



Candice Cryne
AB Enzymes GmbH
Feldbergstrasse 78
D-64293 Darmstadt
GERMANY

Re: GRAS Notice No. GRN 000756

Dear Ms. Cryne:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000756. We received AB Enzymes GmbH's (AB Enzymes) notice on January 24, 2018, and filed it on February 28, 2018.

The subject of the notice is endo-1,4-beta-glucanase overexpressed by a genetically modified *Trichoderma reesei* (endo-1,4-beta-glucanase enzyme preparation) for use as an enzyme in brewing, grain processing, and in the production of potable alcohol at a maximum level of 12 mg Total Organic Solids/kg (TOS/kg) raw material. The notice informs us of AB Enzymes' view that these uses of endo-1,4-beta-glucanase 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 components derived from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. AB Enzymes' notice provides information about each of these components in the endo-1,4-beta-glucanase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, endo-1,4-beta-glucanase is identified by the Enzyme Commission Number 3.2.1.4. The accepted name for the enzyme is cellulase and the systematic name is 4-(1,3;1,4)- β -D-glucan 4-glucanohydrolase. The CAS No. for endo-1,4-beta-glucanase is 9012-54-8. Endo-1,4-beta-glucanase is also known as endo-1,4-beta-D-glucanase; beta-1,4-glucanase; beta-1,4-endoglucan hydrolase; cellulase A; cellulysin AP; endoglucanase D; alkali cellulase; cellulase A 3; celludextrinase; 9.5 cellulase; avicelase; pancellase SS; and, 1,4-(1,3;1,4)-beta-D-glucan 4-glucanohydrolase. Endo-1,4-beta-glucanase catalyzes the endohydrolysis of (1 \rightarrow 4)-beta-D-glucosidic linkages in cellulose, lichenin and cereal beta-D-glucans. AB Enzyme states that the identity of endo-1,4-beta-glucanase was confirmed by N-terminal sequencing and mass spectrometry. AB Enzymes also states that the endo-1,4-beta-glucanase has a signal sequence of 459 amino acids in length.

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AB Enzymes states that *T. reesei* production strain RF11412 is nonpathogenic and nontoxigenic. It is constructed from a mutant strain of *T. reesei* QM6a¹ by introducing a DNA cassette containing the native endo-1,4-beta-glucanase gene, *eg11* (derived from *T. reesei* wild type QM6a), under the control of the *T. reesei* promoter and terminator, along with a selection marker. AB Enzymes states that it confirmed the integration and stability of the inserted DNA by DNA sequencing and Southern Blot analyses, respectively. AB Enzymes also confirmed, that the production strain contains no vector DNA from the expression cassette used during transformation. AB Enzymes states that the transformed DNA does not contain any antibiotic resistance genes, or any known mycotoxins based on testing of fermentation batches.

AB Enzymes states that the endo-1,4-beta-glucanase enzyme preparation is manufactured by submerged fermentation of a pure culture of the production strain, controlled to ensure production strain identity, purity, and enzyme-generating ability. The enzyme is recovered from the supernatant by separating the cell mass from the supernatant after the addition of flocculant and/or filter aids and pH adjustment, by filtration or centrifugation and concentration steps. AB Enzymes states that the enzyme is formulated as a liquid enzyme preparation with the addition of sodium benzoate and water. AB Enzymes states that the entire process is performed using food grade raw materials, and in accordance with current good manufacturing practices. AB Enzymes also states that the final enzyme preparation does not contain any major food allergens from the fermentation medium.

AB Enzymes has established food grade specifications and notes that the endo-1,4-beta-glucanase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 10th 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). AB Enzymes provides analytical data from three batches of endo-1,4-beta-glucanase enzyme preparation to demonstrate manufacturing consistency. AB Enzymes confirms the absence of the production microorganism in the commercial product with an established specification.

AB Enzymes intends to use the endo-1,4-beta-glucanase enzyme preparation as an enzyme in brewing, grain processing, and in the production of potable alcohol at a maximum level of 12 mg TOS/kg raw material. AB Enzymes estimates the dietary exposure from endo-1,4-beta-glucanase enzyme preparation by assuming that the endo-1,4-beta-glucanase enzyme preparation will be used at maximum intended levels and that all the enzyme preparation will remain in the final food. Based on these assumptions, AB Enzymes estimates a maximum dietary exposure of endo-1,4-beta-glucanase enzyme preparation from all intended uses to be 0.05 mg TOS/kg body weight per day (mg TOS/kg bw/d).

AB Enzymes relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the production organism. Additionally, AB Enzymes discusses unpublished toxicological studies using the endo-1,4-beta-glucanase enzyme to corroborate safety; and states that the endo-1,4-beta-glucanase enzyme is neither mutagenic nor clastogenic and that a sub-chronic oral toxicity study conducted in rats shows that consumption of endo-1,4-beta-glucanase enzyme did not cause any treatment-related adverse effects up to the highest dose tested, equivalent to 1000 mg TOS/kg bw/d.

AB Enzymes discusses potential food allergenicity of endo-1,4-beta-glucanase enzyme. AB Enzymes states that no sequence identity matches over 35% were found when endo-1,4-beta-

¹ The identity of *T. reesei* strain QM6a has been confirmed by PCR fingerprinting and sequence analyses.

glucanase enzyme was compared to known allergens stored in the AllergenOnline Database, Allergen Database for Food Safety, and AllerMatch using either an 80-amino acid sequence sliding window or a conventional FASTA overall homology searches. AB Enzymes further discusses publicly available literature as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes. AB Enzymes also states that there is a lack of historical evidence for majority of enzymes being allergens. Based on the totality of the information available, AB Enzymes concludes that it is unlikely that oral consumption of endo-1,4-beta-glucanase enzyme will result in allergenic responses.

Based on the data and information summarized above, AB Enzymes concludes that endo-1,4-beta-glucanase enzyme preparation is GRAS for its intended use.

Section 301(II) of the Federal Food, Drug, and Cosmetic Act (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 AB Enzymes' notice concluding that endo-1,4-beta-glucanase enzyme preparation 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 endo-1,4-beta-glucanase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing endo-1,4-beta-glucanase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(II).

Conclusions

Based on the information that AB Enzymes provided, as well as other information available to FDA, we have no questions at this time regarding AB Enzymes' conclusion that endo-1,4-beta-glucanase over expressed by a genetically modified *T. reesei* is GRAS under its intended conditions of use. This letter is not an affirmation that endo-1,4-beta-glucanase over expressed by a genetically modified *T. reesei* 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 00756 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,
**Dennis M.
Keefe -S**



Digitally signed by Dennis M. Keefe -S
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