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

Re: GRAS Notice No. GRN 001037

Dear Dr. Röhrig:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001037. We received Glycom A/S (Glycom)'s notice on November 8, 2021, and filed it on February 11, 2022. Glycom submitted amendments to the notice on June 17, 2022, and October 4, 2022, that reduced the intended use level in formula for young children (>12 months), revised the dietary exposure assessment, and provided clarification on the microbial specifications, the analytical methods and results from batch analyses, and the construction and safety of the production organism.

The subject of the notice is 3-fucosyllactose (3-FL) for use as an ingredient in non-exempt infant formula for term infants[1] at a level up to 0.75 g/L as consumed; formula and drinks for young children (>12 months) at levels up to 0.90 g/L as consumed; foods for infants and young children at levels up to 6.25 g/kg; and in the following foods at maximum levels ranging from 1.25 to 25 g/kg: meal replacement and nutritional beverages; "sports", "isotonic", and "energy" drinks, soft drinks, and "enhanced" or "fortified" water; meal replacement bars; cereal and nutrition bars; unflavored and flavored milk; buttermilk; yogurt drinks and beverages containing live microorganisms; yogurt; and fruit drinks and ades. The notice informs us of Glycom's view that these uses of 3-FL are GRAS through scientific procedures.

Glycom describes 3-FL as a white to off-white-colored powder or agglomerates containing ≥87 % 3-FL and small amounts of D-lactose and L-fucose. The chemical name for 3-FL is *O*-6-deoxy-α-L-galactopyranosyl-(1→3)-*O*-[β-D-galactopyranosyl-(1→4)]-D-glucose (CAS Registry Number 41312-47-4). 3-FL is a trisaccharide composed of L-fucose, D-galactose, and D-glucose units. Glycom states that their 3-FL product is chemically and structurally identical to the 3-FL present in human milk.

Glycom describes the production organism used in the manufacture of 3-FL. The production organism, *Escherichia coli* K-12 DH1 MDO strain DSM 33416, is genetically

[1] Glycom states that the use of 3-FL in infant formula is not restricted to any specific protein base (e.g., cow milk-based, soy-based, etc.).

engineered from the parent strain *E. coli* K-12 DH1 strain DSM 4235, to produce 3-FL.[2] Glycom constructed the production organism by replacing a promoter element to optimize production of 3-FL, inserting four expression cassettes containing *de novo* synthesized, codon-optimized (when needed) donor genes encoding enzymes for biosynthesis and extracellular transport of 3-FL from two donor species into the genome of the production organism, and deleting four genes to inhibit synthesis of unwanted mixed-acid metabolites. Glycom states that all gene insertions were verified by whole genome sequencing and colony polymerase chain reaction. Glycom states that the production organism does not contain any plasmids, vectors, or antibiotic resistance genes, and that it is stable through 50 generations as determined by whole genome sequencing. Glycom states that *E. coli* K-12 DH1 MDO strain DSM 33416 is non-pathogenic and non-toxigenic and is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) strain collection in Braunschweig, Germany.

Glycom states that 3-FL is manufactured in two stages. In the fermentation stage, the production organism is grown in a medium containing D-glucose as an energy and carbon source and D-lactose as a substrate for the synthesis of 3-FL. In the post-fermentation stage, the secreted 3-FL is isolated, purified and concentrated via a series of ultrafiltration, deionization, decolorization, sterile filtration, and drying steps. Glycom states that crystallization in acetic acid may be optionally employed to further remove carbohydrate impurities. Glycom states that all materials used in the manufacturing processes are food-grade and authorized for their respective uses in the U.S., and that 3-FL is manufactured following current good manufacturing practices.

Glycom provides specifications for 3-FL that include the minimum content of 3-FL (≥87 %) and limits for D-lactose (≤5 %), L-fucose (≤1 %), 3-fucosyl-lactulose (≤1.5 %), other carbohydrates (≤5 %), protein (≤0.01 %), ash (≤0.5 %), moisture (≤6 %), lead (≤0.1 mg/kg), and limits for microorganisms, including *Salmonella* serovars (absent in 25 g).[3] Glycom provides the results from the analysis of eight batches to demonstrate that 3-FL can be manufactured to meet the specifications. Glycom states that based on stability studies, 3-FL is expected to be stable over its intended shelf life of five years and under its intended conditions of use.

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[2] Glycom states that the parent strain *E. coli* K-12 DH1 strain DSM 4235 was optimized for general oligosaccharide expression, as described in GRN 000650, which Glycom incorporates into their notice. In GRN 001037, Glycom made further modifications to optimize 3-FL production, resulting in the production organism, *E. coli* K-12 DH1 MDO strain DSM 33416. The subject of GRN 000650 is 2′-FL. We evaluated GRN 000650 and the supplement to GRN 000650, and responded in letters dated November 23, 2016, and September 11, 2020, respectively, stating that we had no questions at that time regarding the notifiers GRAS conclusions.

[3] Glycom provides specifications for microorganisms for 3-FL that is intended for use in infant formula and formula for young children (>12 months) added during the wet-mix stage of the formula manufacturing process (i.e., prior to retort), as well as use in conventional food. Glycom provides additional specifications for microorganisms for 3-FL that is intended for addition during the dry blend stage of infant formula that include limits for *Cronobacter* spp. (absent in 10 g), *Listeria monocytogenes* (absent in 25 g), and *Bacillus cereus* (≤50 colony forming units (CFU)/g).

Glycom provides estimates of dietary exposure to 3-FL based on their intended uses, uses previously notified as GRAS in GRN 000951,[4] and food consumption data from the 2017-2018 National Health and Nutrition Examination Survey. Glycom estimates the mean and 90th percentile eaters-only dietary exposures to 3-FL for infants aged 0 to 6 months to be 0.87 and 1.72 g/person (p)/d (130 and 247 mg/kg body weight (bw)/d), respectively, and for infants aged 7 to 12 months to be 1.99 and 3.51 g/p/d (221 and 413 mg/kg bw/d), respectively. Glycom reports the mean and 90th percentile eaters-only dietary exposures for children aged 1 to 2 years to be 1.52 and 2.46 g/p/d (124 and 211 mg/kg bw/d), respectively. Glycom reports the mean and 90th percentile eaters-only dietary exposures to 3-FL for the total U.S. population 2 years of age and older to be 1.29 and 2.57 g/p/d (22 and 49 mg/kg bw/d), respectively.

Glycom provides data and information supporting the safety of 3-FL and states that a literature search conducted through August 2021 did not identify any published studies which suggest that 3-FL is unsafe as a food ingredient. Glycom states that their 3-FL is structurally identical to its naturally occurring counterpart in human milk, and human milk oligosaccharides (HMOs), such as 3-FL, do not undergo significant digestion in the upper gastrointestinal tract and are only absorbed in small quantities. Glycom notes the absorption of 3-FL added to infant formula would not be different than absorption of 3-FL from human milk. Glycom provides a discussion of a published safety study using 3-FL from another manufacturer as well as an unpublished study[5] on Glycom's 3-FL ingredient. In these reports, 90-day subchronic oral toxicity studies in rats and genotoxicity tests did not demonstrate any toxicologically relevant effects. Glycom summarizes the results of a clinical study in which formula provided to infants was supplemented with an HMO mixture containing 3-FL. Glycom reports that the HMO-supplemented formula supported normal infant growth and was safe and well-tolerated. Glycom notes that recent scientific opinions from the European Food Safety Authority support the general recognition of safety for the use of 3-FL in infant formula.

Glycom includes the statement of a panel of individuals (Glycom's GRAS panel). Based on their review, Glycom's GRAS panel concluded that 3-FL is safe under the conditions of its intended use.

Based on the totality of the data and information, Glycom concludes that 3-FL is GRAS for its intended use.

Standards of Identity

In the notice, Glycom states their intention to use 3-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

[4] 3-FL is the subject of GRN 000951. We evaluated GRN 000951 and responded in a letter dated August 12, 2021, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

[5] During the course of our review, this study was published as Phipps KR, Lozon D, Stannard DR, et al. Neonatal subchronic toxicity and in vitro genotoxicity studies of the human-identical milk oligosaccharide 3-fucosyllactose. *J Appl Toxicol*. 2022;42(10):1671-1687. doi:10.1002/jat.4335.

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 3-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. 3-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 3-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 3-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 3-FL. Accordingly, our response should not be construed to be a statement that foods containing 3-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 3-FL is GRAS under its intended conditions of use. This letter is not an affirmation that 3-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 001037 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2022.11.07 17:32:58 -05'00'

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Director
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