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

Re: GRAS Notice No. GRN 000824

Dear Dr. Smedley:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000824. We received the notice you submitted on behalf of Nomad Bioscience GmbH (Nomad) on November 27, 2018 and filed it on December 20, 2018. Nomad submitted amendments on April 24, 2019, May 3, 2019, and September 25, 2019. The amendments provided additional information about specifications, application method, exposure estimates, efficacy data, and use on fish of the order Siluriformes.

The subject of the notice is bacteriocin preparations specific to Salmonellae (BPSS) for use as an antimicrobial agent on meat, poultry, fish, and egg products at levels of 0.1 - 3 mg per kg (or per L for fluid products). The notice informs us of Nomad's view that these uses of BPSS are GRAS through scientific procedures.

Our use of the term "bacteriocin preparations specific to Salmonellae" or "BPSS" 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 nonstandardized 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 "bacteriocin preparations specific to Salmonellae" or "BPSS."

Nomad describes five bacteriocins derived from and active against Salmonellae: SalE1a, SalE1b, SalE2, SalE3, and SalE7. They are intended to be used singly or in combination in BPSS. The bacteriocin gene sequences were obtained from non-typhoidal, bacteriocinogenic *Salmonella* strains that naturally attack susceptible strains of *S. enterica* and were identified based on sequence similarities to *E. coli* bacteriocins.

Nomad states that BPSS are produced by expressing recombinant proteins in the plant *Nicotiana benthamiana*, as described in GRN 000775. Each protein is expressed using a plant virus-based system, either based on *Tobacco mosaic virus* or on *Potato virus X*. In either case, a virus expression vector is engineered to produce the recombinant

bacteriocin after introduction into the plant. These viruses are nonpathogenic to animals and are inactivated in subsequent protein isolation steps. One method for introducing the virus expression vector into the plant is by infiltrating *Agrobacterium tumefaciens* harboring DNA encoding the virus expression vector directly into *N. benthamiana* leaves. The introduced DNA replicates and is expressed in the leaves, and the virus vector accumulates along with the recombinant bacteriocin. An alternative method involves developing stable, transgenic *N. benthamiana* lines containing the DNA encoding the virus expression vector. The virus expression vector is inducible by ethanol.

After inducing virus vector expression by either method, the plants are incubated for five to ten days to allow for bacteriocin accumulation. Leaves and stems expressing bacteriocins are homogenized, and insoluble material is removed. Protein is enriched by a series of acid precipitation, centrifugation, and filtration steps. Cation exchange chromatography is used to remove additional host cell proteins and plant metabolites such as polyphenols and alkaloids. Individual bacteriocin preparations are standardized into liquid or powdered formulations, which are combined into a blended final product. Nomad states that it manufactures bacteriocin preparations according to current good manufacturing practice and that all raw materials and processing aids are food grade.

Nomad provides food grade specifications for BPSS. Specifications include limits for microorganisms, nicotine (<400 ng/mg), and anabasine (<100 ng/mg); acceptance criteria for specific activity; physical properties; and stability (>6 months). Nomad provides results of batch analyses to demonstrate that BPSS can be manufactured to meet specifications.

Nomad estimates dietary exposure to bacteriocins to be 0.8 mg/person/day based on the intended uses at the maximum application rate of 3 mg/kg of meat, poultry, fish, and whole eggs. Nomad notes that cooking meat, poultry, fish, and egg products to recommended temperatures would inactivate bacteriocins.

Nomad references published data and information supporting the safe use of the BPSS. Nomad states that bacteriocins from Salmonellae have no known activity against beneficial intestinal bacteria and that they attack bacterial structures and cellular targets that do not exist in mammalian tissues. Humans are exposed to bacteriocin-producing bacteria that naturally reside in the gut. Nomad notes that bacteriocins from Salmonellae are rapidly degraded by gastrointestinal enzymes and are not expected to have allergenic potential.

Nomad provides data demonstrating efficacy of BPSS against a wide range of *Salmonella* ecotypes on poultry, meat, fish, and eggs.

Based on the totality of data and information available, Nomad concludes that the intended uses of BPSS are GRAS.

Use in Products under USDA Jurisdiction

As provided under 21 CFR 170.270, during our evaluation of GRN 000824, 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 BPSS as an antimicrobial for the treatment of meat (including Siluriformes fish), poultry, and egg products at 0.1 - 3 mg of BPSS per kg of treated food. FSIS advises that it would consider BPSS a processing aid that does not require labeling under the requested conditions of use. Please contact Ms. Rosalyn Murphy-Jenkins at (301) 504-0879 or via email at Rosalyn.Murphy-Jenkins@fsis.usda.gov if Nomad has questions regarding labeling.

FSIS requested that we advise Nomad to seek regulatory guidance from its Risk Management and Innovations Staff (RMIS) about the use of BPSS in meat, Siluriformes fish, poultry, and egg products. Nomad should direct such an inquiry to Dr. Melvin Carter, Director, RMIS, Office of Policy and Program Development, FSIS by email at Melvin.Carter@fsis.usda.gov.

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 Nomad's notice concluding that BPSS are GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing BPSS. Accordingly, our response should not be construed to be a statement that foods containing BPSS, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

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 BPSS are GRAS under its intended conditions of use. This letter is not an affirmation that BPSS are 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 000824 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2019.10.21 08:44:34 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Food Additive Safety
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cc: Melvin Carter, Ph.D.
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