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

Re: GRAS Notice No. GRN 001034

Dear Dr. Röhrig:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001034. We received Glycom's notice on September 27, 2021 and filed it on February 9, 2022. Glycom submitted amendments to the notice on July 7, 2022 and September 26, 2022 that narrowed the targeted population to only term infants with cow milk protein allergy (CMPA) and provided additional information on the manufacturing process, batch analyses, intended use and use level, and dietary exposure.

The subject of the notice is 2′-fucosyllactose (2′-FL) for use as an ingredient in exempt hypoallergenic extensively hydrolyzed cow milk or goat milk protein- and amino acid-based infant formula for term infants with CMPA at a level of 2.4 g/L infant formula as consumed.[1] The notice informs us of Glycom's view that these uses of 2′-FL are GRAS through scientific procedures.

Glycom describes 2′-FL as a white to off-white powder or powder with agglomerates that contains a minimum of 94% 2′-FL, 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-glucopyranose (CAS Registry Number 41263-94-9). Glycom notes that 2′-FL is qualitatively identical to 2′-FL that is present in human milk.

Glycom states that the two-stage manufacturing process for 2′-FL is described in GRN 000650[2] and that the production organism, *Escherichia coli* K-12 DH1 MDO strain DSM 32775, is described in the supplement to GRN 000650.[3] As described in the supplement to GRN 000650, *E. coli* K-12 DH1 MDO strain DSM 32775 is genetically engineered from *E. coli* K-12 MDO strain DSM 32197[4] by incorporating plasmids that

[1] Our evaluation of GRN 001034 was limited to the CMPA infant population only.
[2] The subject of GRN 000650 is 2′-FL manufactured using *E. coli* K-12 DH1 MDO strain SCR6. We evaluated GRN 000650 and responded in a letter dated November 23, 2016, stating that we had no questions at that time regarding the notifier's GRAS conclusion.
[3] The subject of the supplement to GRN 000650 is 2′-FL manufactured using *E. coli* K-12 DH1 MDO strain DSM 32775. We evaluated the supplement to GRN 000650 and responded in a letter dated September 11, 2020, stating that we had no questions at that time regarding the notifier's GRAS conclusion.
[4] Glycom incorporates the information regarding *E. coli* K-12 MDO strain DSM 32197 from GRN 000650.

contain *de novo* synthesized, codon-optimized donor genes *via* homologous recombination at three site-specific loci involved in sugar metabolism. The production strain does not contain any plasmids, vectors, or antibiotic resistance genes and is non-pathogenic and non-toxigenic.

In the first stage of the manufacturing process, 2′-FL is produced from D-lactose and D-glucose[5] during the fermentation of *E. coli* K-12 DH1 MDO strain DSM 32775. After fermentation is complete, the microbial biomass is removed from the fermentation medium by filtration. 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 optional microfiltration and ion exchange or resin adsorption chromatography to reduce water and remove minerals and small molecules. The filtrate is then decolorized using activated charcoal, subjected to microfiltration, and concentrated. Acetic acid is then added to the resulting aqueous solution to initiate the crystallization of 2′-FL. The 2′-FL crystals are separated by filtration, washed with acetic acid to remove any remaining trace impurities, and then dried to obtain the final 2′-FL. Glycom states that 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 2′-FL that include the minimum content of 2′-FL (≥94% on a dry weight basis), limits on D-lactose (≤1%), L-fucose (≤1%), difucosyllactose (≤1%), 2′-fucosyl-D-lactulose (≤1%), moisture (≤5%), acetic acid (≤1% as free acetic acid and/or sodium acetate), sulfated ash (≤1.5%), lead (≤0.05 mg/kg),[6] residual proteins (≤0.002%), β-lactoglobulin (≤0.05 mg/kg), and casein (≤0.5 mg/kg), as well as limits for microorganisms, including *Cronobacter sakazakii* (absent in 10 g) and *Salmonella* serovars (absent in 25 g). Glycom provides the results from three non-consecutive batch analyses to demonstrate that 2′-FL can be manufactured to meet the specifications. Glycom states that based on the stability studies incorporated by reference from GRN 000650, 2′-FL is expected to be stable for at least 5 years when protected from light and stored at room temperature and ambient humidity.

Glycom addresses the potential for residual milk protein in 2′-FL from the use of milk-derived lactose in the fermentation medium by presenting the analytical results from a modified Bradford assay for total residual protein and enzyme-linked immunosorbent assay (ELISA) for milk protein, casein, and β-lactoglobulin. Based on these results, Glycom concludes that the steps included during the manufacturing process are sufficient to ensure the absence of detectable milk protein.

Glycom estimates the dietary exposure to 2′-FL from the intended uses based on the maximum intended use level in hypoallergenic infant formula and food consumption data from the 2011-2012 National Health and Nutrition Examination Survey

[5] Glycom states that alternatively D-sucrose or glycerol can be used as a carbon and energy source instead of D-glucose.
[6] In the amendment received on July 7, 2022, Glycom revised the specification limit for lead from ≤0.1 mg/kg to ≤0.05 mg/kg.

(NHANES). Glycom states that the highest mean and 90th percentile eaters-only dietary exposures to 2′-FL are in infants aged 0-6 months (2.1 g/person (p)/day (d) and 3.2 g/p/d, respectively). The corresponding mean and 90th percentile eaters-only dietary exposures in infants aged 7 to <12 months are estimated to be 1.8 g/p/d and 2.4 g/p/d, respectively. Glycom estimates the cumulative dietary exposure to 2′-FL from the intended uses and current uses in foods that infants may consume (excluding dairy-based foods) using the maximum use levels and food consumption data from the 2017-2018 NHANES. Glycom estimates the eaters-only cumulative dietary exposures for infants aged 0-6 months to be 2.15 g/p/d (326 mg/kg body weight (bw)/d) at the mean and 3.73 g/p/d (499 mg/kg bw/d) at the 90th percentile. The eaters-only cumulative dietary exposures for infants aged 7 to <12 months are estimated to be 3.31 g/p/d (365 mg/kg bw/d) at the mean and 6.02 g/p/d (664 mg/kg bw/d) at the 90th percentile.

Glycom states that crystallized 2′-FL is of high purity, is identical to 2′-FL naturally present in human milk, and has been the subject of multiple safety reviews by various qualified experts and authoritative bodies. Glycom states that they intend to expand the current use and maximum use level of 2′-FL to include exempt infant formula for infants with CMPA. Glycom incorporates data and information characterizing the identity, quality, manufacturing, and safety of their 2′-FL previously discussed in GRN 000650. Glycom discusses several new animal and human studies identified from the results of the updated literature search through August 2021 since the evaluation of the most recent 2′-FL GRAS conclusion notified to the FDA (GRN 000897).[7] Glycom states that none of these new studies showed findings that the 2′-FL is unsafe or unsuitable for the expanded use in exempt infant formula for infants with CMPA. Glycom states that their analytical data demonstrate that their manufacturing process controls are sufficient to ensure allergic milk protein is not detected in the ingredient at levels that would cause an allergenic response that poses a risk to human health. Glycom discusses several published clinical studies in which infants with clinically diagnosed CMPA were fed extensively hydrolyzed formula containing 2′-FL. Glycom concludes that the results of these studies as well as the fact that breastfed infants with CMPA safely consume milk with 2′-FL support their conclusion that the 2′-FL for the intended use is GRAS.

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

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 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

[7] We evaluated GRN 000897 and responded in a letter dated June 12, 2020, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition (CFSAN). 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. 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 the ONFL in CFSAN.

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 in CFSAN.

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 001034 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2022.10.21 16:22:02 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition