Gary Yingling
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC 20004

Re: GRAS Notice No. GRN 000825

Dear Mr. Yingling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000825. We received the notice you submitted on behalf of DSM on November 27, 2018 and filed it on February 6, 2019. DSM submitted an amendment on September 25, 2019 with clarifications regarding uses, manufacturing and specifications.

The subject of the notice is beta-galactosidase enzyme preparation produced by a genetically engineered strain of *Kluyveromyces lactis* to express a modified synthetic gene encoding beta-galactosidase from *K. lactis* (beta-galactosidase enzyme preparation) for use as an enzyme at up to 130 mg Total Organic Solids/kg raw material (TOS/kg) in the processing of milk, milk powder, fermented milk products and yogurt, fresh cheeses, milk-based desserts, whey, baked goods, confectionary, cereal bars and soft drinks, and at 36 mg TOS/kg raw material in the processing of milk for non-exempt infant formulas for ages 0 to 12 months and milk-based products for ages 12 to 36 months, respectively. The notice informs us of DSM's view that these uses of beta-galactosidase enzyme preparation are GRAS through scientific procedures.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component that catalyzes the chemical reaction as well as substances used as stabilizers, preservatives, or diluents. Enzyme preparations may also contain components derived from the production organism and from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. DSM's notice provides information about the components in the beta-galactosidase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, beta-galactosidase is identified by the Enzyme Commission Number EC 3.2.1.23.[1] DSM states that the amino acid sequence of this modified beta-galactosidase differs by one amino acid from the published amino acid sequence for beta-galactosidase from *K. lactis*.[2] The modification allows for increased efficiency of the beta-galactosidase to hydrolyze lactose. The molecular weight

[1] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/2/1/23.html
[2] http://www.uniprot.org/uniprot/P00723

of the modified beta-galactosidase is 117.6 kDa.

DSM states that the *K. lactis* production strain, KLA (DS 79419), was derived from a strain isolated from cheese (*K. lactis* DS 00332)[3] and that it is non-pathogenic and non-toxigenic. DSM describes the construction of the production strain as the integration of multiple copies of an expression cassette carrying a synthetic beta-galactosidase gene from *K. lactis* into the *K. lactis* recipient strain DS 38549 at specified loci. According to DSM, the transformation results in the deletion of genes coding for the native beta-galactosidase, invertase, and arylsulfatase. DSM states that the genetic modifications, the targeted integration of seven copies of the modified beta-galactosidase into two defined loci, and the absence of the selection markers, ectopic integrations, or vector sequences are confirmed by DNA sequencing. Additionally, the stability of the introduced DNA is confirmed by the consistency of the enzyme production on an industrial scale and PCR analyses, per DSM.

DSM states that beta-galactosidase enzyme preparation is manufactured by controlled submerged fed-batch fermentation of a pure culture of the *K. lactis* production strain. The beta-galactosidase that accumulates inside the cells is released into the fermentation medium using safe and suitable lytic reagents. The enzyme is then recovered by filtration followed by ultrafiltration to concentrate. The article of commerce is a liquid enzyme preparation obtained from the enzyme concentrate after standardizing with glycerol. DSM states that the entire process is performed using food-grade raw materials and in accordance with current good manufacturing practices. DSM also states that no major food allergens from the fermentation medium are expected to be present in the final beta-galactosidase enzyme preparation.

DSM has established food grade specifications and states that the beta-galactosidase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 11th edition, 2016), and to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). DSM provides analytical data from analyses of three batches of beta-galactosidase enzyme preparation to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production strain.

DSM intends to use beta-galactosidase enzyme preparation in the processing of milk for several foods listed above, with a maximum level of 312 mg TOS/kg in the final food. DSM notes that the beta-galactosidase enzyme preparation will be denatured and inactivated during food processing and will not be functional in the final food. However, in estimating dietary exposure, DSM assumes that all the beta-galactosidase enzyme preparation will remain in the final food. DSM estimates dietary exposure to beta-galactosidase enzyme preparation to be 3.7 mg TOS/kg body weight per day (mg

[3] DSM states that *K. lactis* DS 00332 is deposited as CBS 683 at the Centraalbureau voor Schimmelcultures in Utrecht, the Netherlands (now the Westerdijk Fungal Biodiversity Institute); it is a diploid strain and has been used safely to produce beta-galactosidase.

TOS/kg bw/d) from the intended uses.[4] DSM also intends to use beta-galactosidase enzyme preparation in non-exempt infant formulas for use from birth to 12 months, and milk-based products for children 12 to 36 months of age, at a maximum level of 36 mg TOS/kg in the final formula. DSM estimates dietary exposure to beta-galactosidase enzyme preparation to be 9.6 mg TOS/kg bw/d from these intended uses.[5]

DSM relies on published information that discusses the safety of the *K. lactis* production organism and the safety of microbial enzyme preparations used in food processing. DSM discusses published genotoxicity and rat 28-day oral toxicity studies of beta-galactosidase from a related *K. lactis* strain. Additionally, DSM summarizes published and unpublished toxicological studies of related beta-galactosidases from other microorganisms. DSM also discusses the amino acid sequence similarities among related beta-galactosidases and uses the totality of information from these studies to corroborate safety of the subject of this notice.

DSM cites publicly available literature, as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes, to address potential allergenicity due to beta-galactosidase. Further, based on bioinformatic analyses, DSM reports that the beta-galactosidase does not share any biologically meaningful sequence homology or sequence identity to potential oral allergens. Based on the totality of the information available, DSM concludes that it is unlikely that oral consumption of beta-galactosidase will result in allergenic responses.

Based on the data and information summarized above, DSM concludes that beta-galactosidase enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, DSM states its intention to use beta-galactosidase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. 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.

Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (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

[4] DSM uses dairy products and baked goods intake reported in NHANES 2011-12, and beverage intake reported in USDA's 1994-1995 in their calculation of estimated daily intake of beta-galactosidase enzyme preparation.

[5] DSM uses average consumption data for infants 1 month old who are exclusively milk-fed as reported in the 2005 report of Institute of Medicine, for their calculation of estimated daily exposure of beta-galactosidase enzyme preparation in infants and toddlers up to 2 years of age.

have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of DSM's notice concluding that beta-galactosidase enzyme preparation 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 beta-galactosidase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing beta-galactosidase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that DSM provided, as well as other information available to FDA, we have no questions at this time regarding DSM's conclusion that beta-galactosidase enzyme preparation produced by a genetically engineered *K. lactis* expressing a modified synthetic gene encoding beta-galactosidase from *K. lactis* is GRAS under its intended conditions of use. This letter is not an affirmation that beta-galactosidase enzyme preparation produced by a genetically engineered *K. lactis* expressing a modified synthetic gene encoding beta-galactosidase from *K. lactis* 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 000825 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2019.11.07 11:04:13 -05'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition