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

Re: GRAS Notice No. GRN 001070

Dear Dr. Smedley:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001070. We received the notice that you submitted on behalf of FINK TEC GmbH (FINK) on May 13, 2022, and filed it on September 2, 2022. FINK submitted amendments to the notice on November 10, 2022, November 21, 2022, January 6, 2023[1], and February 27, 2023, providing clarifying information on the identity, intended use, manufacturing process, specifications, and safety narrative, including results from the analysis of three non-consecutive batches, and information supporting the intended use as an antimicrobial.

The subject of the notice is preparations containing six bacteriophages[2] (phage) specific to *Salmonella* serovars (*Salmonella* phage preparation) for use as an antimicrobial at levels ranging from 1 x 10^5 to 1 x 10^7 plaque-forming units (PFU)/g of fresh and processed fruits and vegetables. The notice informs us of FINK's view that these uses of *Salmonella* phage preparation are GRAS through scientific procedures.

FINK describes *Salmonella* phage preparation as a colorless to light yellowish liquid, and describes the identity of the 11 pages, designated ELB17, MP82, KAZ99a, RMP11k, RMS3b, TAT2F, DIN2, MP75, FV7M4, RMP9, and OBO18, as double-stranded DNA, lytic monophages specific to *Salmonella* serovars, which are produced and purified separately, and then subsequently mixed in equal proportions. FINK states that the phages are deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) in Braunschweig, Germany with the deposit designations DSM 26158, DSM 26173, DSM 33039, DSM 33040, DSM 33043, DSM 33044, DSM 33045, DSM 104023, DSM 26125, DSM 26157, and DSM 33041, respectively. FINK explains that the genomes of the 11 phages were fully characterized using next generation sequencing and analyzed using standard bioinformatics methods. FINK states that the 11 phages are not genetically engineered, and do not contain genes encoding any known

[1] FDA notes that the January 6, 2023, amendment was originally transmitted as a Word document on December 22, 2022. At FDA's request, the notifier transmitted a PDF copy of the same amendment on January 6, 2023.

[2] FINK intends to produce *Salmonella* phage preparations containing mixtures of six phages from eleven different phages described and identified in GRN 001070.

Escherichia coli toxin genes and virulence factors, other toxin genes, antibiotic resistance genes, or genes encoding for proteins with allergenic properties. FINK screened the lytic activity and specificity of the 11 phages to *Salmonella* serovars against 41 *Salmonella* serovars and a strain of *E. coli* K-12, noting that six of the 11 phages did also exhibit antimicrobial activity against *E. coli* K-12. FINK states that no additional experiments to detect cross reactivity against distantly related genera were performed.

FINK describes the method of manufacture for *Salmonella* phage preparation. Each monophage is propagated using one of three host strains, *Salmonella* Paratyphi B var. Java strain ATCC BAA-1584, *Salmonella bongori* strain ATCC 43975, and *E. coli* K-12 strain ATCC 47076. FINK explains that *Salmonella* Paratyphi B var. Java and *S. bongori* were selected as host strains as they are known to cause fewer reported infections in humans and they do not produce any enterotoxins, as reported in the literature. Further, FINK states that, wherever possible, the non-pathogenic, non-toxigenic *E. coli* K-12 strain ATCC 47076 host strain is used for propagation of the phages.

The 11 monophages are produced individually by aerobic fermentation. For each phage, a specified host strain is grown to a target optical density before each monophage stock is added at a predetermined multiplicity of infection and incubated under aerobic conditions. After fermentation and lysis are complete, the lysate is titered to determine the concentration of the progeny phages. Following this, the lysate is clarified by an initial continuous centrifugation process and filtered using tangential flow filtration to remove unlysed host cells and host cell debris. The lysate is then concentrated, filtered to remove the fermentation media, exchanged with phosphate-buffered saline, and sterilized using filtration. Each monophage is tested for titer (> 5 x 10^9 PFU/mL), microbial sterility (no growth), and identity (conforms to reference profile) prior to combining. After the monophages pass the quality control step, equal proportions of six monophages are combined, filtered using tangential flow filtration, blended, filtered using sterile filtration, packaged, and stored. FINK states the applied *Salmonella* phage preparation will contain a total phage concentration between 5 x 10^9 and 10^{10} PFU/mL of solution. FINK explains that *Salmonella* phage preparation is diluted with water at the application site, yielding a working solution of 10^5 to 10^7 PFU/mL, depending on the application.

FINK provides the following specifications for *Salmonella* phage preparation, including, concentration (> 10^9 PFU/mL); limits for microorganisms, including total plate count (< 50 colony forming units (CFU)/g), yeast and mold (< 100 CFU/g), *Enterobacteriaceae* (< 100 CFU/g), sulfite-reducing *Clostridia* spp. (< 1000 CFU/g), *Staphylococcus* spp. (< 10 CFU/g), and *Salmonella* serovars (absent/25 g); and a limit for lead (< 0.01 mg/L). FINK provides the results from three non-consecutive lots to demonstrate that *Salmonella* phage preparation can be manufactured to conform with the provided specifications. FINK states that all raw materials used in the manufacture of *Salmonella* phage preparation are food grade, are used in accordance with U.S. regulations or were concluded to be GRAS for their respective uses, and are not derived from allergens or

allergenic sources.[3]

FINK estimates the dietary exposure to *Salmonella* phage preparation based on the maximum use level of 1 x 10^7 PFU/g of food and the average per capita daily consumption of fresh and processed fruits and vegetables reported in the Food Availability (Per Capita) Data System (Economic Research Service, U.S. Department of Agriculture, 2022). FINK estimates the dietary exposure to *Salmonella* phage preparation from the intended uses to be 1.6 µg/person/d.

FINK discusses the safety of phages in general, noting that phages are ubiquitous in the environment, and are found in the human and mammalian gut, and are common components of water and various foods consumed by humans. Further, FINK describes the differences between lytic and temperate phages, noting that lytic phages lack the genes responsible for lysogenic conversion, and are therefore unable to passively invade a host bacterium and transfer genes from one host bacterium to another. FINK concludes that infection of a host bacterium by a lytic phage results in the death of the bacterial host, and explains that all of the phages in *Salmonella* phage preparation are strictly lytic. FINK conducted a literature review through November 2022 and states that no new information contradicting their GRAS conclusion was identified. FINK states that, despite six of the 11 phages displaying antimicrobial activity against the assessed *Salmonella* serovars and a strain of *E. coli* K-12, this does not impact their safety conclusion. FINK explains that some phages, as reported in the literature, exhibit cross reactivity between *Salmonella* serovars and *E. coli*, which are closely related genera belonging to the *Enterobacteriaceae* family. FINK notes that other commercially available phage preparations have GRAS status, and *Salmonella* phage preparation is equivalent to these products.[4]

FINK provides data demonstrating the antimicrobial effects of *Salmonella* phage preparation when applied to fruits (apples), and vegetables (lettuce) at an application titer of 10^7 PFU/cm^2 and 2.5 x 10^6 PFU/cm^2 of food, respectively.

Based on the data and information provided in the submission, FINK concludes that *Salmonella* phage preparation is GRAS for its intended use.

Section 301(ll) of the 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

[3] In the November 10, 2022, amendment, FINK clarifies that the fermentation media does not contain soy peptone.

[4] FINK references GRNs 000218, 000435, 000468, 000528, 000724, 000752, 000757, 000827, 000834, 000917, and 000966. We evaluated these GRNs, and responded in letters respectively dated June 22, 2007, February 22, 2013, December 23, 2013, December 23, 2014, April 10, 2018, July 13, 2018, August 3, 2018, August 12, 2019, November 8, 2019, September 10, 2020, and October 6, 2021, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of FINK's notice concluding that *Salmonella* phage 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 *Salmonella* phage preparation. Accordingly, our response should not be construed to be a statement that foods containing *Salmonella* phage preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that FINK provided, as well as other information available to FDA, we have no questions at this time regarding FINK's conclusion that *Salmonella* phage preparation is GRAS under its intended conditions of use. This letter is not an affirmation that *Salmonella* phage 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 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 001070 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.03.09 17:57:06 -05'00'

Susan J. Carlson, Ph.D.
Director
Division of Food Ingredients
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