Marta H. Mikš, Ph.D., D.Sc.
Glycom A/S
Kogle Allé 4
2970 Hørsholm
DENMARK

Re: GRAS Notice No. GRN 001060

Dear Dr. Mikš:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001060. We received Glycom A/S (Glycom)'s notice on January 21, 2022, and filed it on June 17, 2022. Glycom submitted amendments to the notice on November 18, 2022, January 23, 2023, February 10, 2023, March 16, 2023, and April 3, 2023, that clarified the intended use, manufacturing, specifications, dietary exposure, 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 for term infants[1] at a maximum level of 2.4 g/L as consumed, and in other food categories at maximum levels shown in Table 1. The notice informs us of Glycom'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]

Food Categories	Serving Size	Maximum use levels (g/serving)	Maximum use levels (g/kg or g/L)
Meal replacement drinks, for weight reduction	240 mL	1.2	5
Sports, isotonic, and energy drinks	360 mL	0.43	1.2
Imitation milk	240 mL	0.28	1.2
Non-dairy yogurt	170 g	0.9	5.3
Formula-type drinks for young children	100 mL	0.24	2.4
Other drinks for young children	120 mL	0.14	1.2
Baby foods for infants and young	7 to 170 g	0.084 – 2.04	12

[1] Glycom 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] Glycom states that 2′-FL is not intended for use in foods for which standards of identity do not permit its addition.

children			
Meal replacement bars, for weight reduction	40 g	1.6	40
Unflavored pasteurized and sterilized milk	240 mL	0.28	1.2
Buttermilk	240 mL	0.28	1.2
Flavored milk	240 mL	0.28	1.2
Yogurt	170 g	0.9	5.3
Fruit juices and nectars	240 mL	0.28	1.2

Glycom describes the identity and composition of 2′-FL, stating that 2′-FL is a white to off-white powder containing a minimum of 85% 2′-FL. Glycom 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. Glycom states that 2′-FL is chemically and structurally identical to 2′-FL in human milk.

Glycom describes the production organism used in the manufacture of 2′-FL. The production organism, *Escherichia coli* K-12 DH1 MDO strain DSM 33313 (*E. coli* K-12 DH1 MDO DSM 33313), is genetically engineered from the parent strain *E. coli* K-12 DH1 MDO MAP1001d[3] to produce 2′-FL. Glycom states that *E. coli* K-12 DH1 MDO DSM 33313 is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen culture collection.

Glycom constructed the production organism by inserting one copy of a heterologous gene encoding a Major Facilitator Superfamily (MFS) into *E. coli* K-12 DH1 MDO MAP1001d. In general, MFS proteins facilitate the movement of nutrients across cell membranes. Glycom states that the synthetic DNA of the MFS gene was codon optimized at the DNA sequence level to increase the expression of the MFS protein in the production organism. Glycom states that *E. coli* K-12 DH1 MDO DSM 33313 is non-pathogenic and non-toxigenic.

Glycom describes the two-stage manufacturing process, noting 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 U.S. regulations or are GRAS for their respective uses. In the first stage of the manufacturing process, 2′-FL is produced from D-lactose and D-glucose during fermentation and is secreted into the fermentation medium. After fermentation is complete, the microbial biomass is removed from the fermentation medium by ultrafiltration and the biomass is deactivated by heating. The second stage of the manufacturing process consists of a series of purification steps. The filtrate is concentrated by additional filtration steps, including nanofiltration to remove minerals and small biomolecules (e.g., amino acids,

[3] GRNs 000650 and 000815 describe the construction of *E. coli* K12 MDO MAP1001d. The subjects of GRNs 000650 and 000815 are 2′-O-fucosyllactose and 2′-fucosyllactose/difucosyllactose, respectively. We responded in letters dated November 23, 2016, and September 11, 2020, respectively, stating that we had no questions at that time regarding Glycom's GRAS conclusions.

carbohydrate metabolites), microfiltration (as needed) for microorganism removal, and ion exchange chromatography or electrodialysis to remove water, minerals, and charged or ionizable molecules. The solution is concentrated by evaporation or nanofiltration and then decolored using activated charcoal and filtration. The solution is then further concentrated by evaporation and/or nanofiltration and drying, without crystallization, to produce an amorphous solid. Glycom analyzes the results of 2′-FL produced with and without crystallization to support its conclusion that, while higher levels of D-lactose and difucosyllactose were observed in the final product, process-related residues (e.g., proteins, DNA) remained below the limits of quantitation without the crystallization step.

Glycom provides specifications for 2′-FL, which include the minimum content of 2′-FL (≥85% on a dry matter basis); sum of 2′-FL, L-fucose, D-lactose, and difucosyllactose (≥92%); and limits on difucosyllactose (≤5%), 2′-fucosyl-D-lactulose (≤1.5%), D-lactose (≤10%), L-fucose (≤1%), sum of other carbohydrates (≤6.0%),[4] moisture (≤7%), lead (≤0.05 mg/kg), residual proteins (≤0.01%), sulphated ash (≤0.8%), and microorganisms, including *Salmonella* serovars (absent in 25 g) and *Cronobacter sakazakii* (absent in 10 g).[5] Glycom provides the results of three non-consecutive batch analyses to demonstrate that 2′-FL can be manufactured to meet the specifications.

Glycom incorporates into the notice information from GRN 000546[6] and estimates the dietary exposure to 2′-FL for non-exempt infant formula for term infants and formula-type drinks for young children, based on the maximum intended use level of 2.4 g/L and food consumption data from the 2009-2010 National Health and Nutrition Examination Survey (NHANES). Glycom estimates the eaters-only mean and 90th percentile dietary exposures to 2′-FL for infants 0 to 6 months of age to be 2.02 and 2.91 g/person (p)/day (d) (332.8 and 535.6 mg/kg body weight (bw)/d), respectively. The mean and 90th percentile dietary exposures to 2′-FL for infants 7 to 12 months of age are estimated to be 1.70 and 2.63 g/p/d (188.9 and 295.8 mg/kg bw/d), respectively.

Glycom states that the intended uses of 2′-FL are substitutional for those described in GRN 000650,[7] and incorporates into the notice the dietary exposure estimates from GRN 000650 for 2′-FL for the total population (all ages) based on all intended uses and food consumption data from the 2011-2012 NHANES. The mean and 90th percentile dietary exposures to 2′-FL for the total population are estimated to be 0.64 and 1.31 g/p/d (20.0 and 33.1 mg/kg bw/d), respectively, and for children 1-3 years of age to be 1.12 and 1.97 g/p/d (84.9 and 146.0 mg/kg bw/d), respectively.

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[4] Other carbohydrates present in minor amounts (~2-3% based on batch analyses) include glucose and thermal degradation products of glucose, 2-fucosyl-galactose, 3-fucosyllactose, lactitol, and other fermentation byproducts.

[5] Glycom's *C. sakazakii* specification is intended for use of 2′-FL in powdered infant formulas with dry-blending. We note that a comparable limit for 2′-FL for use in liquid infant formulas that require a retort step is not needed.

[6] 2′-O-fucosyllactose is the subject of GRN 000546. We responded in a letter dated September 16, 2015, stating that we had no questions at that time regarding Glycom's GRAS conclusion.

[7] In GRN 000650, Glycom incorporated into the notice the estimated dietary exposure to 2′-FL for infants from GRN 000546, whereas for ages >1 year, they revised the dietary exposure estimates to reflect intended uses in only a subset of conventional foods that were described in GRN 000546.

Glycom acknowledges that new uses of 2′-FL, as well as increased use levels in specific food categories, have been introduced in recent GRAS notices and would contribute to the overall 2′-FL dietary exposure in the U.S. Therefore, Glycom provides a cumulative dietary exposure estimate to 2′-FL including uses in this notice and in other food categories from prior GRNs (i.e., 000735, 000815, 000897, 001014),[8] as well as food consumption data from the 2017-2018 NHANES. Glycom estimates the cumulative mean and 90th percentile dietary exposures to 2′-FL for the total population ≥2 years of age to be 3.01 and 6.51 g/p/d (50 and 112 mg/kg bw/d), respectively. Glycom also estimates the cumulative mean and 90th percentile dietary exposures to 2′-FL for infants 0 to 6 months of age to be 2.16 and 3.73 g/p/d (328 and 499 mg/kg bw/d), respectively, and 7 to 12 months of age to be 3.63 and 6.28 g/p/d (401 and 678 mg/kg bw/d), respectively. The cumulative mean and 90th percentile dietary exposures for children 1-2 years of age are estimated to be 2.84 and 5.24 g/p/d (230 and 469 mg/kg bw/d), respectively.

Glycom provides data and information supporting the safe use of 2′-FL and states that a literature search conducted through December 2021 did not identify information that would contradict its safety conclusion. Glycom discusses changes to the specifications for non-crystallized 2′-FL and provides justifications for why these changes will not impact the safety of the ingredient. Additionally, Glycom states that any potential differences in dissolution properties between non-crystallized and crystalline 2′-FL are equalized through food formulation processes such that there will be no difference in the absorption, distribution, metabolism, and excretion profiles of the ingredients. To support the safe use of 2′-FL overall, summaries of published studies are incorporated into the notice from GRNs 000546, 000650, 000815, and 000897, which demonstrate that 2′-FL is not genotoxic and has a low potential for toxicity. To support the safe use of non-crystallized 2′-FL, Glycom provides data from unpublished genotoxicity studies and a 90-day repeated dose toxicity study demonstrating that 2′-FL is non-mutagenic, not clastogenic or aneugenic, and is not associated with any adverse effects at doses up to 5,000 mg/kg bw/d, the highest dose tested, in neonatal rats. Glycom incorporates summaries of clinical studies using 2′-FL into the notice from GRNs 000650, 000815, and 000897 and provides a discussion of recently published clinical studies in infants, children, and adults that did not identify any safety concerns. Glycom states that the compositional changes in 2′-FL do not impact the GRAS conclusion.

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

Standards of Identity

In the notice, Glycom 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

[8] 2′-FL is the subject of GRNs 000735, 000897, and 001014. We responded in letters dated April 6, 2018, June 12, 2020, and July 15, 2022, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

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 Glycom'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 Glycom'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 Glycom provided, as well as other information available to FDA, we have no questions at this time regarding Glycom'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 001060 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.04.04 10:11:40 -04'00'

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