Joeri Beauprez, Ph.D.
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BELGIUM

Re: GRAS Notice No. GRN 001091

Dear Dr. Beauprez:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001091. We received Inbiose N.V. (Inbiose)'s notice on March 15, 2022, and filed it on December 5, 2022. Inbiose submitted an amendment to the notice on March 17, 2023, that revised the specifications and dietary exposure and provided additional details about the intended uses, self-limiting nature, and literature search.

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

Table 1: Intended food categories and use levels for 2′-FL[2,3]

Food Categories	**Maximum use levels (g/kg or g/L)**
Non-dairy smoothies & meal replacement beverages	5
Sports, isotonic, and "energy" drinks	1.2
Enhanced or fortified waters	1.2
Hot cereals	31
Ready-to-eat (RTE) cereals - puffed	80
RTE cereals – high fiber	40
RTE cereals – biscuit type	40

[1] Inbiose states that the use of 2′-FL in infant formula is not restricted to any specific protein base (e.g., cow milk-based, soy-based).
[2] Inbiose states that 2′-FL is not intended for use in foods for which standards of identity do not permit its addition.
[3] Inbiose states that the intended uses of 2′-FL are substitutional for those described in GRNs 000735 and 000897, combined. 2′-FL was the subject of GRNs 000735 and 000897; we evaluated these notices and responded in letters dated April 6, 2018, and June 12, 2020, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

Food Categories	Maximum use levels (g/kg or g/L)
Milk substitutes such as soy milk and imitation milks	1.2
Non-dairy yogurts	12
Frozen desserts including ice creams and frozen yogurts, frozen novelties	17
Dairy-based puddings, custards and mousses	17
Fruit pie filling	14.1
"Fruit prep" such as fruit filling in bars, cookies, yogurt and cakes	30
Cereal bars, nutrition bars, and meal replacement bars	30
Non-exempt infant formulas for term infants	2.4
Formula intended for young children (>12 months)	2.4
Other foods for infants and young children	12
Yogurt and juice beverages for infants and young children	10
Baby crackers, pretzels, cookies, and snack items	57
Jellies and jams, fruit preserves, and fruit butters	60
Unflavored pasteurized and sterilized milk	1.2
Fermented and flavored milk	1.2
Dairy smoothies and meal replacement beverages	5
Yogurt	12
Nutritional drinks for pregnant women	6
Fruit juices, drinks, and nectars	1.2
Vegetable juices	1.2
Syrups used to flavor milk beverages	7
Enteral and oral tube-feeding formulas intended consumers ≥11 years of age	20

Inbiose describes the identity and composition of 2′-FL, stating that 2′-FL is a white powder containing a minimum of 94% 2′-FL, as well as small quantities of lactose and other related carbohydrates. Inbiose 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-glucopyranose (CAS Registry Number 41263-94-9). Inbiose states that 2′-FL is chemically and structurally identical to the 2′-FL in human milk.

Inbiose describes the production organism used in the manufacture of 2′-FL. The production organism, *Escherichia coli* strain INB-2FL_03, is genetically engineered from the parent strain *E. coli* K-12 MG1655 to produce 2′-FL. Inbiose constructed the production organism using gene knockouts, gene insertions, and the addition of a production plasmid. Inbiose lists five gene insertions that encode functions for sugar

metabolism derived from four donor species to produce 2′-FL.[4] Inbiose states that the production organism is non-pathogenic and non-toxigenic and that all gene insertions and deletions were verified by polymerase chain reaction, Sanger sequencing, and whole genome sequencing.

Inbiose states that 2′-FL is produced in accordance with current good manufacturing practices. First, the production organism is inoculated into a fermentation medium that contains lactose and a carbon source (sucrose), which results in the secretion of 2′-FL into the medium. After fermentation is complete, the fermentation medium is pasteurized and the remaining intracellular 2′-FL is released. The resulting mixture is subjected to successive microfiltration and ultrafiltration steps to remove the production organism, cell components and large molecules (i.e., protein, DNA, lipopolysaccharides). The 2′-FL is then further purified and concentrated by a series of steps that include ion exchange chromatography, nanofiltration, decolorization (activated charcoal), evaporation, sterile filtration, and drying. Inbiose states that all raw materials, processing aids, and food contact articles are used in accordance with U.S. regulations, have previously been determined to be GRAS, or are the subject of an effective food contact notification for their respective uses.

Inbiose provides specifications for 2′-FL, which include the minimum content of 2′-FL (≥94% on a dry matter basis (DM)) and limits on lactose (≤5% DM), difucosyllactose (≤5% DM), sum of other carbohydrates (≤5% DM)[5], moisture (≤5%), heavy metals, including lead (≤0.05 mg/kg) and arsenic (≤0.1 mg/kg), residual proteins (≤100 µg/g), ash (≤0.5%), and microorganisms, including *Salmonella* serovars (absent in 25 g) and *Cronobacter* spp. (absent in 25 g). Inbiose provides the results from the analyses of three non-consecutive batches to demonstrate that 2′-FL can be manufactured to meet these specifications.

Inbiose discusses the results of stability studies conducted with 2′-FL and summarized in prior GRAS notices (e.g., GRN 000546, 000735, and 000749).[6] Inbiose states that the 2′-FL that is the subject of this notice is compositionally similar to the articles tested in these studies and therefore, is expected to have similar stability. The studies include evaluation of the stability of 2′-FL under real-time (25 °C, 60% relative humidity (RH)) and accelerated (40 °C, 75% RH) storage conditions and as a component of powdered infant formula stored under various temperatures. Inbiose concludes that these studies demonstrate that 2′-FL is stable under the tested storage conditions and is expected to be stable under the conditions of its intended use.

Inbiose provides estimates of dietary exposure to 2′-FL based on the intended uses in

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[4] Inbiose states that the production organism contains genes that encode lactose permease, sucrose permease, sucrose phosphorylase, fructokinase, and α(1,2)-fucosyltransferase.

[5] Inbiose states that the specification for the sum of other carbohydrates includes the sum of 3-fucosyllactose, 2-fucosyl-D-lactulose, fucosyl-galactose, glucose, galactose, fucose, sorbitol, galactitol, mannitol, and trihexose.

[6] 2′-FL was the subject of GRNs 000546 and 000749. We evaluated these notices and responded in letters dated September 16, 2015, and April 23, 2018, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

Table 1 and food consumption data from the 2017-2018 National Health and Nutrition Examination Survey. Inbiose estimates the eaters-only dietary exposures to 2′-FL for infants up to 6 months of age to be 2.2 g/person (p)/day (d) (324 mg/kg body weight (bw)/d) at the mean and 3.8 g/p/d (499 mg/kg bw/d) at the 90th percentile, and for infants 7 to 12 months of age to be 3.4 g/p/d (378 mg/kg bw/d) at the mean and 6.3 g/p/d (662 mg/kg bw/d) at the 90th percentile. In addition, Inbiose estimates the eaters-only dietary exposures to 2′-FL for the U.S. population aged 2 years and older to be 2.6 g/p/d (44 mg/kg bw/d) at the mean and 5.6 g/p/d (103 mg/kg bw/d) at the 90th percentile.[7]

Inbiose incorporates into the notice publicly available safety data previously discussed in GRAS notices for 2′-FL, including GRNs 000546, 000571, 000735 and 000932[8]. Inbiose states that 2′-FL produced via fermentation is structurally identical and therefore physiologically equivalent to 2′-FL in human milk in terms of absorption, distribution, metabolism, and excretion. Furthermore, Inbiose states that, despite differences in the manufacturing process, 2′-FL ingredients are compositionally highly similar, and data and information from safety studies conducted with any of these ingredients are generally applicable to all 2′-FL ingredients. Inbiose discusses published toxicological studies and human studies of 2′-FL from other sources and concludes that there was no evidence of toxicity. Inbiose also discusses newly identified published studies since the review of GRN 000932. This included toxicological studies of 2′-FL in combination with other human milk oligosaccharides (HMOs) in neonatal piglets and rats, and studies in infants with 2′-FL alone or in combination with other HMOs.

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[7] The uses of 2′-FL described in GRN 001091 are intended to be substitutional for previously notified uses; however, the uses summarized in Table 1 and considered in the notifier's estimates of dietary exposure do not include some intended uses evaluated in recent GRAS notices for 2′-FL. For example, in GRN 001051, the notifier evaluated additional uses of 2′-FL as well as increased use levels in specific food categories that were introduced in prior GRAS notices and contribute to the overall dietary exposure to 2′-FL in the U.S. The subject of GRN 001051 is 2′-FL and was under evaluation when we filed GRN 001091. 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. Food categories described in prior GRAS notices that are not considered in GRN 001091 include breads and baked goods (gluten-free), carbonated beverages, coffee, tea, beverage whiteners, meal replacement bars for weight management, meal replacement drinks for weight management, meal replacement drinks for children, buttermilk, and tabletop sugar substitutes. The notifier of GRN 001051 provided a cumulative dietary exposure estimate to 2′-FL from all uses based on food consumption data from the 2017-2018 NHANES. Estimates of the cumulative mean and 90th percentile dietary exposures to 2′-FL for infants 0 to 6 months of age were reported to be 2.4 and 4.4 g/p/d (360 and 578 mg/kg bw/d), respectively. The 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. The mean and 90th percentile dietary exposures to 2′-FL for the U.S. population aged 2 years and older are estimated to be 4.2 and 9.1 g/p/d (65 and 146 mg/kg bw/d), respectively, and 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. These estimates of dietary exposure are higher than those presented in GRN 0001091; however, the intended use of 2′-FL described in GRN 001091 is substitutional for other sources of 2′-FL previously determined to be GRAS for their intended uses and the intended uses described in GRN 001091 are not expected to result in an increase in dietary exposure to 2′-FL.

[8] 2′-FL was the subject of GRNs 000571 and 000932. We evaluated these notices and responded in letters dated November 6, 2015, and February 18, 2021, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

Inbiose states that these studies support the safety of 2′-FL for the intended use.

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

Standards of Identity

In the notice, Inbiose states their intention to use 2′-FL in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations (21 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 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.

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 Inbiose'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 Inbiose'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 Inbiose provided, as well as other information available to FDA, we have no questions at this time regarding Inbiose'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 001091 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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