



Kristi Smedley, Ph.D,
Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Road
Woodbridge, VA 22192-5755

Re: GRAS Notice No. GRN 000676

Dear Dr. Smedley:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000676. We received the notice, which you submitted on behalf of Nomad Bioscience GmbH (Nomad), on November 1, 2016 and filed it on November 23, 2016.

The subject of the notice is preparations containing one or more colicin proteins (colicin preparations) for use as an antimicrobial treatment on meat at levels of 1 to 10 mg colicin per kg treated meat. The notice informs us of Nomad's view that this use of colicin preparations is GRAS through scientific procedures. The notice expands Nomad's previous GRAS conclusion (GRN 000593 for the use of colicin preparations on fresh and processed fruits and vegetables¹) to include use on meat.

Our use of "colicin preparations" 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 "colicin preparations."

Nomad describes nine recombinant colicin proteins intended for use singly or in combination in colicin preparations: colicin E1, colicin E7, colicin Ia, colicin M, colicin N, colicin K, colicin U, colicin 5, and colicin B. These proteins belong to the colicin family of bacteriocins and are synthesized by *Escherichia coli* or by other enteric bacteria in the human intestine. Nomad states that the recombinant colicins are identical to the colicins produced by enteric bacteria and provides the CAS number (if applicable), amino acid sequence, UniProt protein sequence database entry, and molecular weight of each colicin (Table 1).

¹ FDA responded to GRN 000593 with a letter dated December 18, 2015

Table 1
Colicins intended for use in colicin preparations

Colicin	CAS Number	UniProt Entry	Molecular Weight (kDa)
E1	11032-88-5	P02978	57.28
E7		Q47112	61.35
Ia	37217-77-9	E9LLU1	69.427
M	39386-24-8	P05820	29.45
N		P08083	41.743
K	1403-40-3	Q47502	59.661
U		O24681	66.289
5		Q47500	53.137
B	11038-10-1	P05819	54.86

Nomad describes the method of manufacture of colicin preparations. Each recombinant colicin protein is produced in leaves of spinach (*Spinacia oleracea*), red beet (*Beta vulgaris*), or lettuce (*Lactuca sativa*). All plants are grown indoors under environmentally controlled conditions under principles of good agriculture and collection practices.² Each protein is expressed from recombinant *Tobacco mosaic virus* or *Potato virus X* engineered to contain a colicin gene. The plant viral vector replicates in the host plant resulting in accumulation of colicin protein. These viruses are nonpathogenic to animals and are inactivated in subsequent protein isolation steps. The viral vector may be introduced to the plant either by *Agrobacterium tumefaciens*-mediated transient expression or by ethanol induction of stably transformed plants. After induction by either method, the plants are incubated for five to ten days to allow for colicin accumulation, and leaves and stems are homogenized. After removing insoluble material, protein is enriched by a series of acid precipitation, centrifugation, and filtration steps. Nomad intends to offer colicin preparations with two different degrees of purity: lower purity “colicin concentrate” estimated to be 30 to 60 percent pure and higher purity “colicin isolate” estimated to be 80 to 90 percent pure. The more purified preparation undergoes an additional ion-exchange chromatography step. Nomad notes that residual materials derive from safely consumed plants and are not expected to raise safety issues. Nomad states that it manufactures colicin preparations according to current good manufacturing practices. All raw materials and processing aids are food grade.

Nomad provides food grade specifications for colicin isolate and colicin concentrate preparations. Specifications include limits for total heavy metals (<30 mg/kg), lead (<5 mg/kg), microbial contaminants (including *Agrobacterium*), and acceptance criteria for specific activity, physical properties, and stability (>6 months).

² Nomad notes that plant-derived residuals are discarded.

Nomad estimates dietary exposure to colicins from intended uses on red meats to be 1.5 mg/person/day (mg/p/d) based on estimated daily consumption of 150 g red meat/p/d and the maximum application rate of 10 mg/kg. Dietary exposure from intended uses on produce is estimated to be 4.1 mg/p/d based on per capita consumption data from the United States Department of Agriculture (USDA) Food Availability (Per Capita) Data System. However, cooking meats to recommended temperatures would destroy colicins. Therefore, according to Nomad, the addition of colicins to meat and the subsequent cooking of the meat to recommended temperatures will not result in an increase in total dietary exposure to colicins.

Nomad discusses published data and information supporting the safety of colicin. Nomad states that humans are continuously exposed to colicin-producing bacteria in the colon and estimates exposure to be 3 mg of colicins per day. Piglets fed high levels of colicin did not present any adverse effects. Nomad notes that colicins are not expected to be allergenic or immunogenic to humans because they are rapidly degraded by gastrointestinal proteases. A comparison of the amino acid sequences of colicins to a database of known allergens found low potential for allergenicity using criteria proposed in Codex Alimentarius guidelines.³ Nomad also notes that consumption of colicins is unlikely to promote colicin resistance among bacteria in the human colon because colicins are rapidly inactivated by the low pH of the stomach and are digested by gastrointestinal proteases.

Nomad provides data from its own studies and from published scientific literature demonstrating the bacteriocidal effects of colicins on several pathogenic strains of *E. coli*, including O157:H7, both in laboratory studies and when applied to red meat.

Based on the data and information described above, Nomad concludes that this additional use of its colicin preparations is GRAS.

Some Uses May Require Regulatory Actions by the United States Environmental Protection Agency (EPA)

Antimicrobial agents used on raw agricultural commodities may require registration as pesticides with EPA under the Federal Insecticide, Fungicide, and Rodenticide Act. FDA's evaluation of this GRAS notice does not relieve the obligation to register colicin preparations as a pesticide for uses regulated by EPA. For information about the regulatory status of your product when used as a pesticide, please contact EPA's Office of Pesticide Products, Antimicrobial Division.

Use in Products under USDA Jurisdiction

As provided under 21 CFR 170.270, during our evaluation of GRN 000676, we coordinated with the Food Safety and Inspection Service (FSIS) of the USDA. Under the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products

³ CODEX Alimentarius. 2003. Report of the Fourth Session of the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology. CODEX Alimentarius, Yokohama, Japan.

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 colicin preparations as an antimicrobial spray application on meat products at levels of 1 to 10 mg/kg.

FSIS requested that you direct any additional questions regarding regulatory guidance from its Risk, Innovations, and Management Staff (RIMS) about the use of colicin preparations in meat, poultry, and egg products to Dr. William K. Shaw Jr., Director, RIMS, Office of Policy and Program Development, FSIS by email at William.Shaw@fsis.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 Nomad's notice concluding that colicin preparations are GRAS under its intended conditions of use, we did not consider whether section 301(II) or any of its exemptions apply to foods containing colicin preparations. Accordingly, our response should not be construed to be a statement that foods containing colicin preparations, 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 colicin preparations are GRAS under its intended conditions of use. This letter is not an affirmation that colicin preparations 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 000676 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
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ou=People, 0.9.2342.19200300.100.1.1=1300042713,
cn=Michael A. Adams -S
Date: 2017.05.15 16:30:33 -04'00'

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

cc: William K. Shaw Jr., Ph.D.
Director
USDA/FSIS/OPPD/RIMS
Stop Code 3782, Patriots Plaza III
1400 Independence Ave. SW
Washington, DC 20250-3700