Dietrich B. Conze, Ph.D.
Spherix Consulting Group, Inc.
11821 Parklawn Drive, Suite 310
Rockville, MD 20852

Re: GRAS Notice No. GRN 000921

Dear Dr. Conze:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000921. We received the GRAS notice that you submitted on behalf of Jennewein Biotechnologie GmbH (Jennewein) on March 23, 2020, and filed it on May 14, 2020. Jennewein submitted amendments to the notice on July 31, 2020, and October 12, 2020, that clarified the identity, manufacturing process, and information presented in the safety narrative.

The subject of the notice is 3′-sialyllactose sodium salt (3′-SL) for use as an ingredient in cow milk-based, non-exempt infant formula for term infants at a level of 0.28 g/L. The notice informs us of Jennewein's view that this use of 3′-SL is GRAS through scientific procedures.

Jennewein provides information on the identity and composition of 3′-SL (CAS Reg. No. 128596-80-5). Jennewein describes 3′-SL as a white to ivory-colored powder that is ≥88% 3′-SL on a dry matter (DM) basis. Additionally, 3′-SL is a trisaccharide containing N-acetylneuraminic acid (NANA, sialic acid)[1] and lactose.

Jennewein describes the production organism used in the three-stage manufacturing process for 3′-SL. The non-pathogenic and non-toxigenic production organism, *Escherichia coli* BL21 (DE3) strain DSM 33492, is genetically engineered to produce 3′-SL. This strain is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) strain collection in Braunschweig, Germany and is a modification of the host strain *E. coli* BL21 (DE3).[2] Jennewein constructed the production strain after

[1] NANA 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.
[2] Jennewein states that the safety of *E. coli* BL21 (D3) is summarized in GRNs 000485 and 000571. Beta-galactosidase enzyme preparation and 2′-FL are the subjects of GRNs 000485 and 000571, respectively. We evaluated these notices and responded in letters dated April 15, 2014, and November 6, 2015, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

making multiple gene deletions[3] in the host strain. Following the deletions, Jennewein made six insertions of synthetic genes encoding functions for sugar metabolism from five donor species to optimize the production of 3′-SL. Jennewein states that all gene deletions and insertions were verified by polymerase chain reaction.

Jennewein states that the manufacturing process for 3′-SL is the same as described in GRN 000571[2] and incorporates that information into this notice. First, the production strain is inoculated into a fermentation medium that contains lactose. The 3′-SL produced is secreted into the fermentation medium. After fermentation is complete, the 3′-SL is purified by a series of filtration, deionization, electrodialysis, and decolorization steps to remove the production organism, water, minerals, and other small molecules. The 3′-SL is further concentrated and spray-dried to a powder. Jennewein 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 determined to be GRAS for their respective uses.

Jennewein provides specifications for 3′-SL that include minimum levels of 3′-SL (≥88% on a DM basis) and limits on other carbohydrates (≤12%), sialic acid (≤10%), lactose (≤5%), sodium (≤4.2%), N-acetylglucosamine (≤5%), moisture (≤9%), lead (≤0.02 mg/kg), protein (≤100 mg/kg), *Salmonella* serovars (absent in 25 g), and *Cronobacter sakazakii* (absent in 10 g). Jennewein provides the results of four non-consecutive batch analyses to demonstrate that 3′-SL can be manufactured to meet these specifications. Jennewein discusses the results of stability studies conducted with 3′-SL and also incorporates into the notice stability studies conducted with 3′-SL that are described in GRNs 000766[4] and 000880.[5] Jennewein concludes that the shelf-life of 3′-SL is one year from the date of production when stored under ambient conditions.

Jennewein estimates the dietary exposure to 3′-SL based on data and information provided in GRN 000766.[6] Due to the relative increase in use level in this notice, Jennewein calculates an increase in the mean and 90th percentile dietary exposures to 3′-SL for infants up to 12 months of age from 187 mg/day (d) (25.9 mg/kg body weight (bw)/d) and 278 mg/d (43.1 mg/kg bw/d) to 219 mg/d (30.3 mg/kg bw/d) and 325 mg/d (50.4 mg/kg bw/d), respectively.

Jennewein provides data and information supporting the safety of 3′-SL and states that a literature search was conducted through February 2020. Jennewein discusses data demonstrating that Jennewein's 3′-SL is structurally identical to 3′-SL found in human milk. Jennewein further states that their 3′-SL is compositionally and quantitatively

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[3] Jennewein states that deletions made include the endogenous genes encoding *N*-acetylmannosamine kinase, *N*-acetylmannosamine 6-phosphate 2 epimerase, a sialic acid transporter, *N*-acetylmannose lyase, and the phosphophenol pyruvate-dependent mannose specific phosphotransferase system.

[4] 3′-SL is the subject of GRN 000766. We evaluated this notice and responded in a corrected response letter dated May 7, 2019, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

[5] We evaluated GRN 000880 and responded in a corrected response letter dated April 13, 2020, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

[6] GRN 000766 includes estimates of dietary exposure to 3′-SL based on food consumption data from the National Health and Nutrition Examination Survey (2013-2014).

similar to the 3′-SL that is the subject of GRN 000880. Jennewein states that the intended use level of 3′-SL is within the established range of 3′-SL that occurs in human milk.[7] Jennewein notes that 3′-SL is resistant to digestive enzymes in the gastrointestinal tract and only a small amount is absorbed intact. The remaining unabsorbed 3′-SL passes through the gastrointestinal tract where it is either fermented by the microbiota or excreted unchanged in the feces. Jennewein incorporates into the notice safety data and information discussed in GRN 000880 and provides summaries of published acute and 28-day and 90-day repeated dose oral toxicity studies in rats, as well as genotoxicity studies demonstrating no toxicologically relevant effects. Jennewein also discusses several corroborative studies in which 3′-SL was tested in combination with other human milk oligosaccharides (HMOs), including a published 90-day oral toxicity study in rats, an unpublished 21-day tolerance study in neonatal piglets, and published genotoxicity studies.[8] Furthermore, Jennewein incorporates into the notice published clinical studies evaluating growth and development in infants and tolerability in adults from GRNs 000766 and 000880 and provides summaries of these studies to support the safe use of 3′-SL.

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

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

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

[7] In an amendment dated October 12, 2020, Jennewein provided an additional analysis of the published levels of 3′-SL in human milk. Jennewein states that the intended use level is within one standard deviation of the means/medians as provided in the updated analysis.
[8] We did not evaluate the use of 3′-SL in combination with other HMO ingredients during our review of GRN 000921.

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 requires labeling under the FD&C Act because it contains 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 Jennewein'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 Jennewein'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 Jennewein provided, as well as other information available to FDA, we have no questions at this time regarding Jennewein'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 000921 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2020.10.30 17:07:21 -04'00'

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