



Gavin Thompson, Ph.D.
Ramboll Environment & Health
2111 East Highland Ave., Suite 402
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Re: GRAS Notice No. GRN 000929

Dear Dr. Thompson:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000929. We received the notice that you submitted on behalf of Jennewein Biotechnologie GmbH (Jennewein) on April 8, 2020 and filed it on June 19, 2020. Jennewein submitted amendments to the notice on October 19, 2020, and December 7, 2020, removing exempt infant formula for pre-term infants from the intended uses, removing the use of cobalt chloride in the fermentation medium, including results from analysis of five non-consecutive batches, amending the microbiological specifications, and providing additional clarifying information regarding the intended use, production organism, manufacturing process, exposure estimate, and safety narrative.

The subject of the notice is 2'-fucosyllactose (2'-FL) for use as an ingredient in exempt hypoallergenic infant formula for term infants and hypoallergenic toddler formula¹ at a level of 2 g/L of formula, as consumed. Jennewein states that hypoallergenic formula includes both extensively hydrolyzed cow milk protein- and amino acid-based formula. The notice informs us of Jennewein's view that this use of 2'-FL is GRAS through scientific procedures.

Jennewein discusses the identity of 2'-FL. The chemical name is α -L-fucopyranosyl-(1 \rightarrow 2)- β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucopyranoside (CAS Registry Number 41263-94-9). The molecular weight is 488.44 atomic mass units. Jennewein further notes that 2'-FL is the same ingredient that was the subject of GRN 000571.² In GRN 000571 Jennewein addressed use of 2'-FL in milk-based, non-exempt infant formula for term infants, and in toddler formula.

¹ While we do not have a regulatory definition for "toddler formula," we recognize it as formula intended for children > 12 months of age. Formulas for older infants (e.g., 9-12 months of age) would be included in the category of infant formula and must comply with the infant formula regulations under Section 412 of the Federal Food, Drug, and Cosmetic Act (FD&C Act).

² 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.

Jennewein describes the production organism used in the manufacturing process for 2'-FL. The production organism, *Escherichia coli* BL21 (DE3) strain DSM 33609,³ is genetically engineered to produce 2'-FL from the host strain, *E. coli* BL21 (DE3).⁴ Jennewein explains that all heterologous genes encoding for sugar transport and metabolism were introduced into the genome of the host strain. Jennewein states that *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. Jennewein states that *E. coli* BL21 (DE3) strain DSM 33609 is non-pathogenic and non-toxicogenic, and is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) strain collection in Braunschweig, Germany.

Jennewein states that the manufacturing process for 2'-FL is the same as described in GRN 000571, and its supplement, and incorporates that information into this notice. First, the production organism is inoculated into a fermentation medium that contains lactose. Once a specified level of 2'-FL is reached, the culture supernatant containing the 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, electro dialysis, ion exchange chromatography, simulated moving bed chromatography (removal of mono- and di-saccharides), and ultrafiltration to further decolorize and remove impurities. The resulting 2'-FL solution is concentrated, subjected to sterile filtration, and spray-dried to a solid white powder.

Jennewein states that 2'-FL is manufactured in accordance with current good manufacturing practices. The filtration materials, fermentation media components, and ion exchange materials are used in accordance with regulations for direct and indirect ingredients.

Jennewein provides specifications for the 2'-FL spray-dried powder,⁵ including level of 2'-FL (≥ 90 % dry weight) and limits on minor carbohydrates (expressed as area %), including lactose (≤ 5 %), 3-fucosyllactose (≤ 5 %), difucosyllactose (≤ 5 %), fucosylgalactose (≤ 3 %), glucose (≤ 3 %), galactose (≤ 3 %), and fucose (≤ 3 %). Additional specifications include moisture (≤ 9 %), lead (≤ 0.02 mg/kg), ash (≤ 0.5 %),

³ Jennewein 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 food-grade lactase at the end of 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.

⁴ Jennewein states that the safety of *E. coli* BL21 (DE3) is summarized in GRNs 000485 and 000571. The subject of GRN 000485 is beta-galactosidase enzyme preparation. We evaluated this notice and responded in a letter dated April 15, 2014, stating that we had no questions at the time regarding the notifier's GRAS conclusion.

⁵ Although a 2'-FL liquid concentrate (45 % dry matter) is included in GRN 000571, the October 19, 2020 amendment removed the liquid concentrate form from the scope of GRN 000929.

protein (≤ 100 mg/kg), and limits on microorganisms including, *Salmonella* serovars (absent in 25 g) and *Cronobacter sakazakii* (absent in 10 g).⁶ Jennewein provides the results of five non-consecutive batch analyses to demonstrate 2'-FL can be manufactured to meet specifications. 2'-FL is stable for 2 years at 25 °C.

Jennewein addresses the potential for residual milk protein in 2'-FL from use of milk-derived lactose in the fermentation medium, by presenting results from silver-staining sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for protein, enzyme-linked immunosorbent assay (ELISA) for casein and whey, and size exclusion chromatography with UV detection for milk protein. Based on these results and the steps included during manufacturing, Jennewein concludes that the allergenic potential of 2'-FL is extremely low and no sensitive populations have been identified or are anticipated.

Jennewein estimates the dietary exposure to 2'-FL based on the maximum intended use in hypoallergenic infant and toddler formulas and consumption data from the 2015-2016 National Health and Nutrition Examination Survey (NHANES). Jennewein states that the highest consumers-only mean and 90th percentile dietary exposures to 2'-FL are in infants aged 0-5 months (1.9 g/person (p)/d and 2.5 g/p/d, respectively). The notifier also reports mean and 90th percentile consumers-only dietary exposures in infants aged 6 to 11 months, and in toddlers (12 to 35 months of age) to be 1.7 g/p/d and 2.4 g/p/d, and 0.8g/p/d and 1.2 g/p/d, respectively. Jennewein estimates cumulative dietary exposure to 2'-FL from the intended uses and current uses in foods, excluding dairy-based foods, consumed by infants and toddlers consuming hypoallergenic formula. Cumulative estimates at the mean and 90th percentile, respectively, are 1.9 g/p/d and 2.6 g/p/d (310 mg/kg body weight (bw)/d and 403 mg/kg bw/d) for infants 0-5 months, 2.0 g/p/d and 2.9 g/p/d (230 mg/kg bw/d and 320 mg/kg bw/d) for infants 6-11 months, and 0.7 g/p/d and 1.4 g/p/d (62 mg/kg bw/d and 130 mg/kg/d) for toddlers 12-35 months of age.

Jennewein discusses the safety of 2'-FL and states that its 2'-FL is chemically equivalent to the 2'-FL present in human milk. Jennewein incorporates the safety data and information discussed in GRN 000571 and GRN 000735⁷ into the notice by reference.

Jennewein discusses absorption, distribution, metabolism and excretion of 2'-FL. Jennewein states that 2'-FL is poorly absorbed, and the majority of ingested 2'-FL becomes directly available to gut microbiota for metabolism into short-chain fatty acids. Jennewein further discusses published toxicological and human studies to demonstrate the safety of 2'-FL. Jennewein concludes that 2'-FL is not genotoxic based on Ames, *in vitro* micronucleus, *in vivo* micronucleus, and mouse lymphoma assays. Jennewein states that 2'-FL from other sources did not induce any toxicologically relevant adverse effects in repeat-dose oral toxicity studies in rats. No Observed Adverse Effects Levels

⁶ In the October 19, 2020 amendment, Jennewein revised the specifications for *Salmonella* serovars and *C. sakazakii* from absent in 100 g to absent in 25 g and absent in 10 g, respectively.

⁷ The subject of GRN 000735 is 2'-FL. We evaluated this notice and responded in a letter dated April 6, 2018, stating that we had no questions at the time regarding the notifier's GRAS conclusion.

(NOAELs) from these studies range from 5 g/kg bw/d to 7.25 g/kg bw/d. Jennewein's 2'-FL showed no treatment-related adverse effects in a published neonatal pig study; the NOAEL from this study was 292 mg/kg bw/d in male piglets and 299 mg/kg bw/d in female piglets. Jennewein further states that in a 90-day rat oral toxicity study with Jennewein's 2'-FL, the NOAEL was determined to be 7.66 g/kg bw/d. Jennewein states that up to 1.2 g 2'-FL/L formula was well-tolerated in infants, and there was no evidence of increased adverse events or alterations in normal growth. Jennewein discusses a published clinical study conducted in infants and young children with cow milk protein allergy who were fed an extensively hydrolyzed formula supplemented with 2'-FL and lacto-N-neotetraose (LNnT). Jennewein states this study provides evidence that a formula supplemented with 1.0 g/L 2'-FL and 0.5 g/L LNnT is well-tolerated and thus suitable for use in formulas for infants, toddlers, and young children with cow milk protein allergy.⁸ Finally, Jennewein states that most breast-fed infants, including infants with cow milk allergy, have a history of dietary exposure to 2'-FL present in human milk.

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

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

Potential Labeling Issues

Under section 403(a) of the 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 (CFSAN). 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 eight foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. 2'-FL derived from

⁸ Jennewein also discussed toxicity and clinical studies of mixtures of 2'-FL and other ingredients, such as difucosyllactose and LNnT. We did not evaluate the use of 2'-FL in combination with other ingredients during our review of GRN 000929.

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 in CFSAN.

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 Jennewein'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 in CFSAN.

Section 301(II) of the FD&C Act

Section 301(II) 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(II)(1)-(4) applies. In our evaluation of Jennewein's notice concluding that 2'-FL is GRAS under its intended conditions of use, we did not consider whether section 301(II) 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(II).

Conclusions

Based on the information that Jennewein provided, as well as other information available to FDA, we have no questions at this time regarding Jennewein'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 000929 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

**Susan J.
Carlson -S**

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Susan Carlson, Ph.D.

Director

Division of Food Ingredients

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Center for Food Safety

and Applied Nutrition