Manki Ho, Ph.D.
Chr. Hansen A/S
Boege Allé 10-12
2970 Hoersholm
DENMARK

Re: GRAS Notice No. GRN 001099

Dear Dr. Ho:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001099. We received Chr. Hansen A/S (Chr. Hansen)'s notice on May 19, 2022, and filed it on January 27, 2023. Chr. Hansen submitted an amendment to the notice on July 26, 2023, that clarified the intended use.

The subject of the notice is 3-fucosyllactose (3-FL) for use as an ingredient in cow milk-, soy-, and partially hydrolyzed protein-based, non-exempt infant formulas for term infants up to 0.9 g/L of formula (as consumed); formula intended for young children aged 1 to 3 years up to 1.2 g/L of formula (as consumed); other drinks and foods for infants and young children under 3 years of age that include yogurt and juice beverages up to 0.44 g/kg and hot cereals, crackers, pretzels, cookies, and snack items up to 4.4 g/kg; cereal, granola, energy, protein, and meal replacement bars; enhanced and "fortified" waters; sports, isotonic and "energy" drinks; breakfast cereals; fermented milk, flavored milk and mixes; smoothies, yogurts, meal replacement beverages (milk- and non-milk based), and milk substitutes; fruit juices and nectars; fruit-flavored beverages and vegetable juices; and gummy candies[1] at maximum levels ranging from 0.26 to 8.8 g/kg; and in oral and enteral tube feeding formulas (11 years and older) up to 6.6 g/L (as consumed).[2] The notice informs us of Chr. Hansen's view that these uses of 3-FL are GRAS through scientific procedures.

Chr. Hansen describes 3-FL as a white to ivory-colored powder containing ≥90% 3-FL and minor amounts of lactose, glucose, galactose, and fucose. The chemical name for 3-FL is 6-deoxy-α-L-galactohexopyranosyl-(1→3)-[β-D-galactohexopyranosyl-(1→4)]-D-glucohexopyranose (CAS Registry Number 41312-47-4). 3-FL is a trisaccharide composed of L-fucose, D-galactose, and D-glucose units. Chr. Hansen states that 3-FL is structurally identical to the 3-FL present in human milk.

[1] We consider the category of gummy candy to be a food use and does not include use in dietary supplement products.
[2] Chr. Hansen states that 3-FL is not for food use where standards of identity do not permit its addition.

Chr. Hansen describes the production organism used in the manufacturing process for 3-FL. The non-pathogenic and non-toxigenic production organism, *Escherichia coli* BL21 (DE3) strain JBT-3FL, is genetically engineered to produce 3-FL. The strain is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) strain collection in Braunschweig, Germany and is a modification of the host strain *E. coli* BL21 (DE3).[3]

Chr. Hansen discusses the manufacture of 3-FL, noting it is manufactured using the same raw materials, processing aids, food contact substances, and processes as in GRN 000925;[2] therefore, they incorporate that information into the notice. Chr. Hansen describes a three-step production process for 3-FL. First, the production strain is inoculated into a minimal medium containing a carbon source (glucose, sucrose, or glycerol) and the substrate cow milk-derived lactose. During fermentation, 3-FL is produced and secreted into the culture medium. Following fermentation, the production strain is removed from the medium. In the second step, 3-FL is purified from the medium in a series of filtration, ion exchange, electrodialysis, and decolorization steps that yield a 3-FL concentrate. In the last step, the 3-FL concentrate is spray dried to obtain the final 3-FL powder. Chr. Hansen states that all materials used in the manufacturing process are authorized for their respective uses in the U.S. and that 3-FL is manufactured following current good manufacturing practices.

Chr. Hansen provides specifications for 3-FL, which include the minimum content of 3-FL (≥90%) and limits for lactose (≤5%), glucose (≤3%), galactose (≤3%), fucose (≤3%), protein (≤100 mg/kg), ash (≤1%), moisture (≤9%), lead (≤0.02 mg/kg), and microorganisms, including *Salmonella* serovars (absent in 25 g) and *Cronobacter sakazakii* (absent in 10 g). Chr. Hansen provides the results of three non-consecutive batch analyses to demonstrate that 3-FL can be manufactured to meet the specifications. Based on stability studies described in GRN 000925 conducted on a mixture of human milk oligosaccharides (HMOs) containing 3-FL as a component, Chr. Hansen states that the shelf-life is two years when stored under ambient conditions.

Chr. Hansen estimates the dietary exposure to 3-FL based on the intended uses and food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES). Chr. Hansen estimates the mean and 90th percentile eaters-only dietary exposures to 3-FL for the U.S. population aged 2 years and older to be 0.7 g/person (p)/d (11 mg/kg body weight (bw)/d) and 1.6 g/p/d (26 mg/kg bw/d), respectively. Chr. Hansen estimates the mean and 90th percentile eaters-only dietary exposures to 3-FL for infants up to 6 months of age to be 0.8 g/p/d (123 mg/kg bw/d) and 1.2 g/p/d (199 mg/kg bw/d), respectively. Chr. Hansen estimates the mean and 90th percentile eaters-only dietary exposures to 3-FL for infants 7 to <12 months of age to be 0.9 g/p/d (97 mg/kg bw/d) and 1.7 g/p/d (187 mg/kg bw/d), respectively. Chr. Hansen estimates the mean and 90th percentile eaters-only dietary exposures to 3-FL for children 1 to 3 years of age to be 0.5 g/p/d (40 mg/kg bw/d) and 1.2 g/p/d (91 mg/kg

[3] Chr. Hansen states that the safety of *E. coli* BL21 (DE3) is summarized in GRNs 000485, 000571, and 000925, the subjects of which are β-galactosidase enzyme preparation, 2′-FL, and 3-FL, respectively. We evaluated these notices and responded in letters dated April 15, 2014, November 6, 2015, and February 8, 2021, respectively, stating that we had no questions at the time regarding the notifiers' GRAS conclusions.

bw/d), respectively.[4]

Chr. Hansen discusses data and information to support the safety of 3-FL, stating that no changes to the manufacturing process or production strain have occurred and that the specifications are identical to the subject of GRN 000925. Chr. Hansen states that considering the structural equivalence of the subject of the notice to 3-FL found in human milk, it is expected that the absorption, distribution, metabolism, and excretion will be similar to that in infants consuming 3-FL through human milk.

Chr. Hansen describes published toxicological studies with the subject of the notice as a component of a mixture of other HMOs to support safety. These include a bacterial reverse mutation assay, *in vitro* micronucleus assay, 13-week oral toxicity study in rats, and a tolerance study in neonatal piglets. In addition, Chr. Hansen describes published toxicological studies with 3-FL from other manufacturers to further support safety. Chr. Hansen further describes human studies with their HMO mixtures containing 3-FL at 0.75 g/L to support their GRAS conclusion, including a published study to evaluate safety and tolerability in healthy term infants and an unpublished growth monitoring study in healthy term infants.

Chr. Hansen states that the intended uses are similar to those described in GRNs 000925 and 000951.[5] Chr. Hansen further states that although the intended use levels for 3-FL in infant formula and formula for children >12 months of age are higher than those indicated in the above GRNs, the use levels continue to be within concentrations reported in human milk and that the 90th percentile estimated daily dietary exposure for infants is within the estimated dietary exposure to 3-FL by infants consuming this HMO through human milk. Chr. Hansen states that the manufacturers of oral and enteral tube feeding formulas will ensure that the products are formulated in a manner that minimizes the potential for adverse events in the specific population for whom the

[4] The intended uses described in GRN 001099 include a food category not previously evaluated (i.e., gummy candies), as well as increased use levels in several food categories (i.e., non-exempt infant formula, formula for young children, and meal replacement beverages) but does not include the higher intended use levels for other food categories that were described in GRN 001037. The subject of GRN 001037 is 3-FL. We evaluated this notice and responded in a letter dated November 7, 2022, stating that we had no questions at that time regarding the notifier's GRAS conclusion. GRN 001037 was still pending when we received GRN 001099. Food categories described in GRN 001037 with higher maximum use levels include foods for infants and young children; enhanced and fortified waters; sports, isotonic, and "energy" drinks; cereal and granola bars; flavored and fermented milks; yogurt; and fruit flavored drinks and ades. We estimated the cumulative dietary exposure to 3-FL based on the food uses in both GRNs 001037 and 001099, the maximum use levels in those food categories, and food consumption data from the 2017-2018 NHANES. The estimated mean and 90th percentile eaters-only dietary exposures for the infant populations are not significantly different from estimates provided by Chr. Hansen in GRN 001099. Higher dietary exposure estimates were observed for children and adults; however, dietary exposures for these populations are similar to the estimates evaluated in GRN 0001037. The mean and 90th percentile, eaters-only, dietary exposures for children 1 to 3 years of age are 0.9 g/p/d (67 mg/kg bw/d) and 1.9 g/p/d (139 mg/kg bw/d), respectively, and for ages 2 years and older are 1.1 g/p/d (19 mg/kg bw/d) and 2.3 g/p/d (43 mg/kg bw/d), respectively. These results do not contradict the notifier's conclusion that 3-FL is GRAS under the intended conditions of use.

[5] 3-FL is the subject of GRN 000951. We evaluated this notice and responded in a letter dated August 12, 2021, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

products are intended.

Based on the totality of the data and information, Chr. Hansen concludes that 3-FL is GRAS for its intended use.

Standards of Identity

In the notice, Chr. Hansen states its intention to use 3-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, & 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 3-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. 3-FL from lactose may require labeling under the FD&C Act because it may contain protein derived from milk from the fermentation process. 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 Chr. Hansen's GRAS notice does not alleviate the responsibility of any infant formula manufacturer that intends to market an infant formula containing 3-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 Chr. Hansen's notice concluding that 3-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 3-FL. Accordingly, our response should not be construed to be a statement that foods containing 3-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 3-FL is GRAS under its intended conditions of use. This letter is not an affirmation that 3-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 001099 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.08.29 17:55:16 -04'00'

Susan J. Carlson, Ph.D.
Director
Division of Food Ingredients
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