



Candice Cryne
AB Enzymes GmbH
Feldbergstr. 78
D-64293 Darmstadt
GERMANY

Re: GRAS Notice No. GRN 000817

Dear Ms. Cryne:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000817. We received AB Enzymes GmbH (AB Enzymes)'s notice on October 5, 2018, and filed it on November 2, 2018. We received an amendment containing additional safety information on May 29, 2019.

The subject of the notice is serine endopeptidase enzyme preparation produced by *Trichoderma reesei* expressing a gene encoding serine endopeptidase from *Malbranchea cinnamomea* (serine endopeptidase enzyme preparation) for use as an enzyme at up to 10 mg Total Organic Solids (TOS)/kg of raw material in the manufacture of vegetable and animal protein hydrolysates. The notice informs us of AB Enzymes' view that these uses of serine endopeptidase 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. AB Enzymes' notice provides information about the components in the serine endopeptidase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, serine endopeptidase is identified by the Enzyme Commission Number 3.4.21.65.¹ AB Enzymes states that the serine endopeptidase is 281 amino acids in length with a corresponding molecular weight of 28.5 kDa.

AB Enzymes states that the *T. reesei* production strain RF8963 is non-pathogenic and non-toxicogenic. AB Enzymes describes the construction of the *T. reesei* production strain by targeted integration of an expression cassette carrying a gene encoding a serine endopeptidase from *M. cinnamomea* fused to a promoter and a terminator from *T.*

¹ <https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/4/21/65.html>

reesei. AB Enzymes states that the plasmid also carries a synthetic *amdS* gene encoding acetamidase, linked to a promoter and a terminator, all from *Aspergillus nidulans* for growth and selection of the *T. reesei* transformants. AB Enzymes states that the stability of the introduced DNA has been confirmed by Southern blot analysis. AB Enzymes also states that the final production strain does not contain any functional or transferable antibiotic resistance genes.

AB Enzymes states that serine endopeptidase enzyme preparation is manufactured by submerged fed-batch fermentation of a pure culture of the production strain. AB Enzymes states that fermentation is carried out under controlled conditions and that the enzyme is secreted into the fermentation media. After fermentation, flocculants and filter aids are added to the media containing the serine endopeptidase enzyme, at controlled pH and temperature to facilitate enzyme separation. The enzyme is then recovered from the fermentation media by filtration or centrifugation and concentrated, followed by polish and germ filtration. The resulting liquid enzyme concentrate is used for the toxicological studies discussed in the notice. The liquid enzyme concentrate is formulated to a preparation with sodium benzoate, glycerol, sorbitol, and water. AB Enzymes states that the entire process is performed in accordance with current good manufacturing practices. AB Enzymes also states that the final serine endopeptidase enzyme preparation does not contain any major food allergens from the fermentation media.

AB Enzymes has established food grade specification and states that the serine endopeptidase 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 serine endopeptidase liquid enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production strain.

AB Enzymes intends to use serine endopeptidase enzyme preparation in the manufacture of vegetable-derived protein hydrolysates such as soy, wheat, maize, etc., and animal-derived protein hydrolysates, such as whey proteins, caseins, meat, fish, collagen, and gelatin. AB Enzymes intends to use serine endopeptidase enzyme preparation at a maximum level of 10 mg TOS/kg of protein raw material. AB Enzymes notes that the serine endopeptidase enzyme preparation will be deactivated or removed during the production of the protein hydrolysates. However, in estimating dietary exposure, AB Enzymes assumes that all of the serine endopeptidase enzyme preparation will remain in the final food. AB Enzymes estimated dietary exposure from all uses of serine endopeptidase enzyme preparation to be 0.0625 mg TOS/kg body weight per day (mg TOS/kg bw/d).²

AB Enzymes relies on published information that discusses the safety of microbial

² AB Enzymes uses the Budget method to calculate estimated dietary exposure to serine endopeptidase enzyme preparation based on consumption of a maximum of 12.5 g of solid foods and 25 g of beverages per kg body weight per day.

enzyme preparations used in food processing, including the safety of the *T. reesei* production organism. Additionally, AB Enzymes summarizes unpublished toxicological studies using serine endopeptidase enzyme liquid concentrate to corroborate safety of the intended uses of this enzyme preparation. These studies include an *in vitro* mammalian cell gene mutation assay in mouse lymphoma and *in vitro* micronucleus assay in cultured human lymphocytes with and without metabolic activation. AB Enzymes also discusses the results from an unpublished 13-week oral toxicity study in rats using the serine endopeptidase liquid enzyme concentrate that did not cause any treatment-related adverse effects up to the highest dose tested, equivalent to 1000 mg TOS/kg bw/d. AB Enzymes calculates a margin of exposure based on the No Observed Adverse Effect Level of 1000 mg TOS/kg/bw/d from this study and the estimated maximum dietary exposure from the intended uses of serine endopeptidase enzyme preparation, to be approximately 16000. FDA notes the margin of exposure is based on unpublished safety studies and is only corroborative of the published information regarding enzyme preparations used in food processing.

AB Enzymes discusses publicly available literature as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes in food to address potential allergenicity due to the proposed uses of serine endopeptidase. Further, based on bioinformatic analyses, AB Enzymes reports that the serine endopeptidase does not share any biologically meaningful sequence homology or sequence identity to potential oral allergens.³ Based on the totality of the information available, AB Enzymes concludes that it is unlikely that oral consumption of serine endopeptidase will result in allergic responses.

Based on the data and information summarized above, AB Enzymes concludes that serine endopeptidase enzyme preparation is GRAS for its intended use.

Use in Products under USDA Jurisdiction

As provided under 21 CFR 170.270, during our evaluation of GRN 000817, we coordinated with the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture. Under the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act, FSIS determines the efficacy and suitability of ingredients used in meat, poultry, and egg products, and prescribes safe conditions of use. Suitability relates to the ingredient's effectiveness in performing its intended technical effect and the assurance that the ingredient's use will not result in products that are adulterated or misleading for consumers.

FSIS has completed its review and has no objection to the use of the serine endopeptidase enzyme preparation for use as an enzyme at up to 10 mg TOS/kg of protein, in meat products for the production of gelatins and animal protein hydrolysates.

³ AB Enzymes identified sequence homology to six putative allergens (Tri m 2, Tri r 2, Pen n 13, Pen n 18 and Asp fl 1) during bioinformatic analyses. Based on further analyses of homology to an 8 amino acid window, potential B cell epitopes, and protease digestion predictions, AB Enzymes concluded that serine endopeptidase is not likely to cause an allergic response from its consumption.

Regarding labeling, FSIS would consider the substance a processing aid that does not require labeling under the requested conditions of use.

Any additional questions regarding regulatory guidance from FSIS should be directed to: Dr. Melanie Abley, Acting Deputy Director, Risk Management and Innovations Staff, Office of Policy and Program Development, Food Safety and Inspection Service, at (202) 690-6573 or via e-mail at Melanie.Abley@usda.gov.

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 serine endopeptidase 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 serine endopeptidase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing serine endopeptidase 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 serine endopeptidase enzyme preparation produced by *T. reesei* expressing a gene encoding serine endopeptidase from *M. cinnamomea* is GRAS under its intended conditions of use. This letter is not an affirmation that serine endopeptidase enzyme preparation produced by *T. reesei* expressing a gene encoding serine endopeptidase from *M. cinnamomea* 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 000817 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.

Carlson -S

Digitally signed by
Susan J. Carlson -S
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Susan Carlson, Ph.D.

Director

Division of Food Ingredients

Office of Food Additive Safety

Center for Food Safety

and Applied Nutrition