U.S. FOOD & DRUG ADMINISTRATION
Human Foods Program

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CHINA

Re: GRAS Notice No. GRN 001298

Dear Dr. Zhang:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001298. We received Beijing Zeno Technology Development Co., Ltd. (Zeno)'s notice on July 16, 2025, and filed it on January 22, 2026. Zeno submitted amendments to the notice on April 15, 2026, and May 5, 2026, containing additional information on the identity, manufacturing process, production organism, specifications, intended use, dietary exposure, and safety narrative.

The subject of the notice is 2'-fucosyllactose (2'-FL), produced by fermentation using *Escherichia coli* "ZNO2F2", for use as an ingredient in the foods, including infant formula, at the maximum use levels shown in Table 1 (excluding use in products under the jurisdiction of the United States Department of Agriculture or in foods where standards of identity preclude its use). The notice informs us of Zeno'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/L or g/kg)**
Non-exempt infant formulas for term infants[1]	2.4
Formula intended for young children (>12 months)	2.4
Enhanced or fortified waters	1.2
Sports, isotonic, and "energy" drinks	1.2
Non-dairy smoothies and meal replacement beverages (ready-to-drink and powder forms)	5
Hot breakfast cereals (prepared)	31
Ready-to-eat (RTE) breakfast cereals – puffed	80
RTE cereals – high fiber	40

[1] Zeno states that the use of 2'-FL in non-exempt infant formula is not restricted to any specific protein base (e.g., cow milk-based, soy-based).

Food Categories	**Maximum Use Levels (g/L or g/kg)**
RTE cereals – biscuit type	40
Milk substitutes (soy milk and imitation milks)	1.2
Non-dairy yogurts	12
Frozen dairy-based desserts	17
Cereal bars including snack bars, granola bars and breakfast bars	30
Meal replacement bars for general use	30
Dairy-based puddings, custards, and mousses	17
Fruit pie filling	14.1
Fruit filling in bars, cookies, yogurt, and cakes	30
Other foods for infants and young children	12
Yogurt and juice beverages for infants and young children	10
Baby snacks (crackers, pretzels, cookies, and other dry snack items)	57
Jellies and jams, fruit preserves, and fruit butters	60
Dairy smoothie and meal replacement beverages	5
Flavored and fermented milk	1.2
Unflavored pasteurized and sterilized milk	1.2
Yogurt	12
Nutritional drinks for pregnant women	6
Fruit juices, drinks, and nectars	1.2
Vegetable juices	1.2
Syrups used to flavor milk beverages	7
Oral nutritional supplements and enteral tube feeding Formulas for ages ≥11 years	20

Zeno provides information on the identity and composition of 2' -FL. Zeno describes 2'-FL as a white to off white powder containing a minimum of 94% 2'-FL (dry weight basis (DW)). Zeno states that 2'-FL is a fucosylated oligosaccharide composed of L-fucose, D-galactose, and D-glucose. The chemical name for 2'-FL is a α-L-fucopyranosyl-(1→2)-ß-D-galactopyranosyl-(1→4)-D-glucopyranose and the CAS registry number is 41263-94-9. Zeno states that 2'-FL-is chemically and structurally identical to the 2'-FL from human milk, as confirmed by proton nuclear magnetic resonance spectroscopy.

Zeno describes the production organism used in the manufacturing process for 2'-FL. The production organism, *E. coli* "ZNO2F2", was constructed through genetic engineering of the *E. coli* C43 (DE3) host strain using a plasmid based expression system to allow for the efficient production of 2'-FL. Zeno states that all genetic modifications are verified by whole genome sequencing and that the strain is non-pathogenic and non-toxigenic.

Zeno describes a two-stage manufacturing process, which includes an upstream fermentation stage and a downstream purification stage. In the first stage, 2'-FL is produced by fermentation of *E. coli* "ZNO2F2" under controlled conditions. After

fermentation is complete, the fermentation broth undergoes a series of purification steps, including sterilization, decolorization with activated carbon, heat treatment, and filtration through a plate-and-frame filter press to remove the production organism and other impurities. The filtrate containing 2'-FL is purified by ultrafiltration to remove DNA and proteins, concentrated using nanofiltration, decolorized using resin, and subjected to ion exchange to remove cations and anions. The resulting solution undergoes additional nanofiltration and low-temperature evaporation to yield a concentrated syrup which is further purified using chromatographic separation, resin decolorization, nanofiltration, and evaporation. The resulting concentrated solution is spray dried to produce the final 2'-FL powder. Zeno states that 2'-FL is manufactured according to current good manufacturing practices, and that all raw materials and processing aids are food-grade and are used in accordance with applicable U.S. regulations, are GRAS for their intended uses, or are the subject of an effective food contact notification.

Zeno provides specifications for 2'-FL, which include the minimum content of 2'-FL (≥94% DW) and limits on 3,2'-difucosyl-D-lactose (≤2.2% DW). D-lactose (≤3.3%), moisture (≤9%), protein (≤100 mg/kg), ash (≤0.5%), heavy metals, including lead (≤0.05 mg/kg), cereulide (<0.05 ug/kg), and microorganisms, including Salmonella serovars (absent in 25 g), Listeria monocytogenes (absent in 25 g) and Cronobacter sakazakii (absent in 10 g). Zeno provides the results from the analyses of three non-consecutive batches to demonstrate that 2'-FL can be manufactured to meet the specifications.

Zeno states that the intended uses of 2'-FL are substitutional for those described in GRN 001091[2] and incorporates the dietary exposure estimates from GRN 001091 into the notice. Zeno states that based on the intended uses and food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES), the estimated eaters-only dietary exposure to 2'-FL is 2.2 g/person (p)/d (324 mg/kg body weight (bw)/d) at the mean and 3.8 g/p/d (499 mg/kg bw/d) at the 90th percentile for infants aged 0-6 months, and 3.4 g/p/d (378 mg/kg bw/d) at the mean and 6.3 g/p/d (662 mg/kg bw/d) at the 90th percentile for infants aged 7-12 months. For children aged 1-3 years, the estimated eaters-only dietary exposure to 2'-FL is 2.6 g/p/d (193 mg/kg bw/d) and 4.9 g/p/d (380 mg/kg bw/d) at the mean and 90th percentile, respectively. For the U.S. population aged 2 years and older, the eaters-only dietary exposure to 2'-FL is 2.6 g/p/d (44 mg/kg bw/d) and 5.6 g/p/d (103 mg/kg bw/d) at the mean and 90th percentile respectively. Zeno states that the most recent cumulative dietary exposure to 2'-FL is reported in GRN 001051[3], which covers all current uses, including those from GRN 001091, and incorporates those cumulative dietary exposure estimates into the notice. Zeno states that because the intended uses are substitutional for the uses in GRN 001091, an increase in the cumulative dietary exposure to 2'-FL is not expected.

[2] 2'-FL was the subject of GRN 001091. We evaluated this notice and responded in letter dated December 01, 2023, stating that we had no questions at that time regarding the notifiers' GRAS conclusion.
[3] 2'-FL was the subject of GRN 001051. We evaluated this notice and responded in letter dated November 21, 2023, stating that we had no questions at that time regarding the notifiers' GRAS conclusion.

Zeno summarizes publicly available scientific data and information to support the safe use of 2'-FL. Zeno characterizes the notified 2'-FL ingredient as chemically and structurally equivalent to 2'-FL naturally present in human milk and compositionally similar to other 2'-FL ingredients that have been the subject of prior GRAS notices.

To support its GRAS conclusion, Zeno discusses information on the absorption, distribution, metabolism, and excretion of 2'-FL; background dietary exposure to 2'-FL through human milk; and toxicological studies previously reviewed in the context of prior GRAS notices for 2'-FL. The toxicological data reviewed include repeated-dose subchronic studies in rats, tolerability studies in neonatal piglets, and clinical studies in both infants and adults. Among these findings, Zeno notes that no adverse effects were observed in the subchronic studies in rats, and 2'-FL was safe and well-tolerated by infants and adults. Zeno also discusses new scientific literature identified through an updated literature search conducted through April 2026, encompassing multiple clinical studies in healthy infants and adults. Across these studies, Zeno notes that 2'-FL was well-tolerated and not associated with any adverse outcomes.

As further corroborative evidence in support of its GRAS conclusion, Zeno discusses unpublished toxicological studies conducted with the notified 2'-FL ingredient. These studies include a bacterial reverse mutation assay, an *in vitro* mammalian chromosomal aberration assay, an *in vivo* mammalian erythrocyte micronucleus study, and both a repeated-dose subchronic toxicity study and developmental toxicity study in rats. Zeno states that the genotoxicity studies demonstrated the notified 2'-FL ingredient is neither mutagenic nor clastogenic. Zeno also notes that no test-article related adverse effects were observed in either the subchronic or developmental toxicity studies.

Based on the available data and information, Zeno concludes that 2'-FL is GRAS for its intended uses.

Standards of Identity

In the notice, Zeno 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 Nutrition Center of Excellence (NCE). The Office of Pre-Market Additive Safety (OPMAS) 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 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 OPMAS. Questions related to food labeling in general should be directed to ONFL in NCE.

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 Zeno'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 the Office of Critical Foods in NCE.

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 Zeno'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 Zeno provided, as well as other information available to FDA, we have no questions at this time regarding Zeno'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 001298 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

SUSAN J. CARLSON -S

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