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

Re: GRAS Notice No. GRN 001074

Dear Dr. Beauprez:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001074. We received Inbiose N.V. (Inbiose)'s notice on January 24, 2022, and filed it on September 30, 2022. Inbiose submitted amendments to the notice on February 15, 2023, March 27, 2023, and March 29, 2023, that clarified the intended use, use level, manufacturing, specifications, dietary exposure, and aspects of the safety narrative.

The subject of the notice is 3′-sialyllactose sodium salt (3′-SL) for use as an ingredient in non-exempt infant formula for term infants[1] at a level up to 0.28 g/L, as consumed, and in other food categories at the maximum levels shown in Table 1.[2] The notice informs us of Inbiose's view that these uses of 3′-SL are GRAS through scientific procedures.

Table 1: Intended food categories and use levels for 3′-SL

Food Categories	Maximum use levels (g/kg or g/L)
Meal replacement and nutritional drinks (milk- and non-milk-based)	0.9
Sports, isotonic, and "energy" drinks	0.45
Enhanced or fortified waters	0.45
Soft drinks (regular and diet)	0.25
Cappuccino, non-fat, with milk, sweetened	0.52
Herbal tea, presweetened with low calorie sweetener or sugar	12.9
Imitation milk	0.12
Non-dairy yogurt	0.55
Frozen yogurt	1.7
Cereal and granola bars	2.5
Meal replacement bars, for weight management	25.9

[1] Inbiose states that the use of 3′-SL in infant formula is not restricted to any specific protein base (e.g., cow milk-based, soy-based, etc.).
[2] Inbiose states that 3′-SL is not intended for use in products under the U.S. Department of Agriculture's jurisdiction or in foods for which standards of identity do not permit its addition.

Formula-type drinks for young children	0.28
Other drinks for young children	0.15
Milk-based meal replacement beverages for children	0.9
Instant cereals for infants and young children	1.66
Other foods for infants and young children	1.25
Unflavored pasteurized and sterilized milk	0.25
Buttermilk	0.25
Flavored milk	0.25
Yogurt	2.5
Fruit flavored drinks and ades	0.25
Herbal extract sugar substitutes	100
Enteral tube feeding (11 years and older)	1.5

Inbiose provides information on the identity and composition of 3′-SL. Inbiose describes 3′-SL as a white powder containing ≥88% 3′-SL on a dry matter (DM) basis. Additionally, Inbiose states that 3′-SL contains small amounts of sialic acid,[3] D-lactose, and other related carbohydrates. 3′-SL has the CAS Registry No. 128596-80-5.

Inbiose describes the production organism used in the manufacturing process for 3′-SL. Inbiose states that *Escherichia coli* K-12 strain LMBP 12731 (*E. coli* K-12 LMBP 12731) is non-pathogenic and non-toxigenic and is deposited in the Belgian Co-ordinated Collections of Micro-organisms in Ghent, Belgium.

E. coli K-12 LMBP 12731 is genetically engineered from the host strain, *E. coli* K-12 strain ATCC 700926, to produce 3′-SL. Inbiose utilizes several modifications to construct *E. coli* K-12 LMBP 12731. Gene knockouts were used to avoid the breakdown of lactose and sialic acid and to prevent the formation of acetate and lactate. Additionally, 11 knock-ins from seven donor species were introduced enabling *E. coli* K-12 LMBP 12731 to biosynthesize 3′-SL, with some knock-ins encoding for the production strain to be able to grow on sucrose. Inbiose discusses the insertion of a plasmid for the overexpression of N-acylneuraminate cytidylyltransferase and sialyltransferase. Inbiose states that no antibiotic resistance genes are present on the plasmid and that *E. coli* K-12 LMBP 12731 is not capable of DNA transfer to other organisms. Inbiose validated the production strain through whole genome sequencing and further confirmed that *E. coli* K-12 LMBP 12731 does not contain any trace of the plasmid using polymerase chain reaction.

Inbiose discusses the two-stage manufacturing process for 3′-SL. In the fermentation stage, the production organism is grown in a medium containing a carbon source (glucose or sucrose) plus lactose. 3′-SL is partly secreted into the medium, which is then pasteurized releasing the remaining 3′-SL. In the purification stage, the 3′-SL is then harvested via removal of the biomass, including production organism, cell components and larger molecules (i.e., DNA, protein, lipopolysaccharides), by microfiltration. 3′-SL is then further purified and selectively concentrated via a series of steps involving

[3] *N*-acetyl-D-neuraminic acid is 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 the notifier's GRAS conclusion.

nanofiltration, ion exchange chromatography, activated charcoal treatment, sterile filtration, evaporation, and drying. Inbiose states that all materials used in the manufacturing process are authorized for their respective uses in the U.S., and that 3′-SL is manufactured following current good manufacturing practices.

Inbiose provides specifications for 3′-SL that include minimum levels (expressed in area percent, DM basis) of 3′-SL (≥88%) and sum of 3′-SL, sialic acid, and lactose (≥90%), as well as limits on other carbohydrates (≤5%),[4] sialic acid (≤5%), D-lactose (≤5%), and 3′-sialyllactulose (≤5%). Inbiose also provides limits (expressed as percent (w/w) basis) for ash (≤8.5%), sodium (≤4.5%), moisture (≤9%), heavy metals (including lead, ≤0.05 mg/kg), protein (≤100 mg/kg), and microorganisms, including *Salmonella* serovars (absent in 25 g) and *Cronobacter* spp. (absent in 25 g). Inbiose provides the results of five non-consecutive batch analyses to demonstrate that 3′-SL can be manufactured to meet the specifications. Inbiose discusses the results of stability studies conducted with 3′-SL and incorporates into the notice stability studies described in GRNs 000766, 000880, and 000921.[5] Inbiose concludes that the shelf-life of 3′-SL is one year from the date of production when stored under ambient conditions.

Inbiose estimates the dietary exposure to 3′-SL and states that all uses, as summarized in Table 1, are substitutional for the uses described in previous notices (i.e., GRNs 000766, 000880, 000921, 001051[6]) at the maximum use levels. Using food consumption data from the 2017-2018 National Health and Nutritional Examination Survey, Inbiose estimates the mean and 90th percentile eaters-only dietary exposures for infants 0-6 months to be 0.28 g/person (p)/day (d) (0.041 g/kg body weight (bw)/d) and 0.53 g/p/d (0.066 g/kg bw/d), respectively, and for infants 7-12 months to be 0.48 g/p/d (0.052 g/kg bw/d) and 0.89 g/p/d (0.096 g/kg bw/d), respectively. Inbiose also estimates the mean and 90th percentile eaters-only dietary exposures for ages 1-3 years to be 0.23 g/p/d (0.017 g/kg bw/d) and 0.42 g/p/d (0.033 g/kg bw/d), respectively, and for ages 4-10 years to be 0.23 g/p/d (0.008 g/kg bw/d) and 0.51 g/p/d (0.017 g/kg bw/d), respectively. For the U.S. population aged 2 years and older, Inbiose estimates the mean and 90th percentile eaters-only dietary exposures to be 0.39 g/p/d (0.006 g/kg bw)/d) and 0.71 g/p/d (0.012 g/kg bw/d), respectively.

Inbiose discusses data and information supporting the safety of 3′-SL and states that a literature search conducted through February 2023 did not identify any published information that would contradict its GRAS conclusion. Inbiose states that 3′-SL is chemically identical to the 3′-SL in human milk and compositionally similar to other 3′-SL ingredients concluded to be GRAS. Therefore, Inbiose incorporates into the notice published and unpublished data and information discussed in GRNs 000766, 000880,

---

[4] Inbiose identifies other carbohydrates as glucose, sucrose, 3-sialylgalactose, 3′-sialyllactulose, and N-acetylglucosamine.

[5] 3′-sialyllactose sodium salt is the subject of GRNs 000766, 000880, 000921. We evaluated these notices and responded in letters dated May 7, 2019, April 13, 2020, and October 30, 2020, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

[6] 3′-sialyllactose sodium salt is the subject of GRN 001015. We evaluated this notice and responded in a letter dated July 15, 2022, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

000921, and 001015. This information includes absorption, distribution, metabolism, and excretion data for 3′-SL, as well as an acute toxicity study in rats; subacute toxicity studies in dogs, rats, and piglets; 90-day subchronic studies in rats; tolerability studies in piglets; genotoxicity tests; and clinical studies all showing no evidence of toxicity. Inbiose discusses unpublished safety data with the subject of the notice, including genotoxicity tests and single dose acute, 21-day range finding, and 90-day repeated dose studies, all in rats, which showed no signs of toxicity. Inbiose summarizes the results of published clinical studies in which 3′-SL was demonstrated to be safe and well tolerated when consumed by healthy term infants as part of a human milk oligosaccharide mixture.

Based on the totality of the data and information, Inbiose concludes that 3′-SL is GRAS for its intended use.

Standards of Identity

In the notice, Inbiose 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 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 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 (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. 3′-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 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 Inbiose'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 Inbiose provided, as well as other information available to FDA, we have no questions at this time regarding Inbiose'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 001074 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.04.05 11:47:25 -04'00'

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