Brian Watson
BLIS Technologies Ltd.
10 Birch Street
P.O. Box 5804
Dunedin, NEW ZEALAND

Re: GRAS Notice No. GRN 000591

Dear Mr. Watson:

This letter corrects our letter signed on January 25, 2016, sent in response to GRN 000591. The purpose of this revised letter is to correct text about allergenic protein.

The Food and Drug Administration (FDA) is responding to the notice, dated July 2, 2015, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on July 17, 2015, filed it on August 5, 2015, and designated it as GRAS Notice No. GRN 000591.

The subject of the notice is *Streptococcus salivarius* K12. The notice informs FDA of the view of BLIS Technologies Ltd. (BLIS) that *S. salivarius* K12 is GRAS, through scientific procedures, for use as an ingredient in a variety of food categories, including infant[1] and toddler foods; baked goods and baking mixes; beverages and beverage bases; breakfast cereals; cheeses; chewing gum; dairy product analogs; frozen dairy desserts and mixes; gelatins, puddings, and fillings; grain products and pastas; hard and soft candy; milk and milk products; nuts and nut products; processed fruits and fruit juices; sweet sauces, toppings, and syrups; and medical foods at levels up to 20 milligrams (mg) per serving[2] (equivalent to $2x10^9$ colony forming units (CFU) per serving).

As part of its notice, BLIS includes the report of a panel of individuals (BLIS' GRAS panel) that evaluated the data and information that are the basis for BLIS' GRAS determination. BLIS considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food. BLIS' GRAS panel evaluated the identity and characteristics, estimates of dietary exposure, method of production, and product specifications, as well as published and unpublished studies supporting the safety of *S. salivarius* K12. Based on this review, BLIS' GRAS panel concluded that *S. salivarius* K12 that meets its established food grade specifications is GRAS under the conditions of its intended use.

[1] BLIS notes that *S. salivarius* K12 is not intended for use in infant formula.
[2] BLIS notes that serving sizes are based on Reference Amounts Customarily Consumed per eating occasion, as defined in 21 CFR 101.12.

BLIS discusses the identity and characteristic properties of *S. salivarius* K12. Streptococci are spherical, non-motile, Gram-positive microorganisms. BLIS states that *S. salivarius* K12 was isolated from a saliva sample obtained from a healthy child and that the strain was deposited with the ATCC under the accession number ATCC BAA 1024. BLIS provides the results of phenotypic and genotypic characterization to confirm the strain's identity. These analyses include carbohydrate fermentation profile, enzyme profile, 16S ribosomal RNA sequencing, Enterobacterial Repetitive Intergenic Consensus (ERIC)-polymerase chain reaction typing, and pulsed field gel electrophoresis. BLIS notes that the genome and megaplasmid sequence of *S. salivarius* K12 aligned with *S. salivarius* (99.8% homologous) and *Streptococcus thermophilus* (99.61% homologous). BLIS notes that *S. salivarius* is genetically distinct from pathogenic streptococci. BLIS states that *S. salivarius* K12 has been shown to be sensitive to antibiotics routinely used for the control of upper respiratory tract infections. BLIS states that although *S. salivarius* K12 is strongly suspected to harbor aminoglycoside antibiotic resistance determinants (i.e., kanamycin), the results of the bioinformatics analysis demonstrated that these genes are not associated with transposable elements and therefore, the likelihood of this antibiotic resistance being transferred to other closely-related species is low.[3]

BLIS states that *S. salivarius* is nonpathogenic. Based on the comparison of published case reports of opportunistic infection with *S. salivarius* and those involving strains of *Lactobacillus* and *Bifidobacterium*, BLIS notes that the risk of opportunistic infection from the intended uses of *S. salivarius* K12 in food would be no greater than that currently posed by strains of *Lactobacillus* and *Bifidobacterium*, whose food uses have been determined to be GRAS. BLIS states that no genes involved in pathogen adhesion, invasion, toxin or super antigen production, or regulation of virulence were identified in the results of *in vitro* and bioinformatic gene analyses.

BLIS discusses the manufacturing process for *S. salivarius* K12. Growth medium, which contains sucrose, skim milk powder, ammonium salts, and yeast extract, is inoculated with seed culture stock of *S. salivarius* K12. The culture is then incubated for 16 hours at 33°C. After fermentation, the cell mass is concentrated. The cell concentrate is mixed with a lyoprotectant (i.e., trehalose, lactitol, maltodextrin, and deionized water), and the pH of the suspension is adjusted with ammonium hydroxide or sodium hydroxide. The cell concentrate suspension is then freeze-dried and milled into a powder. The freeze-dried product is packaged and stored at 2 to 8° C.

BLIS provides specifications for *S. salivarius* K12, including a minimum content of 10^{11} CFU per gram of *S. salivarius* K12. Specifications also include limits on arsenic (≤1 mg per kilogram (kg)), cadmium (≤ 0.2 mg/kg), lead (≤ 5 mg/kg), mercury (≤0.15 mg/kg), and limits on microbial contaminants: negative for coliforms, *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*; aerobic plate count and mesophilic aerobic spores (<200 CFU/g), and yeasts and molds (<50 CFU/g). BLIS provides

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[3] BLIS states that *S. salivarius* K12 has been shown to be sensitive to antibiotics routinely used for the control of upper respiratory tract infections.

analytical data from three non-consecutive production lots of *S. salivarius* K12 to demonstrate compliance with these specifications.

BLIS provides information on the stability of *S. salivarius* K12. BLIS discusses the results of unpublished studies that demonstrate *S. salivarius* K12 is stable for up to three years when stored at 4° C; however, the viability of the organism decreases by several orders of magnitude as the temperature is elevated to 100°C. BLIS also discusses the stability of *S. salivarius* K12 in various beverage applications and reports that the organism is stable in soy milk. However, it is less stable in rice milk and water, with significant reductions in viability occurring after 2 weeks of storage; it is not stable in iced tea and fruit juice. Consequently, BLIS states that the use of novel delivery systems, such as release caps and straws, may increase the stability of *S. salivarius* K12 in foods requiring long-term storage.

BLIS estimates the dietary exposure to *S. salivarius* K12 based on the intended uses and food consumption data from the National Health and Nutrition Examination Survey (NHANES 2003-2006). BLIS reports the mean and 90th percentile (users-only) exposure to *S. salivarius* K12 to be 9.8x10^{9} CFU/person/day (2x10^{8} CFU/kg body weight (bw)/day) and 1.9x10^{10} CFU/person/day (4.5x10^{8} CFU/kg bw/day), respectively.

BLIS discusses the history of safe consumption of *S. salivarius* strains in the diet. BLIS states that *S. salivarius* strains have a history of food use as fermentation organisms (starter cultures) in the manufacture of dairy products (cheese and yogurt) and notes their occurrence in the human oral cavity, particularly during infancy.

BLIS discusses safety data specific to *S. salivarius* K12. The safety of *S. salivarius* K12 for use in food was based on information derived from placebo-controlled studies in humans evaluating established safety endpoints. BLIS summarizes a published randomized, placebo-controlled, double-blind study in human volunteers who received a dose of 10^{10} CFU/day of *S. salivarius* K12 or placebo for 28 days, followed by a 28-day wash out period. BLIS states that the results demonstrated no statistically significant differences between the treated and placebo groups and that the daily ingestion of *S. salivarius* K12 is well tolerated and does not adversely affect humans. BLIS also discusses published findings from additional clinical studies conducted with *S. salivarius* K12 in healthy and unhealthy adults and children to corroborate safety. No adverse events were reported in children or in adults administered doses up to 4x10^{10} CFU of *S. salivarius* K12/day for periods up to 14 days.

Based on the available data and information, BLIS concludes that *S. salivarius* K12 is GRAS under the conditions of its intended use.

Standards of Identity

In the notice, BLIS states its intention to use *S. salivarius* K12 in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. The Office of Food Additive Safety (OFAS) notes 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

In describing the intended use of *S. salivarius* K12 and in describing the information that BLIS relies on to conclude that *S. salivarius* K12 is GRAS under the conditions of its intended use, BLIS raises a potential issue under the labeling provisions of the Federal Food, Drug, and Cosmetic Act (FD&C Act). Under section 403(a) of the FD&C Act, a food is misbranded if its labeling is false or misleading in any particular. Section 403(r) of the FD&C Act lays out the statutory framework for the use of labeling claims that characterize the level of a nutrient in a food or that characterize the relationship of a nutrient to a disease or health-related condition. If products that contain *S. salivarius* K12 bear any claims on the label or in labeling, such claims are the purview of the Office of Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition. OFAS neither consulted with ONFL on this labeling issue nor evaluated the information in your notice to determine whether it would support any claims made about *S. salivarius* K12 on the label or in labeling.

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. *S. salivarius* K12 produced using milk-based medium requires labeling under the FD&C Act because it contains milk protein.

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 its review of BLIS' notice that *S. salivarius* K12 is GRAS for the intended uses, FDA did not consider whether section 301(ll) or any of its exemptions apply to foods containing *S. salivarius* K12. Accordingly, this response should not be construed to be a statement that foods that contain *S. salivarius* K12, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information provided by BLIS, as well as other information available to FDA, the agency has no questions at this time regarding BLIS' conclusion that *S. salivarius* K12 is GRAS as an ingredient in variety of food categories. The agency has not, however, made its own determination regarding the GRAS status of the subject use of *S. salivarius* K12. As always, it is the continuing responsibility of BLIS to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter responding to GRN 000591, as well as a copy of the information in this notice that conforms to the information in the GRAS exemption claim (proposed 21 CFR 170.36(c)(1)), is available for public review and copying at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
Date: 2019.06.05 13:33:15 -04'00'

Dennis M. Keefe, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition