



Dietrich B. Conze, Ph.D.
Spherix Consulting Group, Inc.
11821 Parklawn Drive, Suite 310
Rockville, MD 20852

Re: GRAS Notice No. GRN 000925

Dear Dr. Conze:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000925. We received the notice that you submitted on behalf of Jennewein Biotechnologie GmbH (Jennewein) on March 23, 2020, and filed it on May 15, 2020. We received amendments to the notice on October 23, 2020, November 5, 2020, and November 30, 2020, that reduced the intended use level, removed the use of cobalt chloride in the fermentation medium, updated the dietary exposure assessment, and clarified information about the production organism and safety data.

The subject of the notice is 3-fucosyllactose (3-FL) for use as an ingredient in cow milk-based, non-exempt infant formula for term infants at a level of 0.44 g/L infant formula as consumed. The notice informs us of Jennewein's view that this use of 3-FL is GRAS through scientific procedures.

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

Jennewein describes the production organism used in the manufacturing process for 3-FL. The non-pathogenic and non-toxicogenic production organism, *Escherichia coli* BL21 (DE3) strain DSM 33491 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).¹ Jennewein constructed the production strain after deleting a gene

¹ Jennewein states that the safety of *E. coli* BL21 (DE3) is summarized in GRNs 000485 and 000571. The subject of GRN 000485 is β -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. The subject of GRN 000571 is 2'-fucosyllactose (2'-FL). FDA evaluated GRN 000571 and responded in a letter dated November 6, 2015, stating that the agency had no questions at that time regarding the notifier's GRAS conclusion

fragment² in the host strain. Following the deletion, Jennewein made five insertions of genes encoding functions for sugar metabolism from two donor species to optimize the production of 3-FL. Jennewein states that all gene insertions were verified by polymerase chain reaction.

Jennewein states that 3-FL is manufactured using the same raw materials, processing aids, food contact substances, and processes as those described in GRN 000571 and incorporates that information into this notice. Jennewein explains the three-step production of 3-FL. First, the production strain is inoculated into a minimal medium containing a carbon source (glucose, sucrose, glycerol, or a combination thereof) and the substrate cow milk-derived lactose. Fermentation of lactose results in the production and secretion of 3-FL into the culture medium. Following the fermentation, the production strain is removed yielding 3-FL-containing fermentation medium. The second step involves purification of 3-FL from the medium in a series of filtration, ion exchange, electrodialysis, and decolorization steps. Lactase may be added at the end of the process to degrade excess lactose. In the last step of the production, the resulting 3-FL concentrate is spray dried yielding the final product as a powder. Jennewein states that all materials used in the manufacturing processes are authorized for their respective uses in the U.S. and that 3-FL is manufactured following current good manufacturing practices.

Jennewein provides specifications for 3-FL that include the minimum content of 3-FL ($\geq 90\%$) and limits for lactose ($\leq 5\%$), glucose ($\leq 3\%$), galactose ($\leq 3\%$), fucose ($\leq 3\%$), protein (≤ 100 mg/kg), ash ($\leq 1\%$), moisture ($\leq 9\%$), lead (≤ 0.02 mg/kg), and microorganisms, including *Salmonella* serovars (absent in 25 g) and *Cronobacter sakazakii* (absent in 10 g). Jennewein provides the results of the analysis of three non-consecutive batches to demonstrate that 3-FL can be manufactured to meet the specifications. Jennewein provides the results of stability studies conducted on a mixture of human milk oligosaccharides (HMOs) containing 3-FL as a component and states that the results support a two-year shelf-life for 3-FL when stored under ambient conditions.

Jennewein estimates dietary exposure to 3-FL for infants aged 8–195 days based on published data on daily energy intakes, a typical caloric density of infant formula (670 kcal/L as consumed), and the assumption that infant formula is the sole source of nutrition for infants. The resulting dietary exposure to 3-FL for infants ranges from 0.06 g/kg body weight (bw)/d to 0.08 g/kg bw/d at the mean and from 0.07 g/kg bw/d to 0.09 g/kg bw/d at the 90th percentile. Jennewein notes that the estimates derived for intervals within the age range of 8–195 days indicate that the consumption of 3-FL (on a kg bw basis) from infant formula decreases with infant age. Jennewein further states that, based on published data, the actual total daily energy intake from infant formula in infants aged 6–11.9 months decreases compared to infants aged 0–5.9 months. Based on the above, Jennewein concludes that dietary exposure to 3-FL for infants aged 7–12 months is expected to be lower than that for infants aged 8–195 days.

² Jennewein states that the deletion removes a non-functional fragment of an endogenous β -galactosidase gene.

Jennewein discusses the safety of 3-FL and states that the intended use level is within the established range of 3-FL that naturally occurs in human milk.³ Jennewein notes that 3-FL is resistant to digestive enzymes in the gastrointestinal tract and only small amounts are absorbed intact. The remaining unabsorbed 3-FL passes through the gastrointestinal tract where it is either fermented by the microbiota or excreted unchanged in the feces. Jennewein states that their 3-FL is compositionally and quantitatively similar to the 3-FL from another manufacturer which is the test article in published safety studies. Jennewein, therefore, states that these published safety studies also support the safety of their 3-FL ingredient. Jennewein provides summaries of these studies including an acute oral toxicity study and a 90-day repeated dose feeding toxicity study in rats as well as genotoxicity studies demonstrating no toxicologically relevant effects. Jennewein also discusses several corroborative studies in which 3-FL, manufactured by Jennewein, was tested in combination with other HMOs, including a published 90-day oral toxicity study in rats, an unpublished 21-day tolerance study in neonatal piglets, and published genotoxicity studies.⁴ Jennewein states that all relevant published safety data pertaining to 3-FL were evaluated, noting that a literature search, conducted through January 2020, revealed no clinical studies where 3-FL was administered to humans.

Jennewein includes the statement of a panel of individuals (Jennewein's GRAS panel). Based on its review, Jennewein's GRAS panel concluded that 3-FL is safe under the conditions of its intended use.

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

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 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 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.

³ In an amendment received on October 23, 2020, Jennewein provided an additional analysis of the published levels of 3-FL in human milk and states that the intended use level of 0.44 g/L falls below the mean of the reported means/medians in the included studies.

⁴ We did not evaluate the use of 3-FL in combination with other HMO ingredients during our review of GRN 000925.

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. 3-FL derived from lactose requires labeling under the FD&C Act because it contains protein derived from milk.

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 3-FL to make the submission required by section 412. Infant formulas are the purview of ONFL.

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 3-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 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(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 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

000925 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

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Director
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