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

Re: GRAS Notice No. GRN 000815

Dear Dr. Röhrig:

This letter revises our response letter to GRN 000815 signed on May 7, 2020. The purpose of this revised letter is to include a new Footnote 4 to clarify the limits for Enterobacteriaceae, *Listeria monocytogenes,* and *Cronobacter sakazakii* in paragraph six of our May 7, 2020, letter.

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000815. We received Glycom A/S' (Glycom) notice on September 24, 2018 and filed it on December 4, 2018. Glycom submitted amendments to the notice on January 28, 2019, March 13, 2019, and July 1, 2019, that contained revised specifications, additional information clarifying the specifications, dietary exposure, and safety narrative, and information about the analyses for allergens, respectively.

The subject of the notice is 2'-fucosyllactose/difucosyllactose (2'-FL/DFL), a product of bacterial fermentation, for use as an ingredient in non-exempt infant formulas for term infants at a maximum level of 1.6 grams per liter (g/L); in toddler formula and in other drinks for young children (between 1 and 3 years of age) at 1.2 g/L; in other foods for infants and young children at 10 grams per kilogram (g/kg); and, in beverages and beverage bases, grain products and pastas, milk (whole and skim), and milk products that are conventional food products used by the general population, at levels ranging from 2 to 40 g/kg. The notice informs us of Glycom's view that this use of 2'-FL/DFL is GRAS through scientific procedures.

Our use of the term, "2'-FL/DFL," in this letter is not our recommendation of that term as an appropriate common or usual name for declaring the substance in accordance with FDA's labeling requirements. Under 21 CFR 101.4, each ingredient must be declared by its common or usual name. In addition, 21 CFR 102.5 outlines general principles to use when establishing common or usual names for nonstandardized foods. Issues associated with labeling and the common or usual name of a food ingredient 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 did not consult with ONFL regarding the appropriate common or usual name for "2'-FL/DFL."

Glycom provides information about the identity and composition of 2'-FL/DFL. Glycom

describes 2'-FL/DFL as a white to off-white amorphous powder or agglomerate, comprised of two structurally and biologically related oligosaccharides that are chemically identical to those found in human milk. The main constituent, 2'-FL, is a neutral trisaccharide comprised of one unit of L-fucose, one unit of D-galactose, and one unit of D-glucose. DFL, the second constituent, is a derivative of 2'-FL and is a neutral tetrasaccharide composed of two units of L-fucose, one unit of D-galactose, and one unit of D-glucose. Glycom states that the approximate average weight ratio of 2'-FL to DFL present in 2'-FL/DFL is 7:1. Glycom notes that this ratio is similar to what is found, on average, in human milk, although there is broad natural variation in the ratio of 2'-FL to DFL in human milk.

Glycom describes the two-stage manufacturing process for 2'-FL/DFL. Specifically, Glycom states that the production strain *E. coli* K-12 MAP1001 is genetically-engineered from *E. coli* K12 MDO[1] by incorporating *de novo* synthesized, codon-optimized genes[2] to express proteins that are "carbohydrate-active enzymes," which can metabolize carbohydrates into oligosaccharides that are identical to human milk oligosaccharides. Glycom states that the production strain is nonpathogenic and nontoxigenic. In the first stage, D-lactose[3] is converted to 2'-FL/DFL *via* fermentation with the *E. coli* K-12 MAP1001 strain. In the second stage, a series of purification, isolation, concentration, and drying steps produce the final 2'-FL/DFL product. Glycom notes that 1H- and 2D-NMR spectroscopy and mass spectrometry have confirmed that the 2'-FL and DFL produced are chemically and structurally identical to the 2'-FL and DFL secreted into human milk. Glycom states that 2'-FL/DFL is produced in compliance with current good manufacturing practices.

Glycom provides specifications for 2'-FL/DFL. These include assay (sum of 2'-FL, DFL, 3'-fucosyllactose, lactose, and fucose; water free) (≥92%), assay (sum of 2'-FL and DFL; water free) (≥85%), D-lactose (≤10%), L-fucose (≤1.0%), 2'-fucosyl-D-lactulose (≤2%), other carbohydrates (≤6%), moisture (≤6%), sulphated ash (≤0.8%), residual protein (≤0.01%), and limits for microorganisms (no detectable *Salmonella* and *Listeria* in 25 g samples, and Enterobacteriaceae and *Cronobacter sakazakii* in 10 g samples)[4]. Glycom provides results of five non-consecutive batch analyses to demonstrate that 2'-FL/DFL can be manufactured to meet these specifications. Additionally, Glycom provides data to demonstrate that 2'-FL/DFL is stable for at least 9 months when stored at ambient room temperature.

Glycom provides estimates of dietary exposure to 2'-FL/DFL. Glycom estimates the per capita and consumer-only exposures of 2'-FL/DFL using food consumption data from

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[1] The construction of *E. coli* K12 MDO was described in GRN 650.

[2] Specifically, the colanic acid operon from *E. coli* and a fucosyltransferase-encoding gene from *Helicobacter pylori.*

[3] Lactose is a disaccharide sourced from cow's milk. Glycom states that the lactose used in the production of 2'-FL/DFL may contain traces of cow milk protein.

[4] Glycom's limits for Enterobacteriaceae, *Listeria monocytogenes*, and *Cronobacter sakazakii* are intended for 2'-FL/DFL used in powdered infant formulas. We note that for 2'-FL/DFL used in liquid infant formulas that require a retort step, the limits for *L. monocytogenes* and *C. sakazakii* are not needed and the limit for Enterobacteriaceae would be <10 CFU/g.

the 2013-2014 National Health and Nutrition Examination Survey. Glycom reports that the dietary exposure to 2'-FL/DFL in infants aged 0 to 6 months is 2.42 g/person/day (g/p/d) at the mean and 4.40 g/p/d at the 90th percentile; for infants aged 7 to <12 months, the mean and 90th percentile exposures are 3.50 g/p/d and 6.56 g/p/d, respectively. Similarly, Glycom reports the dietary exposure to 2'-FL/DFL in toddlers aged 1 to 3 years is 1.80 g/p/d at the mean and 3.75 g/p/d at the 90th percentile. Glycom reports that on a consumer-only basis, the mean and 90th percentile exposures of 2'-FL/DFL by the total U.S. population from all intended food uses are estimated to be 1.65 g/p/d and 3.54 g/p/d, respectively. Glycom also states that there is no known self-limiting level of use associated with 2'-FL/DFL.

Glycom states that both 2'-FL and DFL are natural components in human milk, and that the 2'-FL produced by fermentation is identical to the 2'-FL found in human milk and was the subject of prior GRNs.[5] Furthermore, Glycom states the total exposure to 2'-FL from all current and intended uses in infant formula is not expected to change due to the substitutional nature of the intended uses. Additionally, Glycom states that the total exposure estimated from the intended uses in infant formula are below what breastfeeding infants are normally exposed to and are thus safe.

Glycom states that the majority of ingested 2'-FL/DFL reaches the large intestine undigested, where it serves as substrates for gut microflora or are excreted in feces unchanged. Glycom incorporates into GRN 000815, published toxicity studies cited in GRN000546, GRN000571, GRN000650, GRN000735, and GRN000749 and summarizes the key findings from relevant toxicological studies on chemically synthesized or bacterially produced 2'-FL. In addition, Glycom summarizes and discusses published toxicological studies on 2'-FL and DFL at an 8:1 ratio, including a 90-day repeat dose toxicity study in neonatal rats, a bacterial reverse mutation test, and an *in vitro* micronucleus assay. Glycom states that when 2'-FL/DFL was administered daily by gavage to neonatal rats from day 7 of age for 90-days, no test article-related adverse effects were observed at levels up to 5,000 mg/kg bw/d. Glycom also concludes from these studies that 2'-FL/DFL at an 8:1 ratio was not genotoxic or clastogenic. Finally, Glycom discusses several published human studies that showed that infant formula containing 2'-FL was well-tolerated.

Upon conducting an updated literature search pertinent to the safety of 2'-FL through September 2018, Glycom states that they are not aware of any newly published studies suggesting that 2'-FL/DFL would be unsafe for its intended use as a food ingredient. Glycom states that the conclusion of safe exposure for the general population (excluding infants) from their intended uses of 2'-FL/DFL relies on extrapolations of the safety conclusion for infants.

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[5] GRNs 000546, 000571, 000650, 000735, and 000749 describe the use of 2'-fucosyllactose or 2'-O-fucosyllactose in various infant formulas and conventional food categories. FDA evaluated these notices and responded in letters dated September 16, 2015, November 6, 2015, November 23, 2016, April 6, 2018, and April 23, 2018, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

Glycom includes the report of a panel of individuals (Glycom's GRAS panel). Based on its review, Glycom's GRAS panel concluded that 2'-FL/DFL is safe under the conditions of its intended use.

Based on all the available information, Glycom concludes that 2'-FL/DFL is GRAS for its intended use.[6]

Standards of Identity

In the notice, Glycom states its intention to use 2'-FL/DFL in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. 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 Food, Drug and 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). The notice raises a potential issue under these labeling provisions. Glycom cites studies that describe 2'-FL/DFL as having certain health benefits. If products containing 2'-FL/DFL 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 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/DFL 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.

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[6] In the supplement dated March 26, 2020, Glycom states their conclusion that their corrections to the exposure estimates for infants aged 0 to 6 months and 7 to <12 months do not change the GRAS status of 2'FL/DFL as described previously under GRN 000815. Glycom also states their view that other experts qualified by scientific training and experience in food safety evaluation would agree with their conclusions.

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/DFL 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/DFL 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/DFL. Accordingly, our response should not be construed to be a statement that foods containing 2'-FL/DFL, 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/DFL is GRAS under its intended conditions of use. This letter is not an affirmation that 2'-FL/DFL 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

000815 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 17:47:16 -04'00'

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