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

Re: GRAS Notice No. GRN 001053

Dear Dr. Akiduki:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001053. We received Kyowa Hakka Bio Co., Ltd. (Kyowa)'s notice on January 18, 2022, and filed it on June 21, 2022. Kyowa submitted amendments to the notice on December 7, 2022, and April 24, 2023, that reduced the use level in non-exempt infant formula for term infants and further clarified the intended uses, specifications, analytical methods, aspects of the safety narrative, and safety of the production organism.

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 0.40 g/L of formula as consumed and in several food categories at maximum use levels specified in Table 1. The notice informs us of Kyowa's view that this use of 6′-SL is GRAS through scientific procedures.

Table 1: Intended uses of 6′-SL

Food Category	Intended use level (g/kg or g/L)
Breads and baked goods	10
Soft drinks[2]	0.25
Enhanced, fortified, and flavored waters[2]	0.25
Non-milk-based meal replacement drinks	1.0
Sports, isotonic, and "energy" drinks	0.5
Protein drinks	1.0

[1] Kyowa 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).
[2] Kyowa intends to use 6'-SL in soft drinks and enhanced, fortified, and flavored waters at a maximum level of 0.25 g/L, and Kyowa notes that the intended use in these categories was previously concluded to be GRAS at up to 0.5 g/L in GRN 000881. We note that the cumulative estimated dietary exposure to 6'-SL is not expected to significantly differ as a result of the higher use level in these categories.

Hot breakfast cereals, instant and ready-to-eat (RTE)	1.0
Puffed cereals, RTE	17
High-fiber cereals, RTE	6.2
Biscuit-type cereals, RTE	4.2
Chewing gum	62
Coffee	2.1
Tea	2.1
Milk substitutes (e.g., soy milk and imitation milks)	0.25
Beverage whiteners	125
Non-dairy cream	125
Non-dairy yogurt	2.2
Frozen dairy desserts	3.5
Edible ices, sherbet, and sorbet	3.5
Dairy-based puddings, custards, mousses, and gelatin desserts	3.5
Fruit pie filling	2.9
Fruit filling in bars, cookies, yogurt, and cakes	6.25
Cereal and granola bars, protein and meal replacement bars	10
Term infant formula	0.40
Formula for young children (intended for ages 1 to 3 years)	0.50
Other foods for infants and young children	2.5
Hot cereals (dry and RTE) for infants and young children	2.3
Other drinks for young children	0.25 to 2.1
Desserts for young children	2.3
Crackers, pretzels, cookies, and snack items for infants and young children	12
Jellies and jams, fruit preserves, and fruit butters	12
Unflavored pasteurized and sterilized milk	0.5
Buttermilk	0.25
Flavored milk	0.25
Evaporated and condensed milk	0.25
Milk-based meal replacement beverages for weight management	1.0
Yogurt	5.0
Nutritional drinks intended for pregnant women	12.5
Fruit flavored drinks and ades	0.25
Fruit juices	0.25
Fruit nectars	0.25
Canned fruit	3.5
Fruit-based desserts	3.5
Vegetable juices and nectars	0.25
Table-top sweeteners	62
Syrups used to flavor milk beverages	1.5
Formula for enteral tube feeding (11 years and older)	4.1
Oral nutritional drinks	1.68

Kyowa provides information on the identity and composition of 6′-SL (CAS Registry Number 157574-76-0). Kyowa describes 6′-SL as an off-white to white-colored powder consisting of ≥82% 6′-SL sodium salt on a dry matter (DM) basis and specified lesser amounts of *N*-acetyl-D-neuraminic acid (sialic acid)[3], D-glucose, D-lactose, 6′-sialyllactulose, and 3′-sialyllactose sodium salt. Kyowa states that 6′-SL is a trisaccharide of sialic acid and lactose.

Kyowa describes the production organism used in the manufacture of 6′-SL. The production organism is genetically engineered from the host strain, *Escherichia coli* W (ATCC 9637), to produce 6′-SL. Kyowa constructed the production organism with the insertion of five genes encoding functions for sugar metabolism derived from five donor species to produce 6′-SL.[4] Kyowa states that the production strain is non-pathogenic and non-toxigenic, and the modifications are well-characterized and not associated with any potential toxic or pathogenic traits of the donor organism. Kyowa states that the production strain is deposited at the National Biological Resource Center with the deposition number of NITE SD_00489.

Kyowa describes the manufacturing process for 6′-SL. First, the production organism is inoculated into a sterile fermentation medium that contains food-grade nutrients, including glucose and lactose[5] as carbon sources. The 6′-SL is produced under controlled fermentation conditions and is secreted into the fermentation medium. The production of 6′-SL is terminated by heat treatment (sterilization) and the broth is then cooled and acidified. Production organism cells are removed by microfiltration, and the resulting solution is subjected to a series of cationic and anionic ion exchange resins to remove impurities. The pH of the effluent is adjusted, and the solution concentrated and decolorized with activated carbon. The pH of the resulting solution is adjusted again and filtered using an ultrafiltration membrane that removes endotoxins, residual proteins, impurities, and any remaining production organism. The resulting solution is concentrated, filtered, spray-dried, homogenized, and sieved to obtain the final 6′-SL product. Kyowa states that 6′-SL is produced in accordance with current good manufacturing practices, and all raw materials, processing aids, and fermentation medium ingredients are food grade and are used in accordance with U.S. regulations, are previously concluded to be GRAS for their respective uses, or are the subject of an effective food contact notification.

Kyowa provides specifications for 6′-SL that include minimum levels of 6′-SL sodium salt (≥82% on a DM basis) and limits, expressed on a weight percent basis, for *N*-acetyl-D-neuraminic acid (≤9%), D-glucose (≤3%), D-lactose (≤3%), 6′-sialyllactulose and 3′-sialyllactose sodium salt (combined, ≤5%), moisture (≤10.5%), sodium (≤5% DM),

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[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.

[4] Kyowa states that the production strain contains genes encoding glucosamine 6-phosphate *N*-acetyltransferase, *N*-acylglucosamine 2-epimerase, CMP-*N*-acetylneuraminic acid synthetase, α-2,6-sialyltransferase, and *N*-acetylneuraminic acid synthetase that are inserted into the chromosomal DNA of the host organism.

[5] Kyowa states that the lactose used as a carbon source for the production of 6′-SL is derived from cow milk.

heavy metals including lead (≤0.1 mg/kg), protein (≤100 mg/kg), and limits on microorganisms including Salmonella serovars (absent in 100 g)[6] and *Cronobacter sakazakii* (absent in 100 g).[7] Kyowa provides the results from the analyses of five batches to demonstrate that 6′-SL can be manufactured to meet these specifications.

Kyowa discusses the results of stability studies conducted with 6′-SL under accelerated conditions (40±2 °C and 75±5% relative humidity) over a period of six months and under room temperature conditions (25±2 °C and 60±5% relative humidity). Kyowa states that the results demonstrate that 6′-SL is stable for at least a year at room temperature. In addition, Kyowa states that 6′-SL was stable for 6 months under accelerated conditions, which supports a shelf life of 3 years. Kyowa also incorporates information from GRN 000881[8] on the stability of 6′-SL in a whey-based infant formula powder stored for up to 12 months at temperatures from 4 to 37 °C. Kyowa concludes that the results of this study demonstrate that 6′-SL is stable in powdered infant formula.

Kyowa provides estimates of dietary exposure to 6′-SL based on the intended uses and 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 6′-SL for the U.S. population aged 2 years and older to be 1.95 g/p/d (30 mg/kg body weight (bw)/d) and 3.60 g/p/d (64 mg/kg bw/d), respectively. Kyowa estimates the mean and 90th percentile eaters-only dietary exposures to 6′-SL for infants 0 to 6 months of age to be 0.49 g/p/d (74 mg/kg bw/d) and 0.90 g/p/d (119 mg/kg bw/d), respectively. Kyowa estimates the mean and 90th percentile eaters-only dietary exposures to 6′-SL for infants 7 to <12 months of age to be 1.00 g/p/d (110 mg/kg bw/d) and 1.74 g/p/d (190 mg/kg bw/d), respectively. Kyowa estimates the mean and 90th percentile eaters-only dietary exposures to 6′-SL for children 1 to 3 years of age to be 1.06 g/p/d (77 mg/kg bw/d) and 1.69 g/p/d (134 mg/kg bw/d), respectively.

Kyowa discusses dietary exposure to 6′-SL from the intended use in oral nutritional drinks that are intended for the general population (ages 2 years and older). Kyowa estimates dietary exposure to 6′-SL to be 0.84 g/p/d based on the intended use level of 0.42 g 6′-SL per 45 g serving (250 mL, as consumed) and an estimated consumption rate of two servings per day. Kyowa also discusses dietary exposure to 6′-SL from the intended use in formula for enteral tube feeding for ages 11 years and older. Kyowa estimates dietary exposure to be 2.0 g/p/d based on the intended use level of 4.1 g/L in the ready-to-consume product and an estimated consumption rate of two 250 mL servings per day.

Kyowa discusses the safety of 6′-SL and states that the notified substance is structurally

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[6] Kyowa states that in the analysis of Salmonella, four individual samples of 25 g are analyzed, and all four samples (100 g total) must be negative to meet the specification limit.
[7] Kyowa states that in the analysis of *C. sakazakii*, ten individual samples of 10 g are analyzed, and all ten samples (100 g total) must be negative to meet the specification limit.
[8] The subject of GRN 000881 was 6′-SL sodium salt. 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.

identical to human milk 6′-SL and the subjects of GRN 000881 and GRN 000922[9]; therefore, Kyowa incorporates into the notice the safety data and information from GRN 000881 and GRN 000922. Kyowa 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. Kyowa notes that the proposed use level of 6′-SL is intended to match those levels consumed safely in human milk.

Kyowa 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. Kyowa summarizes published 90-day repeat-dose oral toxicity studies in adult and neonatal rats as well as genotoxicity studies with microbially-produced 6′-SL ingredients, similar to the notified substance, to support the conclusion that 6′-SL is not genotoxic and is safe up to the highest dose tested. Kyowa also discusses other published safety-related studies in rats and in neonatal piglets using a related isomer, 3′-SL, as well as mixtures of HMOs including 6′-SL. To support safety of the notified substance, Kyowa discusses an unpublished bacterial reverse mutation test, *in vivo* micronucleus test, and 90-day oral toxicity study in rats using the notified substance as the test article. Kyowa also discusses published studies in humans supporting safety of the notified substance for the intended use.

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

Standards of Identity

In the notice, Kyowa states their intention to use 6′-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.

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 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

[9] The subject of GRN 000922 was 6′-SL sodium salt. We evaluated this notice and responded in a letter dated April 23, 2021, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

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 Kyowa'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 Kyowa'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 Kyowa provided, as well as other information available to FDA, we have no questions at this time regarding Kyowa'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 001053 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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