



Nobuo Okado
Shin Nihon Chemical Co., Ltd.
19-10 Showa-cho, Anjo Aichi 446-0063
JAPAN

Re: GRAS Notice No. GRN 000965

Dear Mr. Okado:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000965. We received Shin Nihon Chemical Co., Ltd. (Shin Nihon)'s GRAS notice on June 22, 2020 and filed it on December 3, 2020. Shin Nihon submitted amendments to the notice on May 14, 2021, July 2, 2021, and July 11, 2021 providing additional safety information and clarifying information designated confidential.¹

The subject of the notice is endo-arabinase enzyme preparation produced by *Aspergillus tubingensis*, overexpressing the gene encoding endo-arabinase (arabinase enzyme preparation) for use as an enzyme at up to 48 mg Total Organic Solids (TOS)/kg raw material in fruit and vegetable processing, fruit fillings, vegetable purees, non-alcoholic fruit, and as a filter aid in vegetable-based juices and wines. The notice informs us of Shin Nihon's view that this use of arabinase enzyme preparation is 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. Shin Nihon's notice provides information about the components in the arabinase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, endo-arabinase is identified by the Chemical Abstracts Service number 75432-96-1 and the Enzyme Commission Number 3.2.1.99.² Shin Nihon provides the amino acid sequence of arabinase and calculates the molecular mass of the notified enzyme is 34.5 kDa based on the amino acid sequence.

¹ The July 2, 2021 and July 11, 2021 amendments stated that the information contained in GRN 000965 that was designated confidential is not considered confidential by Shin Nihon.

² <https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/2/1/99.html>

Shin Nihon states that the *A. tubingensis* GPA41 production organism is non-pathogenic and non-toxicogenic. Shin Nihon states that the production strain was isolated from a food source and identified based on sequence analysis. Shin Nihon selected the production strain based on its ability to produce arabinase, and the lack of ability to produce mycotoxin, and deposited it at the National Institute of Technology and Evaluation's (NITE) Biological Resource Center under No. NITE SD 00284.

Shin Nihon states that the arabinase enzyme preparation is manufactured by submerged fermentation of a pure culture of the *A. tubingensis* GPA41 production strain grown in a wheat bran- and yeast extract-based fermentation medium under controlled conditions. The arabinase enzyme is secreted into the medium and then recovered by extraction with water. This is followed by membrane separation, concentration, and ultrafiltration steps. The enzyme concentrate is filtered to remove any insoluble materials and residual production strain. The enzyme concentrate is used for the safety studies prior to formulation with glycerol to produce a brown colored liquid arabinase enzyme preparation. Shin Nihon states that the entire process is performed using food grade raw materials and in accordance with current good manufacturing practices. Shin Nihon states that the wheat bran, an ingredient of the fermentation medium, is absent in the final enzyme preparation.

Shin Nihon has established food grade specifications and states that the arabinase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 12th edition, 2021), 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). Shin Nihon provides data from analyses of three batches of ultrafiltered arabinase enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production organism and antibiotic activity. Shin Nihon also provides results to demonstrate that the arabinase enzyme preparation is stable for up to 12 months when stored under ambient temperatures and in airtight containers.

Shin Nihon intends to use arabinase enzyme preparation at a maximum use level of 48 mg TOS/kg raw material in the processing of fruits and vegetables that are added to finished food and beverage products, and during downstream production of vegetable-based juices and wines.³ Shin Nihon states that the arabinase reduces haze in fruit juices and helps as a filtration aid by catalyzing the endohydrolysis of α -(1,5)-arabinofuranosidic linkages in arabinans present in fruit and vegetable cell walls. Shin Nihon notes that the arabinase enzyme preparation will be inactivated, reduced, or removed during processing. Shin Nihon, however, estimates a maximum dietary exposure to arabinase enzyme preparation to be 1.35 mg TOS/kg body weight per day (mg TOS/kg bw/d) from all the intended uses with the assumption that all the arabinase

³ Shin Nihon estimates a cumulative use of 48 mg TOS/kg food based on use during processing of fruits and vegetables (24 mg TOS/kg processed fruit and vegetables) and during production of fruit juice or wine products (24 mg TOS/kg fruit juice or wine).

will remain in the final food.⁴

Shin Nihon relies on published information to demonstrate the safety of the *A. tubingensis* production organism and the safety of microbial enzyme preparations used in food processing. Shin Nihon did not detect any mycotoxins, potential toxic secondary metabolites, or antibiotic activity based on analytical test results of the arabinase enzyme concentrate. Shin Nihon also concludes that their arabinase enzyme concentrate lacks genotoxic potential based on results from bacterial reverse mutation, *in vitro* mammalian chromosomal aberration, *in vivo* mammalian erythrocyte micronucleus, and *in vivo* comet assays. Shin Nihon further states that a 90-day oral toxicity study conducted in rats showed consumption of arabinase enzyme concentrate did not cause any treatment-related adverse effects when dosed at a level corresponding to 1530 mg/kg bw/d, the highest tested. Shin Nihon calculates a margin of exposure of 1133 by comparing the 1530 mg/kg bw/d dose tested in the 90-day rat oral toxicity study to the estimated daily intake for the arabinase enzyme preparation (1.35 mg TOS/kg bw/d).

Shin Nihon discusses publicly available literature, as well as the conclusions of several organizations and working groups, concerning the low risk of allergenicity posed by oral consumption of enzymes to address the potential for allergenicity to arabinase. Based on bioinformatic analyses, Shin Nihon reports no matches between the amino acid sequences of the arabinase and the primary sequences of known allergens based on the guidelines developed by Codex Alimentarius Commission (FAO, 2009). Based on the totality of the information available, Shin Nihon concludes that it is unlikely that oral consumption of arabinase enzyme from the intended use will result in allergenic responses. Shin Nihon further states that arabinase does not share homology or structural similarity with any known animal venom proteins and toxins or virulence factors based on bioinformatic analyses using UniProtKB/Swiss-Prot/TrEMBL databases.

Based on the data and information summarized above, Shin Nihon concludes that arabinase enzyme preparation is GRAS for its intended use.

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 have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of Shin Nihon's notice concluding that arabinase 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 arabinase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing arabinase enzyme preparation, if introduced or

⁴ Shin Nihon uses the Budget method to estimate dietary exposure to arabinase enzyme preparation based on consumption of a maximum of 50 g of solid foods and 100 g of non-milk beverages/kg bw/d.

delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Shin Nihon provided, as well as other information available to FDA, we have no questions at this time regarding Shin Nihon's conclusion that arabinase enzyme preparation produced by *A. tubingensis* overexpressing the gene encoding for arabinase is GRAS under its intended conditions of use. This letter is not an affirmation that arabinase enzyme preparation produced by *A. tubingensis* overexpressing the gene encoding for arabinase 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 000965 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

**Susan J.
Carlson -S**

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Susan Carlson, Ph.D.
Director
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