Saori Akiduki, Ph.D.
Kyowa Hakko Bio Co., Ltd.
4-10-2 Nakano, Nakano-ku,
Tokyo 164-0001,
JAPAN

Re: GRAS Notice No. GRN 001052

Dear Dr. Akiduki:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001052. We received Kyowa Hakka Bio Co., Ltd. (Kyowa)'s notice on January 18, 2022, and filed it on June 9, 2022. Kyowa submitted amendments to the notice on November 16, 2022, January 23, 2023, and March 13, 2023, that clarified the intended uses, specifications, manufacturing process, dietary exposure, aspects of the safety narrative, and the construction and safety of the production organism.

The subject of the notice is 3′-siallylactose sodium salt (3′-SL) for use as an ingredient in non-exempt infant formula for term infants[1] and formulas for young children (>12 months) at a level of 0.24 g/L, as consumed; in drinks for infants and young children at 0.15−1.0 g/L; in hot cereals, desserts, dry snacks, and other foods for infants and young children at 1.1−5.7 g/kg; in non-alcoholic beverages (sports and "energy" drinks, fortified and flavored waters, soft drinks, coffee and tea, fruit juices and nectars, fruit-flavored drinks, vegetable juices and nectars, milk, flavored milk, buttermilk, milk substitutes, meal replacements, and protein drinks) at 0.12–6.0 g/L; in oral nutritional supplements and enteral tube feeding products (≥11 years and older) at 2.0 g/L; in chewing gum at 30 g/kg; in table-top sweeteners at 30 g/kg; in beverage whiteners and cream substitutes at 60 g/kg; and in breads and baked goods; breakfast cereals; frozen dairy desserts; fruit and water ices; gelatins, puddings, and fillings; cereal and granola bars (including meal-replacement bars); jams and jellies; yogurt (including non-dairy yogurt); evaporated and condensed milk; canned fruits; fruit-based desserts; and syrups at 0.12–8.0 g/kg.[2]

Kyowa describes 3′-SL as a white to off-white powder that contains a minimum of 82% 3′-SL on a dry matter (DM) basis and small amounts of *N*-acetyl-D-neuraminic acid (sialic acid)[3], D-glucose, D-lactose, and other carbohydrates structurally related to 3′-SL.

[1] Kyowa 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).
[2] Kyowa states that 3′-SL is not intended for use in products under the jurisdiction of the U.S. Department of Agriculture or in foods for which standards of identity do not permit its addition.
[3] *N*-acetyl-D-neuraminic acid is the subject of GRN 000602. We evaluated this notice and responded in a

3′-SL is a trisaccharide composed of lactose and sialic acid and is designated by CAS Registry Number 128596-80-5.

Kyowa states that 3′-SL is produced by fermentation using a genetically engineered production strain derived from the host strain, *Escherichia coli* W strain ATCC 9637 (*E. coli* W ATCC 9637). Kyowa constructed the production strain, *E. coli* W strain NITE SD_00488 (*E. coli* NITE SD_00488), through the deletion of five genes in the host strain genome in order to optimize the strain's production of 3′-SL. Kyowa stated that they inserted five unique genes from five donor microorganisms to complete the enzyme pathway for the production of 3'-SL. Kyowa confirmed the insertion of these genes using polymerase chain reaction. Kyowa states that *E. coli* W NITE SD_00488 is non-pathogenic, non-toxigenic, and is not capable of DNA transfer to other organisms. Kyowa states that *E. coli* W NITE SD_00488 is deposited at the National Biological Resource Center.

Kyowa states that 3′-SL is manufactured in two main stages. In the first stage of the manufacturing process, the production strain is inoculated into a fermentation medium that contains D-lactose and D-glucose. The 3′-SL is excreted into the fermentation medium during the fermentation under controlled conditions. After fermentation is complete and terminated by heat treatment, the fermentation medium is cooled and acidified. In the second stage of the manufacturing process, the microbial biomass is removed from the fermentation medium by microfiltration. The obtained solution is subjected to a series of purification processes using ion exchange resins, then concentrated, pH-adjusted, decolorized with activated carbon, and further purified by ultrafiltration to remove any remaining impurities. The resulting solution is then concentrated, filtered, spray-dried, and homogenized to yield the final 3′-SL. Kyowa states that 3′-SL is manufactured according to current good manufacturing practices, and that all raw materials, processing aids, and food contact substances are food grade and are approved by U.S. regulations, are the subjects of effective food contact notifications, or were concluded to be GRAS for their respective uses. Kyowa states that, except for D-lactose, none of the raw materials are derived from major allergens or allergenic sources.

Kyowa provides specifications for 3′-SL that include the minimum content of 3′-SL (≥82% DM), and limits on *N*-acetyl-D-neuraminic acid (≤9% w/w), D-glucose (≤3% w/w), D-lactose (≤3% w/w), 3′-sialyllactulose and 6'-sialyllactose sodium salt (≤5% w/w total), residual protein (≤100 mg/kg), moisture (≤10.5% w/w), sodium (≤5% DM), heavy metals, including lead (≤0.1 mg/kg), and microorganisms, including *Cronobacter* spp. (absent in 10 g) and *Salmonella* serovars (absent in 25 g). Kyowa provides the results from the analyses of four non-consecutive batches to demonstrate that 3′-SL can be manufactured to meet these specifications. Kyowa states that the stability study conducted under standard conditions (25 ± 2°C; 60 ± 5% relative humidity) demonstrated that 3′-SL is stable for at least one year.

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letter dated February 1, 2016, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

Kyowa estimates the dietary exposure to 3′-SL using food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES). Kyowa estimates the mean and 90th percentile eaters-only dietary exposures to 3′-SL from the intended uses to be 0.94 g/person (p)/d and 1.73 g/p/d, respectively, for the U.S. population aged 2 years and older. Kyowa estimates the mean and 90th percentile eaters-only dietary exposure to 3′-SL for infants aged 0 to 6 months to be 0.26 g/p/d and 0.56 g/p/d, respectively. The mean and 90th percentile eaters-only dietary exposures for infants aged 7 to <12 months are estimated to be 0.55 g/p/d and 0.98 g/p/d, respectively. Kyowa estimates the mean and 90th percentile eaters-only cumulative dietary exposures to 3′-SL from the intended and current uses for infants aged 0 to 6 months (0.29 g/p/d and 0.56 g/p/d, respectively), infants aged 7 to < 12 months (0.57 g/p/d and 1.0 g/p/d, respectively), and the U.S. population aged 2 years and older (1.56 g/p/d and 3.79 g/p/d, respectively).

Kyowa discusses data and information supporting the safety of 3′-SL and states that a literature search conducted through December 2021 did not identify any published studies which would contradict its GRAS conclusion. Kyowa states that 3′-SL is chemically and structurally identical to the 3′-SL in human milk and has a compositional similarity to other 3′-SL ingredients previously concluded to be GRAS. Therefore, Kyowa states that the safety of 3′-SL is supported by published preclinical and clinical studies conducted with other 3′-SL ingredients. Kyowa incorporates into their notice and provides summaries of information discussed in GRNs 000766, 000880, 000921[4] including absorption, distribution, metabolism, and excretion data for 3′-SL as well as subchronic toxicity studies in rats, gastrointestinal developmental studies in piglets, genotoxicity tests, and clinical studies in adults to support the safe use of 3′-SL. Kyowa discusses corroborative unpublished safety data with the article of commerce, including a 90-day repeated dose toxicity study in rats and genotoxicity studies, which demonstrated no toxicologically relevant effects. Kyowa summarizes a published clinical study 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, Kyowa concludes that 3′-SL is GRAS for its intended use.

Standards of Identity

In the notice, Kyowa states their intention to use 3′-SL in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations (21 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.

[4] 3′-sialyllactose sodium salt is the subject of GRNs 000766, 000880, and 000921. We evaluated these notices and responded in letters or corrected letters dated May 7, 2019; April 13, 2020; and October 30, 2020, respectively, stating that we had no questions at those times regarding the notifiers' GRAS conclusions.

Potential Labeling Issues

Under section 403(a) of the Federal Food, Drug, and Cosmetic Act (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 Kyowa'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 Kyowa'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 Kyowa provided, as well as other information available to FDA, we have no questions at this time regarding Kyowa'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 001052 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.04.18 17:45:16 -04'00'

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Director
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