Marta H. Mikš, Ph.D., D.Sc.
Glycom A/S
Kogle Allé 4
2970 Hørsholm
DENMARK

Re: GRAS Notice No. GRN 000880

Dear Dr. Mikš:

This letter revises our response letter to GRN 000880 signed on February 21, 2020. The purpose of this revised letter is to include a new Footnote 5 to clarify the *Cronobacter sakazakii* specification in paragraph five of our February 21, 2020, response letter.

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000880. We received Glycom A/S (Glycom)'s notice on August 23, 2019, and filed it on October 23, 2019. Glycom submitted amendments to the notice on January 8, 2020, and January 9, 2020, that provided additional information on the intended use, manufacturing, specifications, and evidence presented in the safety narrative.

The subject of the notice is 3'-sialyllactose sodium salt (3'-SL) for use as an ingredient at levels up to 0.2 g/L in cow's milk-based, non-exempt infant formula for term infants;[1] 0.15 g/L in beverages and formula for young children (>12 months of age); 1.25 g/kg in foods for infants and young children; 2.5 g/kg in yogurt; 0.25 g/L in buttermilk and fluid milk (flavored and unflavored); 0.5 g/L in meal replacement drinks; 5 g/kg in meal replacement bars; 2.5 g/kg in cereal and granola bars; and 0.25 g/L in soft drinks, fruit-based drinks, sports drinks, "energy drinks," and enhanced waters. The notice informs us of Glycom's view that these uses of 3'-SL are GRAS through scientific procedures.

Glycom provides information on the identity and composition of 3'-SL (CAS Registry Number 128596-80-5). Glycom describes 3'-SL as a white to off-white amorphous powder or agglomerate that is ≥88% 3'-SL on a dry matter (DM) basis. 3'-SL is a trisaccharide containing N-acetylneuraminic acid (NANA, sialic acid)[2] and lactose.

Glycom describes the two-stage manufacturing process for 3'-SL. First, 3'-SL is produced by fermentation of a pure culture of the genetically engineered production

[1] Glycom states that 3'-SL may be used individually or in combination with other human milk oligosaccharide (HMO) ingredients that were the subjects of previous GRAS notices. Glycom notes that infant formula manufacturers may use different HMO combinations and are ultimately responsible for meeting the requirements of section 412 of the Federal Food, Drug, & Cosmetic (FD&C) Act.
[2] NANA was the subject of GRN 000602. We evaluated this notice and responded in a letter dated February 1, 2016, stating that we had no questions at that time regarding Glycom's GRAS conclusion.

strain *E. coli* K-12 "MAP425"[3] in media containing lactose derived from cow's milk and is secreted into the fermentation media. After fermentation is complete, the 3'-SL is obtained by a series of filtration, deionization, and decolorization steps to remove the production microorganism, water, minerals, and other small molecules, and to concentrate the final 3'-SL product, which is dried to a powder. Glycom states that all processing aids used are food grade and used in accordance with U.S. regulations or are previously determined to be GRAS for their respective uses.

Glycom provides specifications for 3'-SL that include minimum levels of 3'-SL (≥88% on a DM basis) and sodium (2.5 to 4.5%), and limits on D-lactose (≤5%), sialic acid (≤1.5%), 3'-sialyllactulose[4] (≤5%), moisture (≤8%), lead (≤0.1 mg/kg), *Salmonella* serovars (absent in 25 g), and *C. sakazakii* (absent in 10 g).[5] Glycom provides results of five non-consecutive batch analyses to demonstrate that 3'-SL can be manufactured to meet these specifications. Glycom states that the stability of 3'-SL can be calculated to be at least five years when protected from light and stored at room temperature and ambient humidity, based on two-year studies conducted under accelerated conditions.

Glycom provides estimates of dietary exposure to 3'-SL from intended uses in infant formula, conventional foods, and toddler foods using food consumption data from the National Health and Nutrition Examination Survey (2013-2014). Glycom estimates the dietary exposures to 3'-SL for infants up to 6 months of age to be 0.30 g/person (p)/day (d) (44 mg/kg body weight (bw)/d) at the mean and 0.55 g/p/d (75.3 mg/kg bw/d) at the 90th percentile, and for infants 7 to 12 months of age to be 0.44 g/p/d (49.3 mg/kg bw/d) at the mean and 0.82 g/p/d (87.9 mg/kg bw/d) at the 90th percentile. Glycom estimates the dietary exposures to 3'-SL for the total population (consumers only) to be 0.21 g/p/d (4.3 mg/kg bw/d) at the mean and 0.44 g/p/d (8.4 mg/kg bw/d) at the 90th percentile.

Glycom discusses the safety of 3'-SL and states that its 3'-SL is chemically and structurally identical to 3'-SL found in human milk. Glycom notes that the intended use levels of 3'-SL are within reported mean concentrations measured in pooled human milk; therefore, they are not expected to present a safety concern. Glycom provides a general discussion regarding the absorption, distribution, metabolism, and excretion of HMOs, including 3'-SL. Glycom states that 3'-SL does not undergo significant digestion in the upper gastrointestinal tract and that small quantities of ingested HMOs are reported to be absorbed intact and excreted unchanged in the urine.

Glycom summarizes and discusses published toxicological studies on its 3'-SL that include 14-day and 90-day repeat dose toxicity studies in neonatal rats, a bacterial

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[3] *E. coli* K-12 is a non-pathogenic, non-toxigenic, and safe production strain when used in accordance with good manufacturing processes. The *E. coli* K-12 "MAP 425" production strain was derived from *E.* coli K-12 "MDO" described in GRN 000650 by the targeted insertion of α-2,3-sialyltransferase and a gene cluster encoding α-N-acetylglucosame-6-phosphate epimerase, sialic acid synthase, and CMP-Neu5Ac.
[4] Glycom notes that 3'-sialyllactulose is an isomer of 3'-SL that results from the isomerization of the terminal glucose moiety of 3'-SL to fructose.
[5] Glycom's *C. sakazakii* specification is intended for use of 3'-SL in powdered infant formulas. We note that a comparable limit for 3'-SL for use in liquid infant formulas that require a retort step is not needed.

reverse mutation test, and an *in vitro* mammalian cell micronucleus test showing no toxicologically relevant effects. In addition, Glycom discusses published genotoxicity studies and toxicity studies in rats and Beagles, showing no toxicologically relevant effects using 3'-SL manufactured by other methods. Glycom also discusses published tolerability studies in humans using 3'-SL and in neonatal piglets using a combination of 3'-SL and 6'-SL manufactured by other methods to support its safe use. Furthermore, because 6'-SL is a structural isomer to 3'-SL, Glycom discusses published studies on its 6'-SL, as well as 6'-SL from another manufacturer, to support safety.

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

Based on the totality of information discussed above, Glycom concludes that 3'-SL is GRAS for its intended use.

Standards of Identity

In the notice, Glycom states its intention to use 3'-SL 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

Under section 403(a) of the 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'-SL 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 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. 3'-SL 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 notice does not alleviate the responsibility of any infant formula manufacturer that intends to market an infant formula containing 3'-SL 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'-SL 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'-SL. Accordingly, our response should not be construed to be a statement that foods containing 3'-SL, 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'-SL is GRAS under its intended conditions of use. This letter is not an affirmation that 3'-SL 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 000880 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2020.04.13 14:50:30 -04'00'

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