



Kristi O. Smedley, Ph.D.
Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Road
Woodbridge, VA 22192

Re: GRAS Notice No. GRN 000802

Dear Dr. Smedley:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000802. We received the notice that you submitted on behalf of Nomad Bioscience GmbH (Nomad) on July 12, 2018, and filed it on August 20, 2018. On October 23, 2018, April 12, 2019, and April 20, 2019, we received amendments containing additional information about the intended uses, allergenicity potential, and product specifications.

The subject of the notice is a preparation containing six endolysin proteins singly or in combination (endolysin preparation) for use as an antimicrobial to control *Clostridium perfringens* in cooked meats, including red meats and poultry, meat- and poultry-derived products such as gravies and sauces, and other categories of cooked products that contain meat.¹ The endolysin preparation will be used at levels up to 10 mg/kg of cooked food. Endolysin preparation is intended to be used in retail and institutional food operations, such as grocery stores, food trucks, catering operations, buffet restaurants, and school cafeterias. The notice informs us of Nomad's view that these uses of endolysin preparation are GRAS through scientific procedures.

Our use of "endolysin preparation" in this letter is not our recommendation of that term as an appropriate common or usual name for declaring the substance in accordance with FDA's labeling requirements. Under 21 CFR 101.4, each ingredient must be declared by its common or usual name. In addition, 21 CFR 102.5 outlines general principles to use when establishing common or usual names for non-standardized foods. Issues associated with labeling and the common or usual name of a food ingredient are under the purview of the Office of Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition. The Office of Food Additive Safety did not consult with ONFL regarding the appropriate common or usual name for "endolysin preparation."²

¹ In an amendment dated April 20, 2019, Nomad states that the product is not intended to substitute standard methods for the safe handling and preparation of food and is intended for use in addition to standard practices for preventing temperature abuse in cooked foods that can lead to the growth of *C. perfringens*.

² "Endolysin preparation" labels for retail and other uses should clearly identify the common and usual name for the ingredient as well as any other components and should provide instructions for use.

Nomad describes six recombinant endolysins that will either singly or in combination comprise the endolysin preparation: PlyCP26F, PlyCP39O, psm, CP25L, ZP173, and ZP278. Endolysins are peptidoglycan-degrading enzymes found in bacteriophages (phages); they are utilized by phages to lyse and exit the bacterial hosts at the end of the lytic cycle. Nomad states that the recombinant endolysins are derived from phages that infect *Clostridium* and have high specificity for *C. perfringens*. Nomad provides the sources, NCBI accession numbers, and the amino acid sequences of the endolysins. Nomad states these endolysins will be produced in *Nicotiana benthamiana* or in food plant species of spinach (*Spinacia oleracea*), red beet (*Beta vulgaris*), or lettuce (*Lactuca sativa*). These food plant species were used to produce colicins that were the subjects of GRN 000593 and GRN 000676.³

Nomad describes the method of manufacture for the endolysin preparation. Nomad states that each recombinant endolysin was expressed from an engineered tobacco mosaic virus containing the specific endolysin gene. Nomad describes two methods used to induce the expression of endolysin genes in host plants: *Agrobacterium tumefaciens*-mediated induction and ethanol induction of transgenic plants. After induction by either method, the plants are incubated for 5–10 days to allow for accumulation of the endolysins. The leaves and part of the stems are harvested and homogenized. After removal of insoluble material, endolysins are concentrated and purified by a series of precipitation, centrifugation, and filtration steps. The resulting concentrate containing a single type of endolysin can be used singly or blended with other endolysins to produce the final bulk product, which can be spray-dried or lyophilized for ease of storage. Nomad states that if the endolysins are produced using *N. benthamiana*, further purification is performed using hydrophobic exchange chromatography followed by ion exchange chromatography. Nomad states that the manufacturing processes of the endolysin preparation is conducted according to current good manufacturing practice and that all raw materials and processing aids are food-grade. All plant materials are produced and handled according to the principles of good agriculture and collection practices.

Nomad provides food grade specifications for endolysin preparation. These specifications include limits for total heavy metals (≤ 30 mg/kg), lead (≤ 5 mg/kg), cadmium (≤ 5 mg/kg) and microorganisms. Nomad also provides the acceptance criteria for specific activity, physical properties, and stability (>6 months). Additionally, for endolysin preparation produced using *N. benthamiana*, Nomad provides specifications for nicotine (≤ 90 ng/mg) and anabasine (≤ 15 ng/mg). Nomad provides results of batch analyses to demonstrate that the endolysin preparation can be manufactured to meet specifications.

Nomad states that the estimated dietary exposure to endolysins from intended uses of the endolysin preparation on food is 2.6 mg/person/day (mg/p/d) based on the maximum application rate of 10 mg/kg. Nomad also states that the exposure to

³ GRN 000593 and GRN 000676 describe the use of colicin preparations produced in spinach, red beet, and lettuce for use as an antimicrobial on fresh and processed fruits and vegetables, and on meat, respectively. FDA evaluated these notices and responded in letters dated December 18, 2015, and May 15, 2017, stating that we had no questions at that time regarding the notifier's GRAS conclusions.

endolysins from bacteriophage antimicrobial treatments and all background sources is 0.4 mg/p/d.

Nomad discusses published studies to show that phages and, by extension, components of phages such as endolysins are ubiquitous in nature and that humans are exposed to phages and their components from birth. Nomad states that endolysins have high specificity to target structures of the bacterial cell wall. Furthermore, ingested endolysins will be digested and metabolized in the gastrointestinal tract similarly to any other protein. Nomad discusses allergenicity potential of the endolysins and concludes that endolysins are not expected to be allergenic. Nomad states that there are no reports in the literature of adverse events related to ingestion of phages or phage-produced endolysins. Nomad incorporates the published data and information contained in GRN 000593, GRN 000676, and GRN 000738⁴ and other published data to show that impurities from the plant hosts present a low safety risk to consumers.

Nomad provides data from its own unpublished studies demonstrating the bactericidal effects of endolysin preparation in the laboratory and when applied to cooked poultry and beef.

Based on the totality of data and information available, Nomad concludes that the intended use of endolysin preparation is GRAS.

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 Nomad's notice concluding that endolysin 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 endolysin preparation. Accordingly, our response should not be construed to be a statement that foods containing endolysin preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(II).

Conclusions

Based on the information that Nomad provided, as well as other information available to FDA, we have no questions at this time regarding Nomad's conclusion that endolysin preparation is GRAS under its intended conditions of use. This letter is not an affirmation that endolysin preparation is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient


⁴ GRN 000738 describes the use of thaumatin I, thaumatin II or combinations thereof as a sweetener in several food categories. FDA evaluated this notice and responded in a letter dated April 18, 2018, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

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 000802 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,
**Michael A.
Adams -S**

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

 Digitally signed by Michael A.
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