



Susan S. Cho, Ph.D.
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Re: GRAS Notice No. GRN 000932

Dear Dr. Cho:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000932. We received the notice that you submitted on behalf of Advanced Protein Technologies Corp. (APTech) on April 20, 2020 and filed it on July 22, 2020. APTech submitted amendments to the notice on October 23, 2020, and December 3, 2020, providing information regarding the production organism, purification processes, specifications, estimates of exposure, clarification on the intended uses and safety information.

The subject of the notice is 2'-fucosyllactose (2'-FL) for use as an ingredient in milk- and soy-based, non-exempt infant formula for term infants at a maximum level of 2.4 g/L of formula as consumed; in toddler formulas¹ and meal replacement drinks for children ages 1-3 years at a maximum level of 2.4 g/L, as consumed; in infant and toddler foods at maximum levels of 10.0 g/L in drinks, 10.9 g/kg in cereals and desserts, 57 g/kg in dry snacks; in beverages (sports and "energy" drinks, flavored waters, fruit juices and drinks, milk drinks, dairy analogs, milk-based meal replacements) at maximum levels ranging from 0.8-6 g/L; and in the following foods, at maximum levels ranging from 4.8-80 g/kg: breakfast cereals; frozen dairy desserts; puddings, fillings, mousses; yogurt; meal replacement and snack bars; syrups; and jams and jellies. The notice informs us of APTech's view that these uses of 2'-FL are GRAS through scientific procedures.

APTech characterizes the identity of 2'-FL, a trisaccharide composed of L-fucose and lactose (D-galactose and D-glucose). APTech notes that the systematic name for 2'-FL is α -L-fucopyranosyl-(1 \rightarrow 2)- β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucopyranose (CAS Registry Number 41263-94-9). The molecular weight is 488.44 atomic mass units.

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).

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APTech describes the two-stage manufacturing process for 2'-FL. The production organism, *Corynebacterium glutamicum* strain KCTC 13735BP, is genetically engineered to produce 2'-FL from the host strain, *C. glutamicum* strain ATCC 13032. APTech constructed the production organism by transforming a plasmid into the host strain, which contains four heterologous genes encoding functions for sugar metabolism and transport from two non-pathogenic and non-toxicogenic donor species² to optimize the production of 2'-FL. APTech states that the four inserted genes are well-characterized.³ APTech states that the production organism was assessed to be stable through 32 generations of fermentation, based on sequencing plasmid DNA extracted from multiple generations. APTech states that *C. glutamicum* strain KCTC 13735BP is non-pathogenic and non-toxicogenic, and is deposited in the strain collection of the Korean Collection for Type Cultures (KCTC) in the Republic of Korea.

APTech describes the production of 2'-FL from lactose and glucose by fermentation of *C. glutamicum* strain KCTC 13735BP in a medium that also contains yeast extract and minerals. APTech states that all raw materials and ingredients used in the fermentation medium are food grade. Upon completion of fermentation, the production organism is removed by microfiltration. The filtered supernatant containing 2'-FL is then subjected to further downstream purification processes including activated carbon treatment and ultrafiltration to remove color and residual cellular macromolecules, followed by nanofiltration to remove small molecules such as mono- and di-saccharides, amino acids, organic acids, minerals, and salts. An optional second activated carbon treatment may be performed, followed by ion-exchange chromatography, using both cationic and anionic exchange resins, to remove peptides, inorganic salts, and other impurities. The purified 2'-FL solution is then microfiltered, concentrated under vacuum, and crystallized by addition of acetic acid or ethanol. The 2'-FL crystals are dried, yielding a white to off-white/ivory powder.

APTech states that 2'-FL is produced in accordance with current good manufacturing practices. APTech further notes that filtration aids and ion exchange resins are authorized and suitable for their intended uses.

APTech provides specifications for 2'-FL, including the minimum content of 2'-FL (≥ 94 % on a dry weight basis) and limits for minor carbohydrates, expressed as area percent of total carbohydrates, including lactose (≤ 1 %), difucosyllactose (≤ 2 %), glucose (≤ 3 %), and galactose (≤ 3 %). Additionally, APTech provides limits for moisture (≤ 9 %), ash (≤ 0.5 %), protein (≤ 50 $\mu\text{g/g}$), heavy metals, including lead (≤ 0.02 mg/kg), and microorganisms, including *Cronobacter sakazakii* (absent in 10 g), and *Salmonella* serovars (absent in 25 g). APTech provides the results of five non-consecutive batch

² APTech states that the gene encoding α -1,2-fucosyltransferase (*α -1,2-ft*) is from *Pseudopedobacter saltans* strain ATCC 51119, while the remaining three genes encoding GDP-D-mannose-4,6-dehydratase (*gmd*), GDP-L-fucose synthase (*wcaG*), and lactose permease (*lacY*) are from *Escherichia coli* strain ATCC 700926 (also referred to as *E. coli* strain K12 MG1655).

³ Introduced enzymes GDP-D-mannose-4,6-dehydratase and GDP-L-fucose synthase enable conversion of GDP-D-mannose into GDP-L-fucose, lactose permease enables uptake of lactose into *C. glutamicum*, and α -1,2-fucosyltransferase enables transfer of a fucose moiety of GDP-L-fucose to lactose.

analyses of 2'-FL to demonstrate the ability to meet the stated specifications.

APTech summarizes its 9-month stability studies conducted under accelerated and room temperature storage conditions. APTech concluded that 2'-FL was stable in powder form and in 45 % solution over 9 months of storage, even under accelerated conditions.

APTech notes that the intended uses of 2'-FL are identical to those listed previously in GRN 000735,⁴ and cites the dietary exposure estimate presented in that notice. APTech also presents its own comprehensive dietary exposure estimates, including uses in GRN 000932 as well as background levels of 2'-FL from limited uses not included in GRN 000932 but addressed in earlier GRAS notifications for 2'-FL, and thus potentially part of the background diet. Using NHANES 2017-18 food consumption data and assuming maximum intended use levels, APTech estimates dietary exposures (eaters-only) to be 2.06 g/person (p)/d (0.33 g/kg body weight (bw)/d) at the mean and 3.17 g/p/d (0.53 g/kg bw/d) at the 90th percentile for infants 0-5 months of age, and 2.60 g/p/d (0.29 g/kg bw/d) at the mean and 4.95 g/p/d (0.53 g/kg bw/d) at the 90th percentile for infants 6-11 months of age. For children 1-3 years of age, the estimated dietary exposures to 2'-FL are 1.45 g/p/d (0.12 g/kg bw/d) and 2.23 g/p/d (0.19 g/kg bw/d) at the mean and 90th percentile, respectively. For the total population (all ages), estimates of dietary exposure are 1.77 g/p/d (0.034 g/kg bw/d) and 3.59 g/p/d (0.074 g/kg bw/d) at the mean and 90th percentile, respectively. Based on the similarity of cumulative dietary exposure estimates (GRN 000932 and background uses) to the dietary exposure estimates from intended uses in GRN 000932 only, APTech concludes that inclusion of potential uses outside the scope of the notice does not impact the overall dietary exposure to 2'-FL.

APTech states that its 2'-FL is chemically and structurally identical to the 2'-FL found in human milk and 2'-FL that were the subject of previous GRAS notices (GRNs 000546, 000571, 000650, 000735, and 000749).⁵ APTech notes that the proposed maximum use levels of 2'-FL in infant formula is comparable to the mean concentration of 2'-FL reported in human milk, and that the use levels in other food categories are similar to those proposed in previous 2'-FL GRAS notices. APTech states that an updated literature search through March 2020 did not identify any safety concerns of 2'-FL.

APTech states that 2'-FL is poorly absorbed via oral ingestion due to its resistance to digestion, and the majority of ingested 2'-FL is either partially fermented by intestinal microflora or excreted unchanged in the feces. APTech incorporates the safety data and information discussed in the previous 2'-FL GRNs, including two published 90-day toxicological studies in young rats. In one study, 2'-FL administered by gavage did not produce any treatment-related adverse effects at doses up to 5000 mg/kg bw/d. In the

⁴ 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.

⁵ The subjects of GRNs 000546, 000571, 000650, and 000749 are 2'-FL. We evaluated these notices and responded in letters respectively dated September 16, 2015, November 6, 2015, November 23, 2016, and April 23, 2018, stating that we had no questions at the time regarding the notifiers' GRAS conclusions.

other, 2'-FL did not elicit any toxicologically relevant effects at concentrations up to 10% in the diet, equivalent to an oral dose of ≥ 7250 mg/kg bw/d. Unpublished rat studies on APTech's 2'-FL showed similar results that supported the published studies. APTech also discusses a published 20-day oral study in neonatal piglets, in which the administration of 2'-FL in a milk replacement formula was well tolerated and supported normal growth with no adverse effects at concentrations up to 2000 mg/L.⁶

Based on published genotoxicity studies and three unpublished studies with APTech's 2'-FL, APTech concludes that 2'-FL is neither mutagenic nor genotoxic. Additionally, APTech summarizes corroborating safety information from published clinical studies in infants and adults in which no adverse effects were noted. APTech also evaluated the allergenicity potential of its 2'-FL and states that it is not likely to cause allergenic reactions. APTech states that the production microorganism, *C. glutamicum*, has been safely used in the production of an amino acid (GRN 000523)⁷ and a monosaccharide (GRNs 000400 and 000693)⁸.

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

Based on the totality of information, APTech concludes that 2'-FL is GRAS for its intended use.

Standards of Identity

In the notice, APTech 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 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

⁶ APTech also discussed toxicity and clinical studies with mixtures of 2'-FL and other ingredients, such as difucosyllactose. We did not evaluate the use of 2'-FL in combination with other ingredients during our review of GRN 000932.

⁷ The subject of GRN 000523 is L-leucine. We evaluated this notice and responded in a letter dated December 11, 2014, stating that we had no questions at the time regarding the notifier's GRAS conclusion.

⁸ The subject of GRNs 000400 and 000693 is D-psicose. We evaluated these notices and responded in letters respectively dated June 18, 2012 and August 28, 2017, stating that we had no questions at the time regarding the notifiers' GRAS conclusions.

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 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 APTech’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 APTech’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 APTech provided, as well as other information available to FDA, we have no questions at this time regarding APTech'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 000932 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

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Carlson -S

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Director

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