Annette Lau
Glycom A/S
Kogle Allé 4
2970 Hørsholm
DENMARK

Re: GRAS Notice No. GRN 000650

Dear Ms. Lau:

The Food and Drug Administration (FDA, we) completed our evaluation of Glycom A/S's (Glycom) supplement to GRN 000650. We received the supplement on July 31, 2019. The supplement addresses a change in the production organism for the production of 2′-*O*-fucosyllactose (2′-FL). Glycom submitted clarifying information on August 3, 2020 and August 18, 2020, which included additional information regarding the identity of the production organism, the genetic stability of the production organism, the manufacturing process, and an updated literature search.

We previously responded to GRN 000650 on November 23, 2016. We stated that we had no questions at that time regarding Glycom's conclusion that 2′-FL is GRAS for use as an ingredient in non-exempt infant formula for term infants at a maximum use level of 2.4 g/L of reconstituted formula; and, in beverages and beverages bases, dairy product analogs, infant and toddler foods including follow-on formulas, grain products and pastas, milk and milk products, and processed fruits and fruit juices at use levels ranging from 0.084 to 2.04 g/serving.[1]

In the supplement dated July 31, 2019, Glycom informs us of its view that 2′-FL is GRAS, through scientific procedures, for the intended uses described in GRN 000650 and describes a change in the organism used for the production of 2′-FL. Glycom states that the genetically engineered, non-pathogenic and non-toxigenic *Escherichia coli* K-12 DH1 MDO strain "SCR6" is replaced with the genetically engineered, non-pathogenic and non-toxigenic *E. coli* K-12 DH1 MDO strain DSM 32775.

In the supplement, Glycom incorporates the description of the genetic engineering of *E. coli* K-12 DH1 MDO strain DSM 32775 from GRN 000815, [2] which describes this strain. Glycom states that the strain is deposited in the strain collection of the Deutsche

[1] Serving sizes are based on Reference Amounts Customarily Consumed as described in 21 CFR 101.12.

[2] The subject of GRN 000815 is 2′-FL and difucosyllactose (DFL). We evaluated GRN 000815 and responded in a letter dated August 20, 2019, stating that we had no questions at that time regarding the notifier's GRAS conclusion. Although the production strain in this supplement is the same as that discussed in GRN 000815, Glycom discusses how the production of DFL can be limited or how DFL can be removed during the manufacture of 2′-FL.

Sammlung von Mikroorganismen und Zellkulturen (DSMZ) in Braunschweig, Germany. Glycom explains that both *E. coli* K-12 DH1 MDO strain "SCR6" and *E. coli* K-12 DH1 MDO strain DSM 32775 are genetically engineered from *E. coli* K-12 DH1 MDO strain DSM 32197.[3, 4] Glycom describes the construction of *E. coli* K-12 DH1 MDO strain DSM 32775 by incorporating plasmids containing *de novo* synthesized, codon-optimized donor genes into the genome of *E. coli* K-12 DH1 MDO strain DSM 32197 via homologous recombination at three site-specific loci involved in sugar metabolism.[5] Glycom states that the production strain does not contain any plasmids, vectors or antibiotic resistance genes, and that it is stable through 56.3 generations as determined by colony polymerase chain reaction. Glycom explains that, as the genes were integrated chromosomally, *E. coli* K-12 DH1 MDO strain DSM 32775 exhibits more stable performance, growth physiology, and provides higher yields of 2′-FL when compared to the plasmid-based *E. coli* K-12 DH1 MDO strain "SCR6."

Glycom states that the manufacturing described in GRN 000650 is in two-stages; it includes upstream (fermentation) and downstream (purification) processing. Glycom explains that changes to the upstream stage of the process include replacement of the production strain and omission of the use of isopropyl-β-D-1-thiogalactoside as an inducing agent during fermentation. Glycom also states that downstream processing is the same as described in GRN 000650.

Glycom states that the specifications for 2′-FL are the same as described in GRN 000650.[6] Glycom provides results from analyses of three non-consecutive lots to demonstrate that 2′-FL can be manufactured to conform with the specifications in GRN 000650, and comply with the purity profile of the batch used in the toxicology study supporting the safety of 2′-FL as described in GRN 000815. Glycom also confirms via sequencing that the genetic modifications do not introduce unknown proteins, and thereby any new potential allergens. Glycom also conducted an updated review of the scientific literature through July 2019 and concludes that the safety of 2′-FL continues to be confirmed and that there is an absence of any reported adverse effects.

Based on the totality of data and information summarized above, Glycom concludes that 2′-FL produced as a result of a proposed change in manufacturing is GRAS for its intended uses.

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[3] Glycom states that the host strain used to engineer *E. coli* K-12 DH1 MDO strain DSM 32197 is *E. coli* K-12 DH1 strain DSM 4235 or ATCC 33849; it is deposited in the culture collection of the DSMZ and the American Type Culture Collection (ATCC), respectively.

[4] Glycom describes the genetic construction of *E. coli* K12 MDO strain DSM 32197 in GRN 000650.

[5] Glycom states that the introduced genes encoding enzymes responsible for the synthesis of 2′-FL are identical in both *E. coli* K-12 DH1 MDO strain "SCR6" and *E. coli* K-12 DH1 MDO strain DSM 32775. Glycom states that no new genes from new donor organisms were introduced into the production strain, and therefore no additional by-products or impurities are expected.

[6] Glycom states that analysis for residual DNA using qPCR demonstrated that DNA from the production strain is not carried over from the fermentation process into the 2′-FL final product.

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 Code of Federal Regulations (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 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 Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition (CFSAN). The Office of Food Additive Safety 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 eight foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. 2′-FL derived from lactose requires labeling under the FD&C Act because it contains protein derived from milk.

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 supplement 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 Federal Food, Drug, and Cosmetic Act (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 supplement 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 the supplement to GRN 000650 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2020.09.11 17:35:25 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
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