Joeri Beauprez, Ph.D.
Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent
BELGIUM

Re: GRAS Notice No. GRN 001075

Dear Dr. Beauprez:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001075. We received Inbiose N.V. (Inbiose)'s notice on January 24, 2022, and filed it on September 14, 2022. Inbiose submitted an amendment to the notice on January 23, 2023, that clarified the intended use, specifications, and details of the safety studies.

The subject of the notice is 6′-sialyllactose sodium salt (6′-SL) for use as an ingredient in non-exempt infant formula for term infants[1] at a maximum level of 0.4 g/L. 6′-SL will also be used as an ingredient at a maximum level of 0.3 g/L in beverages and formula for young children (>12 months of age); 2.5 g/kg in foods for infants and young children; 5 g/kg in yogurt; 0.5 g/L in buttermilk and fluid milk (flavored and unflavored); 1 g/L in meal replacement drinks; 10 g/kg in meal replacement bars; 5 g/kg in cereal and granola bars; and 0.5 g/L in soft drinks, fruit-based drinks, sports drinks, "energy drinks," and enhanced waters. The notice informs us of Inbiose's view that this use of 6′-SL is GRAS through scientific procedures.

Inbiose provides information on the identity and composition of 6′-SL (CAS Registry Number 157574-76-0). Inbiose describes 6′-SL as a white powder consisting of ≥88% 6′-SL on a dry matter (DM) basis and small quantities of lactose, sialic acid, and other related carbohydrates. 6′-SL is a trisaccharide of *N*-acetylneuraminic acid (NANA, sialic acid) and lactose and has the chemical name *N*-acetyl-α-neuraminic-(2→6)-β-D-galactose-(1→4)-D-glucose sodium salt.

Inbiose describes two production organisms, referred to as strains INB-6SL_01 and INB-6SL_02, used in the manufacturing process. Strain INB-6SL_01 was used in the production of 6′-SL that was the subject of toxicological studies, and Inbiose states that strain INB-6SL_02 is an optimized variant that is derived from the same strain lineage as INB-6SL_01. Inbiose states that both strains are characterized genotypically and phenotypically and result in an equivalent 6′-SL product that meets the same

[1] Inbiose states that the use of 6′-SL in infant formula is not restricted to any specific protein base (e.g., cow milk-based, soy-based, etc.).

specifications; however, only strain INB-6SL_02 will be utilized commercially. The production organisms are genetically engineered from the host strain, *Escherichia coli* K-12 strain MG1655, to produce 6′-SL. Inbiose describes the construction of the production strains that includes multiple gene deletions in the host strain and insertion of *de novo* synthesized genes encoding functions for sugar metabolism derived from donor species to produce 6′-SL. Inbiose states that all gene deletions and insertions were verified by polymerase chain reaction, Sanger sequencing, and whole genome sequencing. In addition to the genomic deletions and insertions, Inbiose states that they introduced a plasmid containing genes for the overexpression of *Pasteurella multocida N*-acylneuraminate cytidylyltransferase and *Photobacterium damselae* sialyltransferase. Inbiose states that the plasmid does not contain antibiotic resistance genes and the organisms are not capable of DNA transfer to other organisms. Finally, Inbiose states that there are no remaining traces of the helper plasmids used in the engineering of the production strain and that there is no trace of the antibiotic marker genes used during construction of the production strain. Inbiose states that *E. coli* K12 is non-pathogenic and non-toxigenic, and the production organisms are deposited in the Belgian Co-ordinated Collections of Micro-organisms in Gent, Belgium, with strain INB-6SL_01 deposited as number LMBP 12505 and strain INB-6SL_02 as number LMBP 12506.

Inbiose describes the method of manufacture for 6′-SL. The production organism is inoculated into a fermentation medium that contains glycerol as a carbon source, lactose, and glucose or sucrose. Inbiose states that the 6′-SL that is produced is secreted into the fermentation medium. After fermentation is complete, the 6′-SL is purified by a series of filtration, deionization, reverse osmosis, nanofiltration, decolorization, and evaporation steps to remove the production organism, DNA, protein, lipopolysaccharides, minerals, water, and other small molecules. The 6′-SL is concentrated and dried to a powder. Inbiose states that all raw materials, processing aids, and medium ingredients are food grade and are used in accordance with U.S. regulations or are previously concluded to be GRAS for their respective uses or have been the subject of an effective food contact notification.

Inbiose provides specifications for 6′-SL that include minimum levels of 6′-SL (≥88% as the sodium salt on a DM basis) and limits for the sum of other carbohydrates (≤10%),[2] sialic acid (≤5%), lactose (≤5%), ash (≤8.5%), sodium (≤4.5%), moisture (≤7%), lead (≤0.05 mg/kg), protein (≤100 μg/g), and microorganisms, including *Salmonella* serovars (absent in 25 g) and *Cronobacter sakazakii* (absent in 10 g). Inbiose provides the results from six non-consecutive batch analyses[3] to demonstrate that 6′-SL can be manufactured to meet the specifications. Inbiose discusses the results of stability studies conducted with 6′-SL described in GRN 000881.[4] Based on the results of an accelerated

[2] Inbiose states that the other carbohydrates present in 6′-SL are predominantly sialic acid and lactose and may also include traces of other carbohydrates, such as *N*-acetylglucosamine, sucrose, sialyllactulose, glucose, and galactose.

[3] The six production batches of 6′-SL include three non-consecutive batches produced with each of the two production organisms.

[4] 6′-SL is the subject of GRNs 000881 and 000922. We evaluated these notices and responded in letters dated April 13, 2020, and April 23, 2021, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

stability study, Inbiose concludes that 6′-SL is stable for at least 5 years when protected from light and stored at room temperature and ambient humidity.

Inbiose discusses the estimated dietary exposures to 6′-SL and incorporates data and information from GRN 000881 into the notice. Inbiose states that the intended uses of 6′-SL are the same as described in GRN 000881; therefore, they do not expect the dietary exposure to change. GRN 000881 provides the estimated dietary exposures to 6′-SL from the intended uses using food consumption data from the 2013-2014 National Health and Nutrition Examination Survey. The eaters-only dietary exposures to 6′-SL for infants up to 6 months of age were estimated to be 0.61 g/person (p)/d (88 mg/kg body weight (bw)/d) at the mean and 1.10 g/p/d (151 mg/kg bw/d) at the 90th percentile, and for infants 7 to 12 months of age to be 0.88 g/p/d (98.7 mg/kg bw/d) at the mean and 1.64 g/p/d (176 mg/kg bw/d) at the 90th percentile. In addition, the dietary exposures to 6′-SL for the total population (all ages, eaters-only) were estimated to be 0.41 g/p/d (8.6 mg/kg bw/d) at the mean and 0.89 g/p/d (16.8 mg/kg bw/d) at the 90th percentile.

Inbiose discusses the safety of 6′-SL, stating that their ingredient produced via fermentation is structurally identical and physiologically equivalent to 6′-SL in human milk. Inbiose further states that the metabolism of 6′-SL is expected to be identical to that of other human milk oligosaccharides (HMOs). Inbiose notes that 6′-SL is not significantly digested in the upper gastrointestinal tract and, once in the large intestine, is metabolized by the intestinal microbiota. As noted above, Inbiose states there is no expected increase in dietary exposure from the intended uses of 6′-SL. Furthermore, Inbiose notes that despite differences in manufacturing, all 6′-SL ingredients are compositionally similar, so the safety data are generally applicable to all 6′-SL ingredients. As such, Inbiose incorporates into the notice all publicly available information discussed in the safety narrative of GRNs 000881 and 000922.[4] Inbiose discusses published toxicological studies of 6′-SL from other sources, including genotoxicity and 90-day repeated dose oral toxicity studies in adult and neonatal rats. Inbiose also discusses published toxicological studies of 6′-SL in combination with other HMOs from other sources to support safety, as well as a study with neonatal piglets that was published since the evaluation on GRN 000922. To support the safety of their ingredient, Inbiose discusses unpublished genotoxicity and oral toxicity studies in neonatal rats with the article of commerce to conclude that 6′-SL is neither genotoxic nor shows signs of adverse toxicity. Inbiose states that while no human studies with 6′-SL alone were found, several human studies with 6′-SL and its isomer, 3′-SL, used in combination with other HMOs were identified. Inbiose concludes from these studies that the intended use of 6′-SL is safe and well-tolerated in infants and adults. Finally, Inbiose states that a literature search through January 2023 did not reveal any publicly available data that would be considered counter to a GRAS conclusion.

Based on the totality of the data and information, Inbiose concludes that 6′-SL is GRAS for its intended use.

Standards of Identity

In the notice, Inbiose states its intention to use 6′-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 Federal Food, Drug, & 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). If products containing 6′-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 (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) or a food ingredient that contains protein derived from one of those foods. 6′-SL 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 Inbiose's GRAS notice does not alleviate the responsibility of any infant formula manufacturer that intends to market an infant formula containing 6′-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 Inbiose's notice concluding that 6′-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 6′-SL. Accordingly, our response should not be construed to be a statement that foods containing 6′-SL, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Inbiose provided, as well as other information available to FDA, we have no questions at this time regarding Inbiose's conclusion that 6′-SL is GRAS under its intended conditions of use. This letter is not an affirmation that 6′-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 001075 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.03.22 16:24:15 -04'00'

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