



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

Re: GRAS Notice No. GRN 000922

Dear Dr. Conze:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000922. We received the notice that you submitted on behalf of Jennewein Biotechnologie GmbH (Jennewein) on March 23, 2020 and filed it on June 3, 2020. Jennewein submitted an amendment to the notice on November 9, 2020, that provides clarifying information on the identity, production organism, manufacturing process, specifications, and composition of the notified substance.

The subject of the notice is 6'-sialyllactose sodium salt (6'-SL) for use as an ingredient in cow milk-based, non-exempt infant formula for term infants at a level of up to 0.4 g/L. The notice informs us of Jennewein's view that this use of 6'-SL is GRAS through scientific procedures.

Jennewein provides information on the identity and composition of the sodium salt of 6'-SL (CAS Registry Number 157574-76-0). Jennewein describes 6'-SL as a white to ivory-colored powder consisting of $\geq 90\%$ 6'-SL on a dry matter (DM) basis. Additionally, 6'-SL is a trisaccharide of N-acetylneuraminic acid (NANA, sialic acid)¹ and lactose. The chemical name is N-acetylneuraminic-(2 \rightarrow 6)- β -D-galactose-(1 \rightarrow 4)-D-glucose sodium salt.

Jennewein describes the production organism used in the three-stage manufacturing process for 6'-SL. The production organism, *Escherichia coli* BL21 (DE3) strain DSM 33493, is genetically engineered from the host strain, *E. coli* BL21 (DE3) to produce 6'-SL.² Jennewein constructed *E. coli* BL21 (DE3) strain DSM 33493 after making multiple gene deletions³ in the host strain. Following the deletions, Jennewein inserted six

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 (DE3) is summarized in GRNs 000485 and 000571. The subjects of GRNs 000485 and 000571 are beta-galactosidase enzyme preparation and 2'-fucosyllactose, 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 the time regarding the notifiers' GRAS conclusions.

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,

synthetic genes encoding functions for sugar metabolism derived from five donor species to produce 6'-SL. Jennewein states that all gene deletions and insertions were verified by polymerase chain reaction. Jennewein states that *E. coli* BL21 (DE3) strain DSM 33493 does not contain plasmids or other episomal vectors and is not capable of DNA transfer to other organisms. Jennewein states that *E. coli* BL21 (DE3) strain DSM 33493 is non-pathogenic and non-toxicogenic, and is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) strain collection in Braunschweig, Germany.

Jennewein states that the manufacturing process for 6'-SL is the same as described in GRN 000571, and Jennewein incorporates information from this notice. First, the production organism is inoculated into a fermentation medium that contains lactose. 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, electro dialysis, and decolorization steps to remove the production organism, water, minerals, and other small molecules. The 6'-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 concluded to be GRAS for their respective uses.

Jennewein provides specifications for 6'-SL that include minimum levels of 6'-SL ($\geq 90\%$ on a DM basis) and limits, expressed on a weight percent basis, for sum of other carbohydrates ($\leq 10\%$), sialic acid ($\leq 10\%$), lactose ($\leq 5\%$), sodium ($\leq 4.2\%$), N-acetylglucosamine ($\leq 5\%$), moisture ($\leq 9\%$), lead (≤ 0.02 mg/kg), protein (≤ 100 mg/kg), and limits on microorganisms including *Salmonella* serovars (absent in 25 g), and *Cronobacter sakazakii* (absent in 10 g). Jennewein provides the results from five non-consecutive batch analyses to demonstrate that 6'-SL can be manufactured to meet these specifications. Jennewein discusses the results of stability studies conducted with 6'-SL and also incorporates into the notice stability studies conducted with 6'-SL that are described in GRN 000881.⁴ Jennewein concludes that the shelf-life of 6'-SL is one year from the date of production when stored under ambient conditions.

Jennewein discusses the dietary exposure to 6'-SL and incorporates data and information provided in GRN 000881.⁵ Jennewein states that the intended use of 6'-SL in non-exempt infant formula for term infants to be the same as described in GRN 000881 and does not expect dietary exposure to change. In GRN 000881, the notifier estimated the mean and 90th percentile dietary exposures (eaters-only) to 6'-SL for infants up to 6 months of age to be 88 mg/kg body weight (bw)/d and 151 mg/kg bw/d, respectively, and for infants 7 to 12 months of age to be 98.7 mg/kg bw/d and 176 mg/kg bw/d, respectively.

and the phosphophenol pyruvate-dependent mannose specific phosphotransferase system.

⁴ The subject of GRN 000881 was 6'-SL. We evaluated this notice and responded in a revised response letter dated April 13, 2020, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

⁵ GRN 000881 included estimates of dietary exposure to 6'-SL that are based on food consumption data from the National Health and Nutrition Examination Survey (2013-2014).

Jennewein discusses the safety of 6'-SL and states that their microbially produced 6'-SL is structurally identical to human milk 6'-SL and the subject of GRN 000881; therefore, Jennewein incorporates into the notice the safety data and information from GRN 000881. Jennewein further states that 6'-SL is an isomer of 3'-SL, which is also present in human milk, and that microbially produced 3'-SL has been the subject of prior GRNs. Jennewein notes that the proposed use level of its 6'-SL is intended to match those levels consumed safely in human milk. Jennewein states that published studies show levels of 6'-SL in human milk range from 0.1-0.8 g/L.

Jennewein states that all available data suggest that the majority of 6'-SL, like all human milk oligosaccharides (HMOs), is resistant to digestive enzymes, poorly absorbed as it reaches the large intestine undigested, and is either mostly fermented by the microbiota or excreted intact in the feces. Jennewein summarizes published 14-day and 90-day repeat-dose oral toxicity studies in neonatal rats as well as genotoxicity studies with microbially produced 6'-SL, similar to Jennewein's 6'-SL, to support the conclusion that 6'-SL is not genotoxic and is safe up to 5000 mg/kg bw /day, the highest dose tested. Further, Jennewein discusses other published and unpublished studies to support the safety of 6'-SL. These included published oral toxicity studies in rats using enzymatically synthesized 6'-SL, as well as published oral toxicity studies in rats and unpublished tolerability studies in neonatal piglets using a mixture of HMOs including 6'-SL. Finally, Jennewein states that the estimated dietary exposure from the intended uses are below the safe or tolerable level of intake for 6'-SL determined using toxicological studies and supporting studies that quantitated the levels of 6'-SL in human milk.

Jennewein includes the statement of a panel of individuals (Jennewein's GRAS panel). Based on its review, Jennewein's GRAS panel concluded that 6'-SL is safe under the conditions of its intended use.

Based on the totality of the data and information, Jennewein concludes that 6'-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 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, and soybeans) or a food ingredient that contains protein derived from one of those foods. 6'-SL requires labeling under the FD&C Act because it contains protein derived from milk.

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 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 Jennewein provided, as well as other information available to FDA, we have no questions at this time regarding Jennewein’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 000922 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

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