 and 7760 mg/kg bw/d in males and females, respectively) (van Berio *et al*, 2018). Tianjin states that the toxicological study by van Berlo *et al*. was conducted using a 2′-FL test article that was similar in identity, purity, and composition to the subject of this notice. Tianjin also discusses the results of unpublished toxicology studies and human studies that were included as corroborative evidence for the safety of 2′-FL.

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

Standards of Identity

In the notice, Tianjin 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, & Cosmetic (FD&C) Act, a food is misbranded if its labeling is false or misleading in any way. Section 403(r) of the FD&C

[4] 2′-FL was the subject of GRNs 0001091 and 0001060. We evaluated these notices and responded in letters dated December 01, 2023, and April 04, 2023, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

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 Nutrition Center of Excellence. The Office of Pre-Market Additive Safety (OPMAS) 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 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 OPMAS. Questions related to food labeling in general should be directed to ONFL.

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 Tianjin'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 Tianjin'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 Tianjin provided, as well as other information available to FDA, we have no questions at this time regarding Tianjin'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 001274 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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