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

Re: GRAS Notice No. GRN 001051

Dear Dr. Akiduki:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001051. We received Kyowa Hakka Bio Co., Ltd (Kyowa)'s notice on January 6, 2022, and filed it on May 19, 2022. Kyowa submitted amendments to the notice on October 20, 2022, March 22, 2023, September 23, 2023, and November 14, 2023, that revised the specifications, limited the intended uses to be substitutional for other uses (excluding uses in exempt infant formula) previously determined to be GRAS, updated the estimates of dietary exposure, and clarified information about the manufacturing process, minor impurities, production organism, and aspects of the safety narrative.

The subject of the notice is 2′-fucosyllactose (2′-FL) for use as an ingredient in non-exempt infant formula for term infants[1] at a maximum level of 2.4 g/L as consumed, and in other food categories at the maximum levels shown in Table 1.[2] The notice informs us of Kyowa's view that these uses of 2′-FL are GRAS through scientific procedures.

Table 1: Intended food categories and use levels for 2′-FL

Food Categories	**Maximum use levels (g/kg or g/L)**
Breads and baked goods, gluten-free	48
Carbonated beverages	1.2
Enhanced or fortified waters	1.2
Sports, isotonic, and "energy" drinks	6
Hot breakfast cereals, prepared	31
Ready-to-eat (RTE) cereals, puffed	80
RTE cereals, high fiber	40
RTE cereals, biscuit-type	40

[1] Kyowa states that the use of 2′-FL in infant formula is not restricted to any specific protein base (e.g., cow milk-based, soy-based).
[2] Kyowa states that 2′-FL is not intended for use in products under the jurisdiction of the United States Department of Agriculture and in foods for which standards of identity do not permit its addition.

Food Categories	**Maximum use levels (g/kg or g/L)**
Coffee and tea[3]	10
Milk substitutes	1.2
Beverage whiteners (powdered)	600
Beverage whiteners (liquid)	80
Non-dairy yogurt	12
Frozen dairy-based desserts	17
Puddings, custards, and mousses	17
Fruit pie filling	14.1
Fruit filling in bars, cookies, yogurt, cakes	30
Non-exempt infant formula for term infants	2.4
Formula intended for young children (>12 months-3 years)	2.4
Hot cereals for infants and young children, prepared (from dry instant) and ready-to-serve	12
Other baby foods for infants and young children: yogurt, fruits, vegetables, toddler meals, desserts	12
Other drinks for infants and young children: juice and yogurt drinks	10
Baby snacks: crackers, pretzels, cookies, and other dry snack items	57
Jams, jellies, preserves, and fruit butters	60
Meal replacement bars, general use	30
Cereal bars including snack, granola, and breakfast bars	30
Meal replacement bars, for weight management	40
Meal replacement drinks for general use (including nutritional drinks, smoothies), milk and non-milk based	5
Meal replacement drinks for weight management, milk and non-milk based	12
Milk-based meal replacement beverages for children (e.g., pediatric nutritional drinks)	12
Unflavored pasteurized and sterilized milk	1.2
Buttermilk	1.2
Flavored and fermented milks	1.2
Yogurt	12
Fruit juices and nectars	1.2
Fruit-flavored drinks and ades	1.2
Vegetable juices and nectars	1.2
Sugar substitutes: table-top sweeteners	300
Syrups used to flavor milk beverages	7
Nutritional drinks for pregnant women	12
Oral and enteral tube feeding formulas for ages ≥11 years	20

[3] The category of coffee and tea includes ready-to-drink (e.g., bottled, flavored, pre-sweetened) coffee and tea and powder mixes used to prepare coffee and tea. For estimates of dietary exposure, it is assumed that the intended uses of 2′-FL do not include use in plain brewed coffee or tea.

Kyowa describes the identity and composition of 2′-FL, stating that 2′-FL is a white to off-white powder containing a minimum of 92% (dry basis) 2′-FL. Kyowa notes that 2′-FL is a trisaccharide consisting of L-fucose, D-galactose, and D-glucose. The chemical name for 2′-FL is α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose and the CAS registry number is 41263-94-9. Kyowa states that 2′-FL is chemically and structurally identical to 2′-FL in human milk.

Kyowa states that 2′-FL is produced by fermentation using a genetically engineered production strain derived from the host strain, *Escherichia coli* W ATCC 9637. Kyowa constructed the production strain, *E. coli* W NITE SD_00487, through the deletion of five genes in the host strain genome and insertion of five copies of a gene encoding α-1,2-fucosyltransferase[4] at these deletion sites. Kyowa also states that they insert a marker cassette containing the *sacB* gene and the *cat* gene used in strain selection and removed prior to use of the organism for production of 2′-FL. Kyowa states that they confirm all genetic modifications using polymerase chain reaction. Kyowa states that the *E. coli* production strain is deposited in the National Biological Resource Center (NBRC)[5] under deposition number NITE SD_00487. Kyowa states that *E. coli* NITE SD_00487 is non-pathogenic, non-toxigenic, not capable of DNA transfer to other organisms, and does not contain any elements expected to confer resistance to antibiotics. Further, Kyowa concludes that based on the long history of safe use of the host strain in food manufacturing and the well-characterized genetic changes, the production strain is not expected to produce antimicrobials or secondary metabolites.

Kyowa describes the two-stage manufacturing process: fermentation and purification. Kyowa states that 2′-FL is manufactured according to current good manufacturing practices, and all raw materials and processing aids are food-grade and are used in accordance with applicable U.S. regulations, are GRAS for their respective uses, or are the subject of an effective food contact notification. In the first stage of the manufacturing process, 2′-FL is produced from D-lactose and D-glucose during fermentation with *E. coli* NITE SD_00487 under controlled conditions and is secreted into the fermentation medium. After fermentation is complete, the production of 2′-FL is stopped by heat treatment (sterilization) and the broth is cooled and acidified. The microbial biomass is removed from the fermentation medium by microfiltration. The second stage of the manufacturing process consists of a series of purification steps. The filtrate is subjected to a series of cationic and anionic resins to remove minerals and small biomolecules (e.g., nucleic acids, protein, and organic impurities). The solution is concentrated, pH adjusted, and decolorized using activated carbon. Finally, the solution is concentrated by additional filtration steps, including microfiltration and ultrafiltration to remove residual proteins and other impurities. The solution is then further concentrated by evaporation, spray dried, homogenized using an air blender, and sieved to produce the 2′-FL powder.

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[4] The gene was cloned from chromosomal DNA from *Helicobacter mustelae* (ATCC 43772) by polymerase chain reaction and modified by site-directed mutagenesis. Kyowa states that the DNA insert is well-characterized and contains only the desired sequence.

[5] NBRC is located at the National Institute of Technology and Evaluation, Japan.

Kyowa provides specifications for 2′-FL, which include the minimum content of 2′-FL (≥92% on a dry weight basis (DW)), and limits on 3,2′-difucosyl-D-lactose (≤3% DW), D-lactose (≤5% DW), L-fucose (≤1% DW), D-glucose and D-galactose (≤1% DW), fucosylgalactose (≤3% DW), moisture (≤9%), residual proteins (≤0.01%), ash (≤0.5%), pH 4.0-9.0, heavy metals, including lead (≤0.1 mg/kg), and microorganisms, including *Salmonella* serovars (absent in 25 g) and *Cronobacter spp*. (absent in 10 g). Purity specifications are consistent with the 2′-FL monograph in the 13th edition of the Food Chemicals Codex (FCC, 2023). Kyowa provides the results from the analyses of five batches (four of which are non-consecutive) of 2′-FL to demonstrate that the ingredient can be manufactured to meet the specifications.

Kyowa presents the results of stability tests conducted on 3 lots of 2′-FL, held for six months under accelerated storage conditions (40±2 °C, 75±5% relative humidity). From this study, Kyowa concludes that the shelf-life of 2′-FL is 3 years at room temperature.

Kyowa estimates the dietary exposure to 2′-FL for all intended uses, including use in non-exempt infant formula for term infants and several additional food categories. Kyowa states that the intended uses of 2′-FL are substitutional for those described in previous GRNs: GRNs 000546, 000571, 000650, 000735, 000749, 000852, 000897, 001014, and 001060.[6]

Using food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES), Kyowa estimates the eaters-only mean and 90th percentile dietary exposures to 2′-FL for infants 0 to 6 months of age to be 2.4 and 4.4 g/person (p)/day (d) (360 and 578 mg/kg body weight (bw)/d), respectively. The mean and 90th percentile dietary exposures to 2′-FL for infants 7 to 12 months of age are estimated to be 4.3 and 7.7 g/p/d (474 and 812 mg/kg bw/d), respectively. The mean and 90th percentile dietary exposures to 2′-FL for the total population (aged 2 years and older) are estimated to be 4.2 and 9.1 g/p/d (65 and 146 mg/kg bw/d), respectively, and for children 1-2 years of age to be 2.9 and 5.7 g/p/d (237 and 477 mg/kg bw/d), respectively. The highest estimates of dietary exposure were observed for adults 65 years of age and older where the mean and 90th percentile eaters-only dietary exposures are 5.2 and 11.0 g/p/d (67 and 148 mg/kg bw/d), respectively.

Kyowa compares its estimates of dietary exposure to the cumulative estimate of dietary exposure for 2′-FL reported in GRN 001060. Kyowa notes that, while the cumulative estimates of dietary exposure for infants, toddlers, children, and teens were similar to those reported in GRN 001060, the estimates in GRN 001051 are slightly higher for adults than those reported in GRN 001060. Kyowa notes that these slight differences likely arise from inclusion of certain foods (e.g., tea, coffee, beverage whiteners, table-

[6] 2′-FL (referred to as for use in non-exempt infant formula for term infants, and in multiple additional food categories including infant and toddler foods, is the subject of GRNs 000546, 000571, 000650 000735,000749, 000852, 000897, 001014, and 001060

top sugar substitutes, and gluten-free baked goods)[7] in the GRN 001051 estimate that do not appear to have been included in the GRN 001060 cumulative dietary exposure estimate. Despite this slight increase in cumulative estimates of dietary exposure in GRN 001051, the uses in GRN 001051 are substitutional for other sources of 2′-FL previously determined to be GRAS for their intended uses, and the uses described in GRN 001051 will not result in an increase in dietary exposure to 2′-FL.

Kyowa discusses the data and information supporting the safety of 2′-FL and states that a literature search conducted through December 2021 did not identify any studies which would contradict its GRAS conclusion. Kyowa states that 2′-FL is structurally and chemically identical to the 2′-FL in human milk and has a compositional similarity to other 2′-FL ingredients previously concluded to be GRAS. Therefore, Kyowa states that the safety of 2′-FL is supported by preclinical and clinical studies conducted with other 2′-FL ingredients. Kyowa incorporates into the notice and provides summaries of published and unpublished information discussed in GRNs 000546, 000571, 000650, 00735, 000749, 000815[8], 000852, 000897, and 000929[9] including absorption, distribution, metabolism, and excretion data for 2′-FL as well as single dose acute and repeated dose subchronic studies in rats, tolerability studies in piglets, genotoxicity tests, and clinical studies in infants and adults. Kyowa also summarizes published safety studies not discussed in previous GRAS notices for 2′-FL and 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 also provides summaries of published clinical studies demonstrating safe consumption of 2′-FL by adults with irritable bowel syndrome, ulcerative colitis, or celiac disease, and the safe consumption of 2′-FL by infants. Kyowa states that these clinical studies demonstrate that 2′-FL is safe and well tolerated when consumed by adults and infants.

Kyowa concludes that 2′-FL has low allergic potential, noting that while the D-lactose starting material is obtained from milk, there are multiple filtration steps in the purification of 2′-FL that result in removal of protein. Kyowa further notes that, based on the results of the analyses of five lots of 2′-FL, residual protein levels were equal to or below the limit of detection of 1 mg/kg using two different enzyme-linked immunosorbent assays specific for milk proteins.

Based on the totality of the data and information, Kyowa concludes that 2′-FL is GRAS for its intended use.

Standards of Identity

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[7] FDA notes that these food categories were listed in GRN 000546 but have not been included in other GRNs.

[8] 2′-fucosyllactose and difucosyllactose is the subject of GRN 000815. We evaluated this notice and responded in a letter dated September 11, 2020, stating that we did not have questions at that time regarding the notifier's GRAS conclusion.

[9] 2′-FL is the subject of GRN 000929. We evaluated this notice and responded in a letter dated February 26, 2021, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

In the notice, Kyowa states their intention to use 2′-FL 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 2′-FL 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. 2′-FL 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 2′-FL 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 2′-FL 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 2′-FL. Accordingly, our response should not be construed to be a statement that foods containing 2′-FL 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 2′-FL is GRAS under its intended conditions of use. This letter is not an affirmation that 2′-FL 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 001051 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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