Dietrich B. Conze, Ph.D.
Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Re: GRAS Notice No. GRN 001014

Dear Dr. Conze:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001014. We received the notice that you submitted on behalf of Chr. Hansen A/S (Chr. Hansen) on June 8, 2021 and filed it on October 18, 2021. Chr. Hansen submitted amendments to the notice on January 21, 2022, April 26, 2022, and May 31, 2022, removing the intended use in oral electrolyte solutions, and providing additional clarifying information regarding the intended use, specifications, dietary exposure estimate, safety narrative, and an updated literature search.

The subject of the notice is 2′-fucosyllactose (2′-FL) for use as an ingredient at a level of 2.4 g/L in formula for young children (> 12 months); at a level of 12 g/kg (or g/L) in milk- and soy-based meal replacement beverages for children, ready-to-serve and dry instant cereals for infants and young children, meal replacement drinks, meal replacement bars, snack bars, and breakfast bars; at a level of 6 g/L in non-carbonated sports drinks and flavored waters; and at a level of 20 g/L in enteral tube feeding formulas. The notice informs us of Chr. Hansen's view that these uses of 2′-FL are GRAS through scientific procedures.

Chr. Hansen states that 2′-FL is found in human milk. 2′-FL is a fucosylated, neutral trisaccharide composed of L-fucose, D-galactose, and D-glucose. Chr. Hansen describes 2′-FL as a white to ivory-colored powder containing a minimum of 90% 2′-FL. The chemical name for 2′-FL is α-L-Fucopyranosyl-(1→2) β-D-galactopyranosyl-(1→4)-D-glucopyranoside (CAS Registry Number 41263-94-9). Chr. Hansen further notes that 2′-FL is the same ingredient that was the subject of GRN 000571.[1]

Chr. Hansen describes the production organism used in the manufacturing process for 2′-FL. The production organism, *Escherichia coli* BL21(DE3) strain DSM 33609,[2] is

[1] We evaluated GRN 000571 and responded in a letter dated November 6, 2015, stating that we had no questions at the time regarding the notifier's GRAS conclusion.

[2] Chr. Hansen states that the safety of *E. coli* BL21(DE3) strain DSM 33609 is summarized in the supplement to GRN 000571. The subject of the supplement to GRN 000571 is 2′-FL. It describes the construction of *E. coli* BL21(DE3) strain DSM 33609 which is used to produce 2′-FL, and the addition of

genetically engineered to produce 2′-FL from the host strain, *E. coli* BL21(DE3).[3] As described in GRN 000571, and incorporated into this notice, all heterologous genes encoding for sugar transport and metabolism were introduced into the genome of the host strain. As described further in GRN 000571, *E. coli* BL21(DE3) strain DSM 33609 does not contain plasmids or other episomal vectors and is not capable of DNA transfer to other organisms. Chr. Hansen states that *E. coli* BL21(DE3) strain DSM 33609 is non-pathogenic and non-toxigenic, and is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) strain collection in Braunschweig, Germany.

Chr. Hansen states that the manufacturing process for 2′-FL is the same as described in GRN 000571 and its supplement, except cobalt is no longer used in the fermentation medium, and incorporates that information into this notice. First, the production organism is inoculated into a fermentation medium that contains lactose. Fermentation of lactose results in the production and secretion of 2′-FL into the fermentation medium. Once a specified level of 2′-FL is reached, the culture supernatant containing 2′-FL is separated from the microbial biomass by filtration. Food-grade lactase may be used to degrade excess lactose. The filtrate is subjected to a series of cationic and anionic ion exchange resins to remove impurities (e.g., proteins, DNA, organic acids, and inorganic salts). The eluent, which contains 2′-FL, is then concentrated by evaporation and subjected to multiple purification steps including treatment with activated carbon, electrodialysis, ion exchange chromatography, and simulated moving bed chromatography (removal of mono- and di-saccharides) to decolorize and remove impurities. The resulting 2′-FL solution is concentrated, subjected to sterile filtration, and spray-dried. Chr. Hansen states that 2′-FL is manufactured in accordance with good manufacturing practices. Chr. Hansen explains that all starting materials, processing aids, and food contact materials are the same as described in GRN 000571 and are food-grade and used in accordance with U.S. regulations.

Chr. Hansen provides specifications for 2′-FL, including the amount of 2′-FL (≥ 90%, dry weight); and limits for carbohydrates (expressed as area %), including lactose (≤ 5 %), 3-fucosyllactose (≤ 5 %), difucosyllactose (≤ 5 %), fucosylgalactose (≤ 3 %), glucose (≤ 3 %), galactose (≤ 3 %), and fucose (≤ 3 %). Additional specifications include protein content (≤ 100 µg/g), ash (≤ 0.5 %), moisture (≤ 9.0 %); limits for heavy metals including, lead (≤ 0.02 mg/kg); and limits for microorganisms including, *Salmonella* serovars (absent in 25 g) and *Cronobacter* spp.[4] (absent in 10 g). Chr. Hansen provides analytical results from the analyses of five non-consecutive batches to demonstrate that 2′-FL can be manufactured to meet the specifications. Chr. Hansen states that 2′-FL is

food-grade lactase at the end of the fermentation to remove excess lactose. We evaluated this supplement and responded in a letter dated November 8, 2019, stating that we had no questions at the time regarding the notifier's GRAS conclusion.

[3] Chr. Hansen states that the safety of *E. coli* BL21(DE3) is summarized in GRN 000571 and the supplement to GRN 000571.

[4] In an amendment dated May 31, 2022, Chr. Hansen clarified that the specification is for *Cronobacter* spp., and is performed using ISO 22964. Chr. Hansen stated that if any *Cronobacter* spp. are detected, the batch will not be released, and further analysis will be performed to identify the species.

stable for 2 years when stored under ambient conditions.

Chr. Hansen provides an eaters-only dietary exposure estimate to 2′-FL from the intended uses using food consumption data from the 2015-2016 National Health and Nutrition Examination Survey. Chr. Hansen estimates dietary exposure to 2′-FL to be 0.10 g/person (p)/d at the mean and 0.18 g/p/d at the 90th percentile for infants 0-6 months of age, and 0.33 g/p/d at the mean and 0.78 g/p/d at the 90th percentile for infants 7-12 months of age. Chr. Hansen estimates dietary exposure to 2′-FL to be 0.97 g/p/d at the mean and 2.19 g/p/d at the 90th percentile for young children aged 13 months to years and 2.16 g/p/d at the mean and 5.26 g/p/d at the 90th percentile for the U.S. population aged 2 years and older.

Chr. Hansen indicates that the intended uses of 2′-FL include new uses, as well as some uses that will be substitutional for other forms of 2′-FL currently in use in conventional foods, and also increases in use levels for some current uses. Chr. Hansen applied the maximum use level for all food uses of 2′-FL, including uses in GRN 001014 as well as uses in earlier GRNs for 2′-FL (GRNs 000546, 000571, 000650, 000735, 000749, 000852, and 000897)[5] to estimate a cumulative dietary exposure to 2′-FL. For the U.S. population aged 2 years and older, the cumulative dietary exposure to 2′-FL is estimated to be 2.50 g/p/d at the mean and 5.16 mg/p/d at the 90th percentile. Chr. Hansen concludes that the intended uses do not significantly increase the dietary exposure to 2′-FL.

Chr. Hansen states that published *in vivo* studies in rats and human infants demonstrate that 2′-FL is highly resistant to digestion by digestive enzymes in the gut; only small amounts are absorbed intact and are excreted unchanged in the urine. 2′-FL is partially fermented by gut microbiota and is mostly excreted unchanged in the feces. Chr. Hansen notes that several 90-day oral subchronic studies in rats show that up to 5 g/kg body weight (bw)/d of 2′-FL is safe when orally consumed. A published study in two day old Yorkshire crossbred piglets demonstrates that the piglets tolerated up to 8 g/L of a mixture of human milk oligosaccharides (HMOs), 2′-FL, 3-fucosyllactose, lacto-*N*-tetraose, 3′-sialyllactose, and 6′-sialyllactose when fed for 21 days; the HMO mix contained 49.1% 2′-FL.[6] Chr. Hansen states that several published studies demonstrate that 2′-FL is not mutagenic or genotoxic.

Chr. Hansen briefly summarizes several published human studies designed to investigate the tolerability of 2′-FL. Collectively, these studies show that HMOs, including 2′-FL, are well tolerated in adults up to 20 g/d. Citing published studies that were discussed in several previous GRAS notices, Chr. Hansen states that the ingestion of up to 20 g/d of either 2′-FL, lacto-*N*-neotetraose (LNnT), or a combination of 2′-FL

[5] The subjects of GRNs 000546, 000650, 000735, 000749, 000852, and 000897 are 2′-FL. We evaluated these notices and responded in letters dated September 16, 2015, November 23, 2016, April 6, 2018, April 23, 2018, November 15, 2019, and June 12, 2020, respectively, stating that we had no questions at the time regarding the notifiers' GRAS conclusions.

[6] FDA did not evaluate the use of 2′-FL in combination with other HMOs during our evaluation of GRN 001014.

and LNnT in healthy adults as well as in adults with inflammatory bowel disease, ulcerative colitis, Crohn's disease, or celiac disease is generally well tolerated.

Chr. Hansen includes the statement of a panel of individuals (Chr. Hansen's GRAS panel). Based on its review, Chr. Hansen's GRAS panel concluded that 2′-FL is safe under the conditions of its intended use.

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

Standards of Identity

In the notice, Chr. Hansen states its intention to use 2′-FL in several food categories, including foods for which standards of identity exist, located in Title 21 of the 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 (effective January 1, 2023)) or a food ingredient that contains protein derived from one of those foods. 2′-FL derived from lactose 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 Chr. Hansen'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 Chr. Hansen provided, as well as other information available to FDA, we have no questions at this time regarding Chr. Hansen'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 001014 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2022.07.15 15:36:55 -04'00'

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