Alexander Sulakvelidze, Ph.D.
Intralytix, Inc.
8681 Robert Fulton Drive
Columbia, MD 21046

Re: GRAS Notice No. GRN 000966

Dear Dr. Sulakvelidze:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000966. We received Intralytix, Inc.'s (Intralytix) notice on August 18, 2020 and filed it on December 3, 2020. Intralytix submitted amendments to the notice on February 4, 2021, March 5, 2021, April 30, 2021, May 6, 2021, May 12, 2021, and July 27, 2021, providing clarifying information on the identity, intended use, manufacturing process, specifications, and safety narrative, including results from the analysis of four non-consecutive batches, information supporting the intended use as an antimicrobial, and the removal of ground poultry from the intended uses.

The subject of the notice is preparations containing three to eight bacteriophages (phage) specific to *Campylobacter jejuni* (*Campylobacter* phage preparation) for use as an antimicrobial on raw red meat, including whole carcasses, primals, subprimals, cuts, trimmings, and organs; and raw poultry, including carcasses and parts at levels up to 10^8 plaque-forming units (PFU)/g of food. The notice informs us of Intralytix's view that this use of *Campylobacter* phage preparation is GRAS through scientific procedures.

Intralytix describes *Campylobacter* phage preparation as a clear to opalescent liquid, and describes the identity of three of the pages, designated J350, J375, and J386, as double-stranded DNA, lytic monophages specific to *C. jejuni*, which are produced and purified separately, and then subsequently mixed.[1] Intralytix states that the phages are deposited in the American Type Culture Collection (ATCC) in Manassas, VA with the deposit designations PTA-126840, PTA-126842, and PTA-126845, respectively. Intralytix explains that the three phages were fully characterized using electron microscopy, genotypic fingerprinting, and whole-genome sequence analysis. Intralytix states that the three phages are not genetically engineered, and do not contain genes encoding any known toxin genes, antibiotic resistance genes and bacterial 16s rRNA genes. Intralytix screened the lytic activity and specificity of the three phages to *C. jejuni* against 61 *C. jejuni* strains and 25 non-target bacterial strains (*Staphylococcus aureus*, *Listeria* spp., *Pseudomonas aeruginosa*, *Salmonella* serovars, and *Escherichia coli*),

[1] The notifier intends to produce *Campylobacter* phage preparations containing a mixture of three to eight double-stranded DNA, lytic phages specific to *C. jejuni*, and subject to the same manufacturing and safety standards described in GRN 000966.

noting that the three phages did not lyse any of the tested, non-target bacterial strains.

Intralytix describes the method of manufacture for *Campylobacter* phage preparation. Each monophage is propagated using a *C. jejuni* host strain. Intralytix explains that the *C. jejuni* host strains used for propagation have been characterized, including the biochemical properties and genomic composition of the strains, and states that the *C. jejuni* host strains do not contain the functionally active genes for any known enterotoxins and were susceptible to all five antibiotics tested.

The three monophages are produced individually by microaerobic (reduced oxygen) fermentation. The host strain is grown to a target concentration before each monophage stock is added at a predetermined multiplicity of infection and incubated under microaerophilic conditions. After fermentation and lysis are complete, the lysate is clarified by sterile filtration, concentrated, washed with phosphate-buffered saline, and sterilized using filtration. Each monophage is tested for potency (PFU/mL), microbial purity, and identity prior to combining. After the monophages pass the quality control step, appropriate amounts of each monophage are combined, filtered using sterile filtration, packaged, and stored at 2-8 °C. Intralytix states the *Campylobacter* phage preparation is diluted with water at the application site, yielding a working solution of 10^9 PFU/mL, and is applied at a rate that ensures the final concentration is no greater than 10^8 PFU/g of food.

Intralytix provides the following specifications for *Campylobacter* phage preparation: potency (≥ 10^{10} PFU/mL), microbial purity (no microbial growth after 14 days), endotoxin content (≤ 25,000 endotoxin units/mL), and identity (all component phages are present). Intralytix provides the results from four non-consecutive lots to demonstrate that *Campylobacter* phage preparation can be manufactured to conform with the provided specifications. Intralytix states that all raw materials used in the manufacture of *Campylobacter* phage preparation are safe for use in food and that the final preparation is free from allergens.

Intralytix estimates the dietary exposure to *Campylobacter* phage preparation based on the intended use level of 10^8 PFU/g of food and the average per capita daily consumption of red meat and poultry estimated using data from the Food Availability (Per Capita) Data System (Economic Research Service, U.S. Department of Agriculture, 2020). Intralytix estimates the dietary exposure to *Campylobacter* phage preparation from the intended uses to be 5.8 µg/person/d.

Intralytix discusses the safety of phages in general, noting that phages are ubiquitous in the environment, and are found in the human and animal gut, the human oral cavity, sewage, are commonly consumed in drinking water, and are natural components of various foods consumed by humans. Further, Intralytix states that studies have demonstrated that lytic bacteriophages are obligate intracellular parasites of bacteria and are not infectious in humans or in other mammals. Intralytix explains that the phages in *Campylobacter* phage preparation are strictly lytic. Intralytix conducted a literature review through July 2020 and summarizes the results from published studies demonstrating that exposure to phages does not elicit any adverse effects in humans and

animals. Intralytix also summarizes the results from published studies evaluating the use of phages to control for the presence of *Campylobacter* spp. in foods. Intralytix notes that other commercially available phage preparations have GRAS status, and its *Campylobacter* phage preparation is equivalent to these products.[2]

Intralytix provides data demonstrating the antimicrobial effects of *Campylobacter* phage preparation when applied to poultry (chicken breast), and red meat (veal loin chops) at ≤ 10^8 PFU/g of food.

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

Use in Products under USDA Jurisdiction

As provided under 21 CFR 170.270, during our evaluation of GRN 000966, 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 *Campylobacter* phage preparation, a preparation containing the three *C. jejuni* specific phages identified and characterized in GRN 000966, as an antimicrobial on raw red meat, including whole carcasses, primals, subprimals, cuts, trimmings, and organs; and raw poultry, including carcasses and parts up to a level of 10^8 PFU/g of food. Regarding labeling, FSIS would consider the substance a processing aid that does not require labeling under the accepted above conditions of use.

As described above, Intralytix intends to produce phages that are not identified in GRN 000966. These additional phages must comply with the conditions described in GRN 000966 to add to the existing phage preparation. FSIS requires an additional review of these phages for inclusion into the FSIS Directive 7120.1 Safe and Suitable Ingredients used in the Production of Meat, Poultry, and Egg Products.

FSIS requested that we advise you to seek regulatory guidance from its Risk Management and Innovations Staff (RMIS) about the use of *Campylobacter* phage preparation in meat, poultry, and egg products. You 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.

[2] Intralytix references GRNs 000218, 000435, 000468, 000528, 000672, 000724, 000752, 000757, 000834. We evaluated these GRNs, and responded in letters respectively dated June 22, 2007, February 22, 2013, December 23, 2013, December 23, 2014, March 27, 2017, April 10, 2018, July 13, 2018, August 3, 2018, November 8, 2019, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

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

Conclusions

Based on the information that Intralytix provided, as well as other information available to FDA, we have no questions at this time regarding Intralytix's conclusion that *Campylobacter* phage preparation is GRAS under its intended conditions of use. This letter is not an affirmation that *Campylobacter* 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 000966 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2021.10.06 17:48:36 -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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