Christoph Röhrig, Ph.D.
Glycom A/S
Kogle Allé 4
2970 Hørsholm
DENMARK

Re: GRAS Notice No. GRN 001035

Dear Dr. Röhrig:

This letter corrects our response letter to GRN 001035 signed on January 19, 2023. The purpose of this revised letter is to correct the name of the production strain in paragraphs 4 and 5 of the original response letter. This error originated from the GRAS notice that was submitted, which stated that the production strain is *Escherichia coli* K-12 DH1 MDO strain MP2176. The correct strain is *Escherichia coli* K-12 DH1 MDO strain MP2173b, which is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) strain collection in Braunschweig, Germany, under deposition number DSM 33417.

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001035. We received Glycom A/S (Glycom)'s notice on October 19, 2021, and filed it on January 28, 2022. Glycom submitted amendments to the notice on May 13, 2022, September 22, 2022, December 22, 2022, and January 12, 2023, that clarified the intended use, manufacturing, specifications, dietary exposure, and reduced the use level for infants and young children >12 months of age.

The subject of the notice is lacto-*N*-fucopentaose I with 2′-fucosyllactose (LNFP-I/2′-FL) for use as an ingredient in non-exempt infant formula for term infants[1] at use levels providing up to 0.8 g LNFP-I/L infant formula as consumed. LNFP-I/2′-FL is also intended for use as an ingredient in other foods at use levels providing LNFP-I up to the maximum levels specified in Table 1.[2] The notice informs us of Glycom's view that these uses of LNFP-I/2′-FL are GRAS through scientific procedures.

[1] Glycom states that the use of LNFP-I/2′-FL in infant formula is not restricted to any specific protein base (e.g., cow milk-based, soy-based, etc.).
[2] Glycom states that LNFP-I/2′-FL is not intended for use in products under the U.S. Department of Agriculture's jurisdiction or in foods for which standards of identity do not permit its addition.

Table 1. LNFP-I/2′-FL food uses and corresponding maximum use levels of LNFP-I.

Foods	Maximum use level LNFP-I (g/kg or g/L)
Non-exempt infant formula for term infants	0.8
Formulas for young children (>12 months)	0.8
Other foods for infants and young children	8.33
Other drinks for young children	1.2
Non-milk-based meal and nutritional beverages	2.0
Sports, isotonic, and "energy" drinks, soft drinks, enhanced or fortified waters	1.0
Meal replacement bars for weight reduction	20.0
Cereal and nutrition bars	20.0
Unflavored pasteurized milk and sterilized milk	1.0
Buttermilk	1.5
Flavored milk	1.5
Milk-based meal replacement and nutritional beverages	2.0
Cultured dairy beverages	1.5
Yogurt	10.0
Fruit drinks and ades	1.0

Glycom describes LNFP-I/2′-FL as a white to off-white powder or powder with agglomerates consisting of a minimum of 50% LNFP-I and 15% 2′-FL (w/w, dry basis (DB)) and containing small amounts of other carbohydrates. LNFP-I is a pentasaccharide composed of L-fucose, D-galactose, D-glucosamine, and a disaccharide D-lactose. The chemical name for LNPF-I is α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-D-glucose (CAS Registry Number 7578-25-8). 2′-FL is a trisaccharide composed 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 (CAS Registry Number 41263-94-9). Glycom notes that LNFP-I and 2′-FL are chemically and structurally identical to LNFP-I and 2′-FL that are present in human milk.

Glycom states that LNFP-I/2′-FL is produced in a single fermentation using *Escherichia coli* K-12 DH1 MDO strain MP2173b. The production strain is genetically engineered from *E. coli* MDO[3] by the introduction of three heterologous genes encoding for enzymes involved in the metabolism of complex carbohydrates. Glycom states that *E. coli* MDO strain MP2173b does not contain antibiotic resistance genes, is non-pathogenic and non-toxigenic, and is deposited in the DSMZ strain collection.

Glycom describes the two-stage manufacturing process for LNFP-I/2′-FL. In the first stage, LNFP-I/2′-FL is produced from D-lactose and D-glucose[4] during the fermentation

[3] Glycom states that the data and information on the construction of *E. coli* K-12 MDO is described in GRN 000650 and is incorporated into this notice. 2′-O-fucosyllactose is the subject of GRN 000650. We evaluated this notice and responded in a letter dated November 23, 2016, stating that we had no questions at that time regarding Glycom's GRAS conclusion.

[4] Glycom states that instead of D-glucose, D-sucrose or glycerol can be used as a carbon and energy source.

of *E. coli* K-12 DH1 MDO strain MP2173b. After fermentation is complete, the microbial biomass is removed from the fermentation medium by ultrafiltration and microfiltration. The second stage of the manufacturing process consists of a series of purification, isolation, and concentration steps. The filtrate is concentrated by nanofiltration, followed by microfiltration and ion exchange chromatography to reduce the water content, and remove minerals and small molecules. The preconcentrated filtrate is then decolorized, subjected to additional nanofiltration and microfiltration and it may be further concentrated by optional evaporation. The concentrate is then dried to obtain the final LNFP-I/2′-FL. Glycom states that LNFP-I/2′-FL is manufactured according to current good manufacturing practice, and that all raw materials, processing aids, and food contact substances are approved by U.S. regulations, are the subjects of effective food contact notifications, or were concluded to be GRAS for their respective uses.

Glycom provides specifications for LNFP-I/2′-FL that include the following: minimum content of LNFP-I and 2′-FL (≥75% w/w DB); sum of specified saccharides (≥90% w/w DB), including LNFP-I (≥50% w/w DB), 2′-FL (≥15% w/w DB), D-lactose (≤10%), lacto-*N*-tetraose (≤5%), difucosyl-D-lactose (≤2%), LNFP-I fructose isomer (≤1.5%), 2′-fucosyl-D-lactulose (≤1%), 3-fucosyllactose (≤1%), and sum of L-fucose and 2′-fucosyl-lactitol (≤1%); other carbohydrates (≤6%); pH (4-7; 20 °C, 5% solution); moisture (≤8%); sulfated ash (≤0.5%); lead (≤0.1 mg/kg); residual proteins (≤0.01%); and limits for microorganisms, including *Salmonella* serovars (absent in 25 g), *Cronobacter sakazakii* (absent in 10 g), *Listeria monocytogenes* (absent in 25 g), and *Bacillus cereus* (≤50 colony forming units/g).[5] Glycom provides the results from six non-consecutive batch analyses to demonstrate that LNFP-I/2′-FL can be manufactured to meet the specifications. Glycom provides the results of real-time, accelerated, and stress and forced stability studies performed on LNFP-I/2′-FL in the solid form and in aqueous solution and states that the substance is stable for at least 3 years.

Glycom estimates the dietary exposure to LNFP-I from the intended uses of LNFP-I/2′-FL based on the maximum use levels of LNFP-I and food consumption data from the 2017-2018 National Health and Nutrition Examination Survey. The mean and 90th percentile eaters-only dietary exposures to LNFP-1 for infants aged 0-6 months are estimated to be 1.01 g/person (p)/day (d) (150 mg/kg body weight (bw)/d) and 2.15 g/p/d (308 mg/kg bw/d), respectively. The mean and 90th percentile eaters-only dietary exposures to LNFP-I for infants aged 7 to <12 months are estimated to be 2.21 g/p/d (246 mg/kg bw/d) and 4.21 g/p/d (503 mg/kg bw/d), respectively. The mean and 90th percentile eaters-only dietary exposures to LNFP-1 for the total U.S. population aged 2 years and older are estimated to be 0.76 g/p/d (13 mg/kg bw/d) and 1.66 g/p/d (29 mg/kg bw/d), respectively. Glycom also provides estimates of dietary exposure to LNFP-I/2′-FL and to 2′-FL from the intended uses of LNFP-I/2′-FL. Based on theoretical maximum use levels,[6] Glycom estimates that the mean and 90th percentile eaters-only

[5] Glycom states that the specification limits for *Cronobacter sakazakii*, *Listeria monocytogenes*, and *Bacillus cereus* are for LNFP-I/2′-FL added during the dry-blending stage of infant formula manufacturing.

[6] Glycom calculates the theoretical maximum use levels for 2′-FL and LNFP-I/2′-FL based on the assumption that LNFP-I/2′-FL contains 50% LNFP-I (i.e., the minimum level that is required to meet the

dietary exposures to 2′-FL are the same as those to LNFP-I, and that the corresponding dietary exposures to LNFP-I/2′-FL are twice as much as those to LNFP-I. Glycom states that the dietary exposure to 2′-FL from the intended uses of LNFP-I/2′-FL is substitutional for other sources of 2′-FL that were the subjects of previous GRAS notices.

Glycom discusses publicly available data and information that support the safe use of LNFP-I/2′-FL. Glycom states that the LNFP-I and 2′-FL components of the final ingredient are structurally identical to their naturally occurring counterparts in human milk, and upon oral exposure, LNFP-I and 2′-FL do not undergo significant digestion in the upper gastrointestinal tract. Glycom states that while small quantities of ingested human milk oligosaccharides (HMO)s are absorbed intact, they are excreted unchanged in the urine; thus, oral ingestion of HMOs, in general, is not considered a safety concern. Glycom discusses published toxicological studies with test articles containing LNFP-I/2′-FL that are representative of the product it intends to commercially market. These studies include a bacterial reverse mutation assay, an *in vitro* mammalian cell micronucleus test in human lymphocytes, and a sub-chronic 90-day repeat dose toxicity study in neonatal Crl:CD(SD) rats by oral gavage. Based on these results, Glycom concludes that LNFP-I/2′-FL is not genotoxic. Additionally, Glycom notes that there were no test article-related, toxicologically relevant adverse effects, even at the highest dose tested. Glycom states that since infants comprise the most sensitive age group, the safe use of LNFP-I/2′-FL can be extended to older age groups consuming similar levels in conventional foods.

Glycom notes that it did not identify any published human studies specifically evaluating LNFP-I/2′-FL consumption. However, Glycom incorporates into the notice human intervention studies with 2′-FL discussed in previous GRNs[7] and concludes that clinical data from randomized controlled studies support the safety and tolerability of 2′-FL.

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

Standards of Identity

In the notice, Glycom states its intention to use LNFP-I/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

established specifications). Therefore, 2′-FL would be present at a theoretical maximum use level of 50%.

[7] Glycom incorporates data and information from GRNs 000546, 000571, 000650, 000735, 000749, 000815, 000852, and 000897 into the notice. We evaluated these notices and responded in letters stating that we had no questions at that time regarding the notifiers' GRAS conclusions. These letters are available on our GRAS Notice inventory at https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices.

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 LNFP-I/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 (effective January 1, 2023)) or a food ingredient that contains protein derived from one of those foods. LNFP-I/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 LNFP-I/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 LNFP-I/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 LNFP-I/2′-FL. Accordingly, our response should not be construed to be a statement that foods containing LNFP-I/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 LNFP-I/2′-FL is GRAS under its intended conditions of use. This letter is not an affirmation that LNFP-I/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 001035 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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Date: 2023.03.01 12:00:10 -05'00'

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